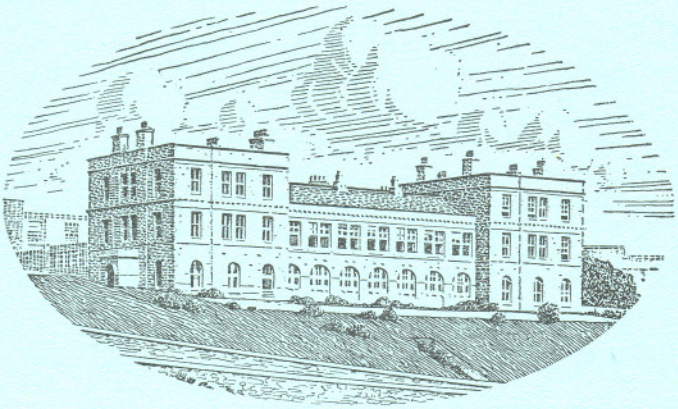
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



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THE BIOMASS OF THE BOTTOM FAUNA IN THE ENGLISH CHANNEL OFF PLYMOUTH

By N. A. Holme

The Plymouth Laboratory

(Text-figs. 1–7)

CONTENTS

INTRODUCTION

The earliest survey of the bottom fauna in the English Channel off Plymouth is that of Allen (1899), who made a large number of dredge hauls in the neighbourhood of the Eddystone. His sampling was necessarily qualitative, and not until a quarter of a century later were any quantitative studies undertaken (Ford, 1923). Following the work of C. G. J. Petersen and others in Denmark, Ford made a survey of the communities near Plymouth, which he found to be *Venus* communities of two types, the occurrence of each type depending upon the grade of deposit.

Many workers have criticized the use of the Petersen grab as a quantitative instrument in deposits other than soft mud. It clearly does not dig deeply enough to sample all the fauna, but it has given valuable results for comparative purposes.

Further quantitative work at Plymouth has been undertaken for more limited areas by Steven (1930) and by Smith (1932), using the Petersen grab and conical dredge respectively. Mare (1942) made a detailed study of the inhabitants of a muddy ground a few miles offshore, with particular reference to the micro-organisms. She gives the only available data on the quantity of living tissue per unit area of the sea-bed at Plymouth.

Elsewhere in the British Isles, Davis (1923, 1925) and Stephen (1923) have studied benthic communities in the North Sea, and more recently Jones (1951), in an account of the communities off the Isle of Man, has given figures for the quantity of living tissue per unit area.

It is well known that in the early nineteen-thirties the productivity of the sea in the western part of the English Channel started to decline. This change, associated with the replacement of a water-mass characterized by the plank-tonic chaetognath *Sagitta elegans* with one characterized by *S. setosa*, may be the cause of a subsequent decline in the numbers of young fish caught in a standard net, and probably also in a decrease in the populations of other organisms. Unfortunately, there are usually insufficient data to record changes in population densities, and a need was felt for further quantitative data by means of which any future changes in productivity could be followed.

The quantity of life to be found in deposits of sands, gravels or muds on the sea-bed can be more readily assessed than most other forms of life in the sea, and it was felt that a survey of the bottom fauna, using a bottom sampler similar to that previously described (Holme, 1949) would provide a basis for following such changes.

I am indebted to Captain C. A. Hoodless, and the crew of R.V. *Sabella* for their skill in operation of the gear at sea. I would like to thank Dr M. N. Hill for loan of his free-fall core sampler, and Prof. W. B. R. King, F.R.S., for identifying the rock samples obtained with this instrument. Mr F. A. J. Armstrong has given much assistance in suggesting methods for dry-weight determinations, and in other ways. The following have kindly identified or checked certain groups: Mr G. M. Spooner (Amphipoda), Miss P. Kott (Tunicata), Dr H. G. Vevers (Echinodermata), and Dr C. Burdon-Jones (Enteropneusta).

Conditions

The area sampled is situated in the English Channel within 16 miles of Plymouth, and lies between 50° 6′ N. and 50° 20′ N., and between 4° 5′ W. and 4° 16′ W. The coastline between Lizard Point in Cornwall and Bolt Head in Devonshire sweeps northward to form a large open bay, thus providing slight protection from Atlantic swell, particularly in the western part of the bay, around Falmouth. Off the Eddystone, however, protection is negligible, but tidal streams in the bay are somewhat weaker than in the main part of the Channel to the southward.

Depth. A mile or two off the coast near Plymouth the bottom shelves away steeply from 20 to 40 m., and then slopes down very gently so that in mid-channel, 60 miles to the south, the depth is only 100 m. Except for three stations near shore, the area covered in this survey is on this gently sloping bottom, in depths of 40–70 m. Twenty stations were worked in the survey; they were placed at 2-mile intervals along three lines running southward from the shore (Fig. 1).

Tidal streams. Tidal streams near shore are variable in direction and strength, but out by the Eddystone they set east and west, reaching a velocity of over 1 knot (1829 m./hr.) at spring tides.

Wave action. It is difficult to assess the effects of wave action on the burrowing fauna. Atlantic swell and waves generated in the Channel must cause considerable disturbance in shallow water—in Whitsand Bay, for example, where large lamellibranchs such as *Ensis* are often washed in alive during storms. In deeper water the effects of wave action must be much reduced. Allen (1899) considered that the abundance of hydroids and the polyzoan *Cellaria*, which root themselves in the sand, was evidence that the grounds in the neighbourhood of the Eddystone were not subjected to violent disturbances at any time. In the immediate vicinity of the Eddystone reef, however, there is evidence of considerable wave action and tidal scour (Allen, 1899, p. 376; Smith, 1932, p. 253).

Deposits. The texture of the deposits is very variable and at certain stations inside the Eddystone a small change in position due to the ship drifting between one haul of the bottom-sampler and the next often results in a marked change in the nature of the sediment brought up (e.g. at stations A3 and A4). Samples of deposit, as brought up in the bottom-sampler, have been mechanically graded. The Wentworth grade scale has been adopted, and the soils have been graded through gauze sieves of the following apertures: 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{5}$ and $\frac{1}{8}$ mm. A separation of particles over and under $\frac{1}{32}$ mm. was made by sedimentation and repeated decantations. (The method is not described here in detail: further work involving mechanical analysis of soils in relation to their lamellibranch faunas is in progress, and a detailed account will be published with this work.)

The results of mechanical analysis are given in Table I, and cumulative curves for some stations are shown in Fig. 2.

Close inshore, in Whitsand Bay, a fairly clean sand is found; farther offshore this gives way to a patchwork of grounds extending out as far as the Eddystone. Off Whitsand Bay is the 'Rame mud' which is in fact largely composed of fine sand (60% between $\frac{1}{8}$ and $\frac{1}{32}$ mm. grade at A2). Farther out the deposits range from coarse muddy sand to fine gravel mixtures: these two types are found both at A3 and A4. Where tidal scour is strong, there may occur patches of gravel, often largely composed of shell-remains and so forming a 'shell-gravel'. A typical shell-gravel occurs close to the Eddystone

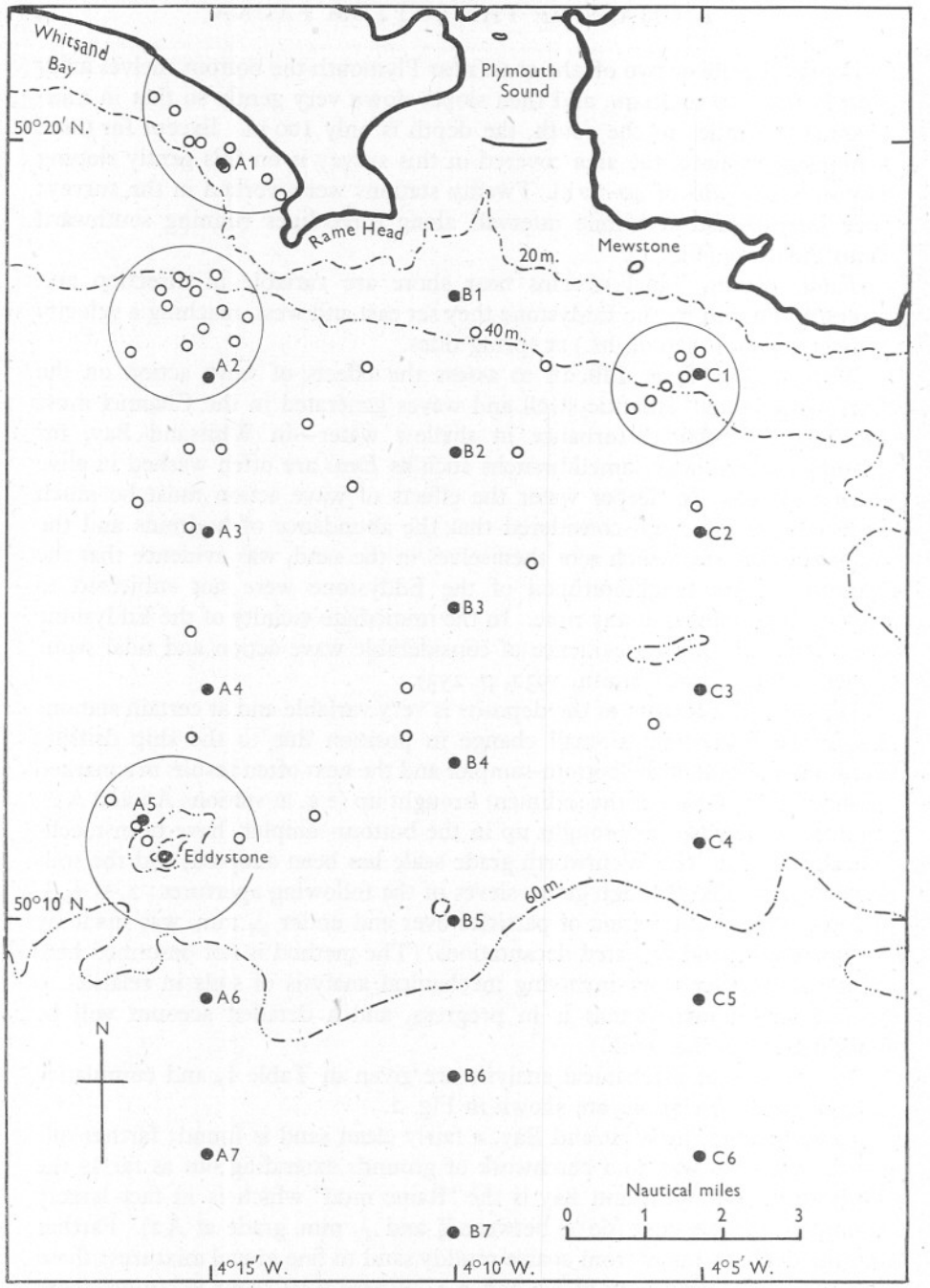

Fig. 1. Chart of the English Channel off Plymouth. Depth contours in metres. ●, 1950 stations; ○, Ford's stations; the four areas where samples have been grouped together (see p. 40) are enclosed in circles.

(A5), and there are other patches off Plymouth Sound (B1) and off the Mewstone (C1). In such places there are probably outcrops of bare rock in pockets of which the shell gravel rests. To the south and east of the Eddystone lies a fairly clean fine sand, its composition varying little from station to station (A6, 7, B4, 5, 6, 7, C4, 5, 6.)

Nowhere was the soil below the surface found to be black from the presence of ferrous sulphide, and the soils therefore may be assumed to be well

TABLE I. SOIL GRADES, EXPRESSED AS PERCENTAGES BY WEIGHT

(Figures in brackets after station numbers indicate the serial number of the haul.)

	Grade (mm.)							
Station	>2	2–1·0	1·0–0·5	0·5–0·25	0·25–0·20	0·20–0·125	0·125–0·0313	<0·0313
A1	0·07	0·37	1·11	6·09	8·62	63·74	19·76	0·22
A2	0·13	1·29	1·87	4·06	1·67	11·14	60·27	19·58
A3 (1–2)	0·71	1·20	10·35	26·54	5·49	35·95	18·24	1·52
A3 (3–4)	28·90	14·18	9·81	9·00	1·62	12·31	17·15	7·03
A4 (1)	1·20	2·99	9·56	30·57	7·77	25·96	19·04	2·90
A4 (2)	27·73	10·86	7·73	13·73	3·29	13·29	17·69	5·69
A5	49·16	38·04	9·36	2·03	0·18	0·35	0·52	0·35
A6	0	0·15	2·37	14·59	7·70	61·63	12·30	1·26
A7	0·07	0·20	1·92	17·98	6·37	63·04	9·36	1·06
B1	48·03	32·05	16·51	1·48	0·09	0·35	0·87	0·61
B2	27·05	8·08	7·03	9·22	2·07	11·48	26·46	8·60
B3	0·32	1·60	4·87	6·85	1·79	44·01	36·19	4·36
B4	0·45	0·53	3·04	10·22	3·82	55·87	23·48	2·59
B5	0·07	0·75	3·40	7·34	10·81	59·28	16·93	1·43
B5*	0·44	0·89	3·34	9·39	12·54	60·01	12·59	0·79
B6	0·06	1·07	3·74	11·87	6·53	62·73	13·00	1·01
B7	0	0·16	1·81	14·90	6·74	67·01	8·11	1·26
C1 (3–4)	14·45	41·80	37·75	4·22	0·25	0·29	0·63	0·63
C2 (3)	6·93	8·45	7·19	14·32	3·10	21·56	32·58	5·88
C3 (2)	21·32	16·73	4·18	2·43	0·47	6·95	36·03	11·88
C4	0	0·15	0·66	2·93	4·75	69·63	19·76	2·12
C4†	0·07	0·82	0·67	1·80	2·10	55·17	38·38	0·97
C5	0	0·05	0·31	6·55	11·27	67·35	13·26	1·21
C6	0	0·15	0·95	11·66	6·82	69·94	9·53	0·95

* 5 December 1950 (hauls 37–38). † 7 November 1950.

oxygenated. I have found this the usual condition of soils in the English Channel. The only soil so far found to be black below the surface was in Torbay (p. 11), where tidal streams are weak and stagnant conditions resulting from deposition of organic matter may arise from time to time.

The low percentage of silt (<0·0313 mm.) in the deposits outside the Eddystone suggests that there is little or no *net* deposition in the area. Thus benthic filter- and deposit-feeding organisms cannot be dependent on a continual rain of dead organisms, faecal pellets or other organic detritus from the overlying water, except possibly during calm weather and at neap tides when there is a minimum of disturbance near the bottom. Normally such detritus must stay in suspension to be fed on by filter-feeding planktonic and

benthic organisms or by bacteria. These conclusions are supported by the work of Armstrong & Harvey (1950), who found that water samples taken very close to the bottom had a phosphorus content only a little higher than that of the overlying water. From this it was inferred that there was no material deposit of detritus rich in phosphorus.

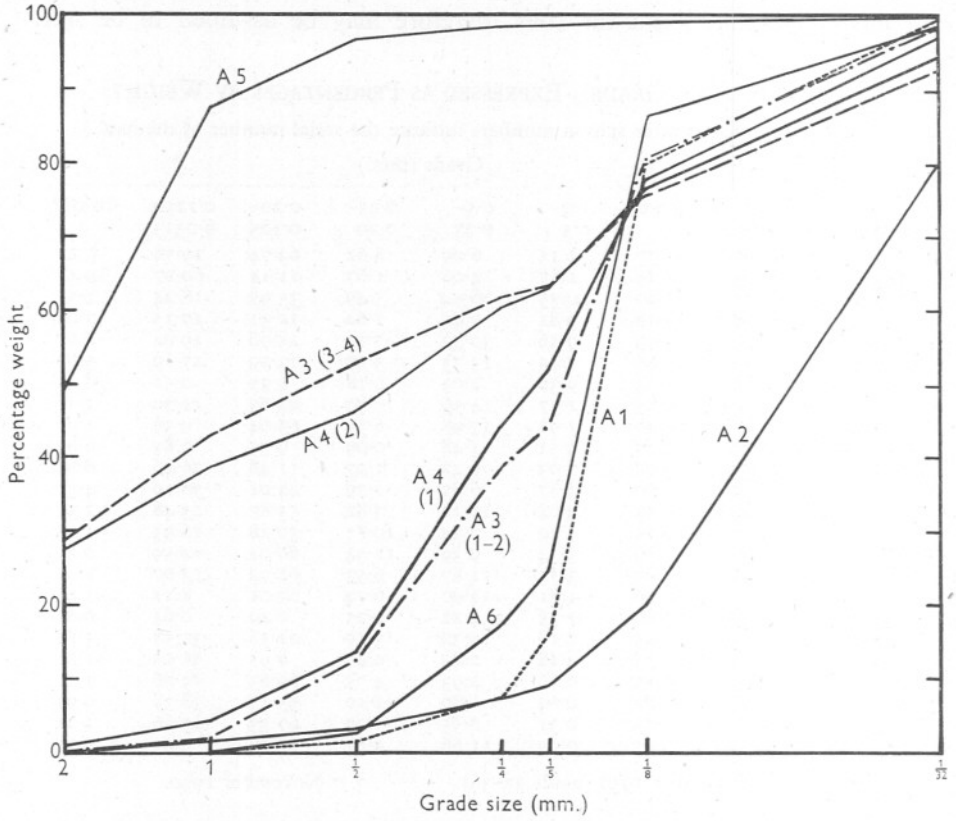

Fig. 2. Cumulative curves of the soil grades of stations on the 'A' line. Note the variation in soil grade in different hauls at A3 and A4. The soil at A7, which is not included, is similar to that at A6.

Depth of sediment. Until lately, nothing was known of the depth of sediment overlying the solid rock forming the floor of the English Channel. Recent work by King (1950) and by M. N. Hill, using a modified Stetson free-fall corer, has shown that there is only a thin veneer of sediment in many places in the western half of the Channel.

Core-samples have been taken at seventeen of the twenty stations worked in the benthos survey, and rock has been found at eleven of these (Table II). When the coring tube strikes rock it often penetrates a short distance, the

rock breaking off and forming a plug in the jaws of the tube. In this way a core of the overlying sediment may be obtained. If the coring tube fails to enter rock a core of sand is often retained by an arrangement of flap-valves near the mouth of the tube. The depth of the sediment has been calculated from the volume of the sample. Where no rock was taken this represents the minimum depth of sediment. Possibly some of the depths recorded at stations where no rock was taken represent the total depth of sediment, the corer not having sufficient momentum after passing through 30 cm. or more of sand to penetrate the rock.

TABLE II. DEPTH OF SEDIMENT AND GEOLOGICAL NATURE OF THE UNDERLYING ROCK

	Depth of sediment (cm.)	Rock
A 1 *a**	*c.* 1	Devonian slates
*b**	18·0	Devonian slates
A 2	10·4	Red rubble, probably N.R.S.
A 3	>71·5	..
A 4	>33·8	..
A 6	21·8	Red rubble breccia with limestone fragments. Almost certainly N.R.S.
A 7	32·2	Fine red rather marly N.R.S.
B 3	19·4	Breccia, mainly small slate fragments. Almost certainly N.R.S.
B 3/4†	>*c.* 50	..
B 4	36·0	Breccia of quartzite fragments. N.R.S.
B 5	33·3	Firm fine N.R.S.
B 6	>25·0	..
B 7‡	21·7	Fairly coarse N.R.S.
C 1	19·4	Fine, typical, N.R.S.
C 2	31·4	Very fine loamy N.R.S.
C 3	0	Mica schist
C 4	>33·3	..
C 5	>38·9	..
C 6	>27·8	..

* Sample *a* from 100 yards west of the wreck in Whitsand Bay, sample *b* from 100 yards north-west of the wreck.
† Mid-way between B 3 and B 4.
‡ Three previous drops had failed to take a rock sample.
N.R.S., New Red Sandstone.

Estimated depths of sediment are shown in Table II. At C 3 rock is exposed at the surface, and at A 1 the sediment was only 1 cm. deep in one haul. At the other stations where rock was taken the minimum depth was 10·4 cm., and the mean 25·0 cm. Although most of the burrowing fauna is believed to be present in the top 10 or 20 cm. of soil, it is evident that the shallowness of the soil may often restrict the occurrence or growth of deeper burrowing species, some of which are thought to penetrate at least 30 cm. into the sediment.

Methods

Collection of samples

Method of collecting

Samples were taken with a modified form of 'scoop-sampler', as already described (Holme, 1949), but having two counter-rotating scoops instead of the single one (Fig. 3). 54 kg. of weights were added to the frame, bringing the total weight to about 115 kg. It was hoped that the use of two scoops would

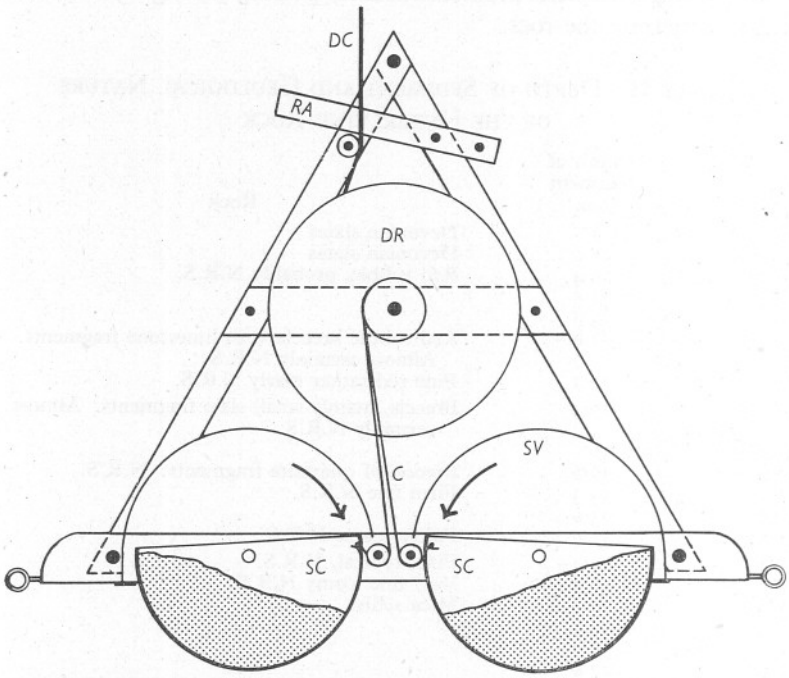

Fig. 3. Diagram of the double-scoop sampler (cf. Holme, 1949, Figs. 1 and 2). The scoops are in the closed position. *C*, small cables which pull the scoops round; *DC*, the main cable which has unwound from the drum and by which the sampler is hoisted; *DR*, drum; *RA*, release arm; *SC*, scoops; *SV*, scoop-covers. The soil sample is stippled.

eliminate the lateral movement of the frame during digging, and so produce a larger sample. Samples taken with the new instrument were, however, not larger than with the previous instrument, but twice the area is sampled at each haul. In addition, the faunas taken by the two scoops can be compared, to give data on the degree of patchiness of the bottom-fauna.

Each scoop samples a rectangular area which in vertical section is nearly semicircular, like half a cheese. If the scoop is digging to its fullest extent an area $36 \times 15{\cdot}3$ cm. is sampled, which is equal to 0·055 m.^{2}, in practice the scoops do not usually dig to the maximum depth of 15 cm., and a correspondingly smaller surface area is covered. In addition, the area sampled

decreases with depth in the sediment. For practical purposes each scoop is assumed to sample 0·05 m.^{2}.

No doubt the scoops would dig more deeply in the firmer sediments if the frame were more heavily weighted, but the weight of the apparatus is limited by the size of the ship and the facilities available for handling it on board.

Five hauls were made with the sampler at each station, covering a total area of 0·5 m.^{2}. The ship was allowed to drift while the hauls were being taken, but as sampling was always carried out in fine weather the amount of drift was not great. Once or twice a marker float was anchored at the original position, and the ship was found to drift not more than $\frac{1}{4}$–$\frac{1}{2}$ mile during the 20–30 min. necessary to take the five samples (see p. 14 and p. 29, station B2).

On grounds within a few miles of the shore it is possible to drift over a variety of types of bottom-soil while sampling at a station. On these grounds the soils are sometimes so patchy that a drift of only a few yards may result in a complete change in the nature of the sample brought up (see also Ford, 1923, p. 167). On such grounds the fauna listed for any one station may therefore represent samples from the different 'communities' characterizing the various grades of bottom soils. For the purpose in hand, of assessing the total quantity of life on the sea-bed, this is no great disadvantage, but it would be unwise to attempt to correlate the distribution of species at these stations with the soil grades as shown in Table I. Under such conditions each haul would have to be treated as a separate sample.

On grounds farther offshore, the drift of the ship while on station has no appreciable effect on the fauna brought up (see p. 30).

Validity of the samples

The volume of soil brought up by the sampler is some indication of the depth to which it is digging, but since there is little information on the vertical distribution of species in the soil its efficiency can only be judged against that of other instruments.

It is well known that on soils other than mud the 1/10 m.^{2} Petersen grab does not dig deep enough to sample all the fauna. Johansen (1927) gives an interesting comparison between the performances of the Petersen grab and the Knudsen sampler, which can dig to a depth of 30 cm. in sand. It has already been shown (Holme, 1949) that the 'scoop-sampler' samples sandy grounds more efficiently than does the Petersen grab, and a further comparison is given in Table III.

From observations on intertidal banks it is known that the majority of individuals are to be found in the top 15 cm. or so, and Johansen (1927), working with the Knudsen sampler in shallow water, considers that 'invertebrates are not taken beyond about 12–25 cm. down in the sea floor'.

A brief survey by Molander (1928) of the vertical distribution of the fauna in Gullmar Fjord, using a square coring instrument with an occluding

shutter, indicates that the majority of species and individuals are to be found in the top 5 cm. of the sediment, few occurring below 10 cm. The maximum depth of sampling was only 15 cm., however, so some deep-living species may have been missed.

In general it is probably safe to assume that most individuals occur in the top 15 cm., but the few living below this depth are mostly rather large forms and sometimes must contribute largely to the total weight of animal tissue per unit area.

Undoubtedly the 'scoop-sampler', which digs to a maximum of 15 cm., misses deep burrowing forms, such as *Upogebia* and *Callianassa*—which are probably much more numerous than the figures given in this paper suggest. For some species, however, the *effective* sampling depth is greater than 15 cm., as with large lamellibranchs (particularly *Lutraria*) whose siphons are from time to time taken in the scoop. These animals live perhaps 30 cm. down in the sediment and are seldom taken, but from the size of the severed siphon the size of the whole animal can be estimated.

It is uncertain to what extent active surface-living forms can evade the sampler. Small fish, prawns, etc., can probably dart out of the way as the sampler descends, but it seems unlikely that crabs or scallops (*Pecten*, *Chlamys*) can move fast enough to escape. Once the sampler reaches the sea-bed its construction makes it difficult for them to escape.

There is a small loss of soil and possibly of animals as the sampler reaches the surface and the water in the scoop-cover empties out around the scoop; and once on board the water with contained animals may slop on the deck, particularly if there is any swell at the time. These losses, however, are considered to have been small.

While testing a new 'suction'-coring apparatus (Fig. 4), a comparison was made between the number of individuals taken against those taken at the same place with the $\frac{1}{20}$ m.^{2} single-scoop sampler and a $\frac{1}{10}$ m.^{2} Petersen grab. Although the suction-corer is not in general use, mainly because it is rather difficult to handle on board ship, a brief description is included here.

The corer consists of coring-tube (*C*) 36 cm. long and of 16·25 cm. internal diameter, the top of which is in communication with an air chamber (*P*). This assembly is supported by a pipe frame, which rests on the sea-bed and through which the assembly slides into the sediment. It is lowered from the stationary ship, and when it strikes the bottom the tap (*T*) is opened, to give a negative pressure in the coring tube as water rushes in to compress the air in the chamber. It takes about 30 sec. for all the water to enter the chamber, and during this time the coring tube is sinking into the sediment, aided by the reduced pressure inside the tube. The release (*R*) at the top of the chamber has disconnected from the main warp when the cable slackened so that, on hauling up, a lifting force is transmitted to the lifting arms (*LA*), which are nearly horizontal at this stage. As the coring tube is pulled out of the sediment the whole sampler turns over and finally swings under its own weight to bring the open end of the coring tube uppermost. The sample is retained in the coring tube by the sheet of

gauze (*G*), but the open end of the tube is not covered. As the sampler comes up, the air in the chamber expands under the reduced pressure. The tap (*T*) has previously closed as the instrument was inverted, and the air is blown off to the exterior through a small valve.

The suction produced was found to increase penetration into the sediment, but penetration would probably have been greatly improved if the coring assembly had been more heavily weighted. The volume of the sample is very little less than would be expected from the measured depth of penetration, there being apparently little loss while the sampler is turning over.

The sampler resembles that of Knudsen (1927), except that suction is produced by hydrostatic pressure instead of by a pump, and that there is a frame supporting the instrument on the bottom.

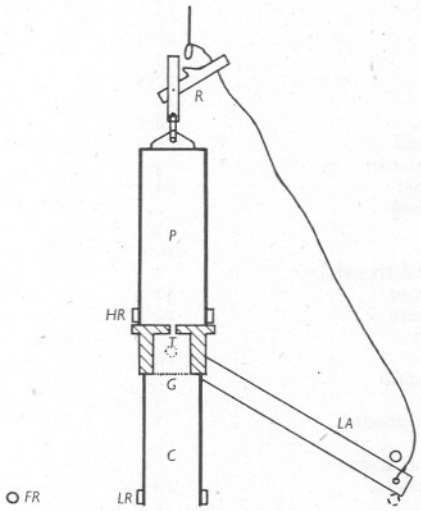

Fig. 4. Diagram of the suction-corer in vertical section. Many details have been omitted. *C*, coring tube; *FR*, pipe frame, which rests on the sea bed, and which is connected to the upper (*HR*) and lower (*LR*) metal rings through which the coring assembly slides. *G*, gauze covering top of coring tube; *LA*, lifting arms, the position of the bolts on which these rotate is dotted. The pipe frame has an upward U-bend to make room for the end of the lifting arms. *P*, pressure chamber; *R*, release; *T*, position of tap mechanism.

The sample taken by this or any other coring tube is more satisfactory in that the cross-sectional area is uniform at all depths in the sample, whereas in the scoop-sampler it decreases with depth.

Details of the comparative hauls are given below:

16. xi. 1949. Brixham Breakwater, light bearing 145° T., 1·25 sea miles (middle of Torbay). Depth: 10·5 m. (from chart).

R.V. *Sabella* anchored. Fine muddy sand, black below top 2–3 cm. 1·2 mm. mesh sieve.

(i) Suction-corer ($\frac{1}{50}$ m.^{2}), fourteen hauls, total area 0·28 m.^{2}. Length of cores (cm.): 20, 14, 13, 29, 20, 15, 7·5, 9, 11·5, 16·5, 21·5, 21·5, 16·5, 18 (the variation in length of the cores is partly due to experimental alteration in the rate and timing of the 'vacuum' release). Mean length: 16·6 cm.

(ii) Petersen grab ($\frac{1}{10}$ m.^{2}), five hauls, total area 0·5 m.^{2}. The mean volume of the sample corresponded to a 'bite' of about 3 cm.

(iii) Single-scoop sampler ($\frac{1}{20}$ m.^{2}), with 50 kg. weights. Total area: 0·25 m.^{2}. Samples about 3 l. each, corresponding to a maximum penetration of 7–10 cm., and a mean penetration of 6 cm.

TABLE III. COMPARISON OF FAUNAS TAKEN BY THE $\frac{1}{50}$ M.^{2} SUCTION-CORER, $\frac{1}{10}$ M.^{2} PETERSEN GRAB, AND THE $\frac{1}{20}$ M.^{2} SCOOP-SAMPLER. DENSITIES PER M.^{2}

	Suction-corer	Petersen grab	Scoop-sampler
Anemones	7	10	12
Aphroditidae	14	..	..
Phyllodocidae	3·5	..	..
Nephthys sp.	28·5	24	44
?*Eone* sp.	7	..	..
Owenia tubes	10·5	..	12
Maldanidae	111	10	148
Notomastus sp.	28·5	2	32
Melinna palmata	278·5	62	268
Amphitrite edwardsi	3·5	..	4
Polychaeta indet.	68	6	96
Phascolion strombi	..	2	..
Nemertinea	7	2	..
Amphipoda	10·5	12	24
Crab (adult and megalopa)	7	..	..
Thyasira flexuosa	32	18	32
Mysella bidentata	10·5	4	8
Venus striatula	..	2	8
Abra alba	10·5	12	8
Cultellus pellucidus	..	2	4
Spisula sp.	3·5	8	..
Lutraria sp. (siphons)	7	..	4
Corbula gibba	..	..	4
Lamellibranchia indet.	..	..	4
Turritella communis	28·5	26	40
?*Eulima glabra*	3·5	..	4
Cylichna sp.	..	..	4
Philine sp.	3·5	2	..
Gastropoda indet.	3·5	..	..
Amphiura filiformis	193	26	216
Synapta	3·5	..	..
Total	883·5	230	976

A comparison of the faunas taken is given in Table III. The number of individuals taken by the suction-corer, which penetrated to about 17 cm., is of the same order as that taken by the scoop-sampler, which had dug half as deeply. This indicates that the majority of species were aggregated in the top 7–10 cm.; *Lutraria*, however, of which only siphons were taken, was out of reach of both samplers. The Petersen grab, which was only digging to about 3 cm. depth, was clearly not sampling the fauna adequately.

Number of samples required

Three series of observations have been made to find the number of hauls necessary for a qualitative evaluation of the fauna. Clearly the number of

hauls required will vary with the objects of any investigation and local conditions. At each station the animals taken in each haul were kept separately and identified. At two stations the ship was anchored while the hauls were made; but at B 5 the ship was drifting over a ground of fairly even faunistic composition (see p. 19). A series of 100 samples taken by Petersen and Boysen Jensen is included for comparison. These were taken with the $\frac{1}{10}$ m.^{2} Petersen grab at various points in Thisted Bredning, the fauna being similar all over the Broad.

(i) Bigbury Bay. 20. iv. 49. Borough Island, bearing 023° T., 1·0 sea miles. Depth: 25·6 m. (from chart). Muddy sand.

R.V. *Sabella* anchored. Fifteen hauls with the single-scoop sampler ($\frac{1}{20}$ m.^{2}) 1·2 mm. sieve.

(ii) Whitsand Bay. 6. v. 49. Rame Head, bearing 144° T., 1·83 sea miles. Depth: *c.* 9 m. (from chart). Sand with shale fragments. R.V. *Sabella* anchored. Fourteen hauls with the single-scoop sampler, then further anchor chain let out so that ship drifted back a little and a further six hauls made. 1·2 mm. sieve.

(iii) Station B 5. 5. xii. 50 (see p. 14).

R.V. *Sabella* drifting. Twenty hauls made with the double-scoop sampler ($2 \times \frac{1}{20}$ m.^{2}). Each scoop-full is considered separately.

(iv) Thisted Bredning. See Petersen & Boysen Jensen (1911), Table I. $\frac{1}{10}$ m.^{2} grab.

The species found in each haul are shown in Tables IV–VI. Not all species have been identified: under 'unidentified Polychaeta' are usually lumped the less easily identifiable worms which are in any case often fragmented after collection, sieving and preserving. The total number of species recorded is therefore a minimum figure.

During identification there is a possibility of bias arising from the order in which the individual samples are examined. Where not every species is identified there is the choice of identifying a species or lumping it with the unidentified forms. Thus an unknown worm in the first haul examined might be identified down to its species, but if the first individual had been found in the last haul it might not have been identified. It is therefore important that the order of the samples be randomized before plotting the total number of species against the area sampled. The records from Whitsand Bay and Bigbury Bay were randomized after identification but before plotting the curves, but the samples from station B 5 were examined in a random order.

The cumulative curves (Fig. 5) record the total number of species taken as the area sampled is increased. The curves rise steeply at first; and clearly a sample of less than $\frac{1}{2}$ m.^{2}, the area adopted in the survey, does not give an adequate qualitative sample.

Williams (1950) has analysed the occurrence of species of plants in quadrats. He has shown that if the number of species taken is plotted against the log of area (or number of quadrats), a straight-line relationship is approached. Fig. 6 shows the results of plotting against log area for the sets of samples under discussion. Each gives a straight-line relationship. From the slope of the lines it is possible to predict the number of species that will be taken in increasingly larger samples. Thus doubling the area sampled increases the

number of species by the following amounts: St B5, 12; Whitsand Bay, 7; Bigbury Bay, $4\frac{1}{2}$; Thisted Bredning, $2\frac{1}{2}$.

It is doubtful if any useful purpose would be served by analysing the occurrence of so-called 'characteristic' species in these samples since these may be by no means common on the grounds. A species may be characteristic of a community without necessarily being at all abundant, and while no doubt the ideal characteristic species are both restricted to a particular set of conditions and very common where they occur at all, it may be necessary to label a community by species of comparatively low density. Thus the *Venus* communities of Ford are based on species which are by no means abundant on the grounds in question, and may frequently be absent from the grab samples at a station.

The curves in Fig. 5 would not reach an asymptote until every part of the ground would have been brought up by the sampler. There are always rare species which are scarcely ever taken, and the number of species on a ground may be added to by immigrants from neighbouring grounds.

Some species, seldom taken in grab hauls, are common in trawl or dredge hauls which cover a much larger area of the bottom. An under-water photographic survey of the sea-bed off Plymouth has recently been made by Vevers (1951), who gives figures for the densities of members of the epifauna identified in the photographs. These are in general relatively scarce; thus at position L4, mid-way between the Eddystone and Plymouth breakwater light, the mean density of certain species was: *Asterias rubens*: 1 individual in 10·4 m.^{2}; *Porania pulvillus*: 1 in 83 m.^{2}; *Ophiura texturata*: 1 in 20·8 m.^{2}; *Chlamys opercularis*: 1 in 7·5 m.^{2}; *Turritella communis*: 1 in 83 m.^{2}; *Eupagurus prideauxi*: 1 in 83 m.^{2}; *Hyalinoecia tubicola*: 1 in 83 m.^{2}; *Cellaria* sp.: 1 in 3 m.^{2}.

In the same way certain members of the *infauna* must occur so rarely as seldom to be taken by the sampler.

Variability of samples at a station

The variability in the samples taken at a station was studied by means of twenty successive hauls taken with the double-scoop sampler at B5. Each scoop-full was kept separately and identified. In this way not only could the fauna of successive hauls be compared, but also that in the two adjacent samples taken in each haul.

Details were as follows:

5. xii. 50. Station B5. Marker float anchored at this position, the hauls being made as the ship drifted away from it. After every five hauls the ship steamed back to the mark, so that the samples are positioned along four radiating lines. The extent of drift was estimated after each set of five hauls: (i) 0·25 mile S. from float; (ii) 0·25 mile S.S.E.; (iii) 0·5 mile S.E.; (iv) 0·5 mile S.E.

Wind: light north breeze, freshening during the sampling.

Sieve: 1·2 mm.

The samples were numbered serially—thus nos. 1–10 represent the first five pairs of samples, and nos. 1 and 2 are the two scoop-fulls from the first haul.

TABLE IV. BIGBURY BAY. SPECIES TAKEN IN SUCCESSIVE HAULS OF THE $\frac{1}{20}$ M.^{2} SCOOP-SAMPLER

(The specific identity of the single specimen of *Phacoides* in haul 7 is uncertain. Names in brackets have not been included as data for Figs. 5 and 6.)

Haul no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Amphiura filiformis	7	19	13	18	22	19	11	4	10	10	13	11	15	14	7
?*Sigalion* sp.	..	1	..	1	1	..	..	1	2	2	..	..	..	..	..
Cirratulidae	..	4	..	..	2	..	..	..	..	1	1	..	..	..	3
Montacuta bidentata	..	2	1	9	10	4	6	7	1	8	5	5	6	9	7
Thyasira flexuosa	..	2	3	..	..	1	1	1	1	4	5	2	1	1	4
Cylichna sp.	..	1	..	..	1	1	..	..	..	1	..	..	1	..	..
?*Owenia* sp.	..	..	1	1	..	..	..	..	..	..	..	..	..	..	1
Chlamys opercularis	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..
Nucula nitida	..	..	1	1	..	..	..	..	..	..	1	..	..	..	1
Echinocardium cordatum	..	..	..	2	1	1	1	..	1	..	..	1	2	1	..
Abra sp.	..	..	..	1	2	..	1	1	..	1	..	..	1	..	..
[Amphipoda]	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1
Goniada sp.	..	..	..	..	1	..	1	..	1	..	..	1	..	..	..
[Polychaeta]	..	..	..	..	1	..	..	..	..	..	1	..	2	..	..
Phacoides borealis	..	..	..	..	1	..	1	..	..	..	..	..	..	..	..
Melinna sp.	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..
?*Spisula* sp.	..	..	..	..	..	..	..	..	1	..	..	..	..	..	1
[Nemertinea]	..	..	..	..	..	..	..	..	1	..	..	..	..	1	..
Aphroditidae	..	..	..	..	..	..	..	..	..	..	3	..	..	..	..
Venus striatula	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..
Nassa sp.	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..
Nephthys sp.	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1
Upogebia deltaura	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
Synapta	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1

TABLE V. WHITSAND BAY. SPECIES TAKEN IN SUCCESSIVE HAULS OF THE $\frac{1}{20}$ M.^{2} SCOOP-SAMPLER

(Names in brackets have not been included as data for Figs. 5 and 6. Numbers in brackets indicate that the specific identity is uncertain.)

Haul no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
?*Cardium echinatum*	1	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cochlodesma praetenue	1	..	1	..	2	1	1	..	..	..	..	..	..	1	2	..	..	1	..	1
Mactra corallina	1	..	..	..	..	..	2	..	..	(1)	2	..	1	3	..	..	2	..	..	1
Nephthys sp.	1	2	1	1	..	1	1	1	1	..	..	..	1	1	1	1	..	2	1	1
Magelona papillicornis	8	2	1	5	1	..	6	6	8	3	3	2	4	2	2	2	2	2	..	..
Bathyporeia tenuipes	(1)	..	..	..	..	1	..	1	1	..	..	..	..	..	..	..	..	2	..	..
Urothoë grimaldii var. *poseidonis*	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1
Tellina fabula	..	1	1	..	..	..	..	..	..	..	1	..	1	1	..	..	..	..	..	..
?*Amphiura filiformis*	..	1	1	1	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..
Natica poliana	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
[Nemertinea]	..	..	1	1	1	..	1	..	..	..	..	..	..	..	..	..	1	..	..	..
[Polychaeta]	..	..	1	2	3	1	4	2	1	1	3	1	2	2	1	1	1	..	2	1
Nucula nitida	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Eteone picta	..	..	..	(1)	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
[Amphipoda]	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Eunicidae	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
?*Owenia* sp.	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
?*Abra* sp.	..	..	..	1	..	..	..	..	1	..	..	..	..	1	..	..	1	..	..	..
Nototropis vedlomensis	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Siphonoecetes colletti	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Leucothoë lilljeborgi	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Dosinia lupinus	..	..	..	..	..	..	(1)	..	..	1	..	..	2	..	..	..	..	1	..	..
[Lamellibranchia]	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..
Bathyporeia elegans	..	..	..	..	..	..	..	..	..	..	1	..	..	1	1	..	1	..	1	1
Echinocardium cordatum	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..
Montacuta ferruginosa	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..
Phyllodocidae	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..
Phascolion strombi	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..
Cirratulidae	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..
Ensis arcuatus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Leucothoë spinicarpa	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Cumacea	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Gari fervensis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1

Table VI. Fauna of Successive Samples at B5, 5 December 1950

Sample no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Total
COELENTERATA																																									
[Hydroid]	..	+	..	..	+	..	..	+	..	+	..	+	..	+	+	+	..	..	+	+	..	..	..	..	+	..	..	..	..	..	+	+	..	+	+	..	+	..	..	..	+
Virgularia mirabilis	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Cerianthus lloydi	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1
Edwardsia sp.	..	..	1	..	..	2	..	2	1	..	1	..	..	..	..	3	1	..	1	2	..	2	1	..	1	2	3	..	1	1	..	..	1	..	1	..	..	1	1	..	29
POLYCHAETA																																								
Lumbriconereis sp.	3	10	7	2	3	8	5	10	3	7	4	3	9	10	2	15	5	15	6	9	10	4	4	4	7	1	7	10	4	9	9	3	5	4	7	10	2	8	4	3	251
Magelona papillicornis	3	1	..	..	7	..	..	1	3	..	4	1	4	..	1	..	..	4	..	4	1	3	4	..	2	1	3	1	1	4	1	..	1	1	..	3	..	1	4	1	65
Magelona cincta	3	..	..	1	1	1	3	3	1	..	3	1	1	4	2	3	1	..	2	..	1	1	2	..	2	1	..	1	..	..	..	..	..	..	1	1	4	2	..	..	46
Chaetopterus variopedatus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	3
Maldanidae	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	1	1	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	1	1	..	10
Pectinaria auricoma	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Pectinaria koreni	..	..	..	..	1	..	..	..	1	1	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	6
Amphicteis gunneri	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Polychaeta indet.]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GEPHYREA																																								
Phascolion strombi	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
ARTHROPODA																																								
Ampelisca tenuicornis	3	3	1	..	1	1	2	2	..	..	1	..	..	1	2	1	1	4	2	5	..	1	3	3	3	..	4	2	3	4	4	1	2	..	3	2	3	2	1	..	71
Ampelisca brevicornis	..	2	..	..	..	..	..	..	..	..	1	..	..	1	..	..	1	..	1	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	1	..	9
Ampelisca spinipes	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Bathyporeia tenuipes	..	..	..	..	..	..	..	..	2	1	..	..	..	1	..	..	1	..	..	1	1	1	4	..	..	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	14
Urothoë elegans	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	5
Harpinia antennaria	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	2
Cheirocratus intermedius	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	(1)	..	..	..	1	..	3
Maera othonis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	1	..	..	..	..	..	2
Acidostoma obesum	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Leucothoë sp.*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Erichthonius brasiliensis	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Nebalia bipes	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1
[Decapoda, callianassid]	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Decapod larva]	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Mysidacea]	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
['Prawn']	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Galathea sp.	..	..	..	1	..	1	..	..	..	..	..	..	..	1	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1	..	..	..	..	..	7
Porcellana longicornis	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	(1)	..	..	..	..	..	..	..	..	..	..	..	..	..	3
Portunus holsatus	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Portunus marmoreus	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Ebalia sp.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
[Pycnogonida]	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Total
MOLLUSCA																																									
Acteon tornatilis	..	..	..	..	..	..	..	..	1	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
Cylichna cylindracea	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	2
Nucula nucleus	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Nucula turgida	..	..	1	..	..	..	1	1	..	..	..	..	..	..	1	..	..	..	..	..	..	1	..	..	1	..	..	1	..	..	1	1	2	1	..	..	..	..	..	..	12
Musculus marmoratus	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Chlamys sp.†	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4†
Thyasira flexuosa	..	1	..	..	..	1	1	..	..	2	..	..	..	..	1	..	1	1	..	1	..	1	..	2	..	..	1	..	..	..	..	..	..	..	..	..	1	..	..	2	16
Phacoides borealis†	1	..	..	1	..	..	2	..	1	4	1	1	..	..	..	..	1	..	1	..	1	1	3	..	1	2	..	..	..	2	1	..	..	..	..	1	2	1	1	..	26†
Montacuta ferruginosa	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4
Cyprina islandica	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Cardium echinatum	..	..	..	..	..	..	(1)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	2
Dosinia lupinus	..	1	..	..	1	..	1	1	3	3	1	..	..	..	..	..	2	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	16
Venus ovata	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Venus striatula	..	1	..	..	..	..	..	..	..	..	..	1	1	..	..	1	..	..	..	..	..	..	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	6
Venerupis rhomboides	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Abra alba	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	3
Abra prismatica	..	..	..	1	1	..	..	..	1	..	2	..	..	..	..	..	..	..	1	2	1	..	1	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	11
Gari fervensis	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	3
Cultellus pellucidus	1	..	1	..	2	1	..	3	1	3	..	..	2	..	1	..	..	1	..	2	3	..	1	1	2	1	1	1	1	..	..	1	1	1	..	3	1	2	1	1	40
Hiatella arctica	..	..	..	..	..	..	..	2	..	1	..	..	..	..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	6
Cochlodesma praetenue	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	3
Lyonsia norwegica	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
POLYZOA																																								
Cellaria sp.	..	..	..	..	..	+	..	..	..	+	..	+	..	+	..	+	..	+	+	+	+	..	..	..	..	..	+	..	+	..	+	..	+	..	..	..	..	..	..	..	+
ECHINODERMATA																																								
Ophiothrix fragilis	..	..	..	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Amphiura filiformis	1	..	1	1	2	..	..	..	..	..	2	2	..	1	..	6	1	1	..	1	..	1	1	..	6	2	1	3	1	2	..	1	..	..	1	..	..	..	1	2	41
Acrocnida brachiata	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1
Ophiura affinis	..	..	1	..	..	..	(1)	5	..	2	..	..	3	3	1	2	1	2	1	1	2	..	..	1	4	..	..	2	3	3	4	..	..	..	..	2	1	..	1	2	48
Ophiura texturata	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Echinocyamus pusillus	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4
Echinocardium cordatum	..	1	..	..	..	..	..	(1)	..	..	..	(1)	..	..	(1)	..	1	..	..	..	(2)	..	..	(1)	..	..	(1)	(1)	..	(1)	..	..	(1)	..	..	(2)	1,	(3)	..	..	18
Labidoplax digitata	..	..	..	1	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	1	1	..	..	..	..	6
TUNICATA																																								
Ascidia mentula	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1
Ascidiella aspersa	..	..	..	..	..	..	..	1	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
Diplosoma listerianum	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
ENTEROPNEUSTA																																								
Glossobalanus sarniensis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	..	..	..	..	..	..	..	..	..	2
Total species taken	9	17	21	25	28	31	32	39	42	43	44	44	47	48	50	52	52	53	54	55	55	55	57	57	57	57	58	60	61	61	61	62	62	62	62	62	62	63	63	64	

* See p. 25.
† See corrections at foot of Table XXIII. The totals in the right-hand column are corrected.
Names in brackets have not been used as data for Figs. 5 and 6. Numbers in brackets indicate that the specific identity is uncertain.

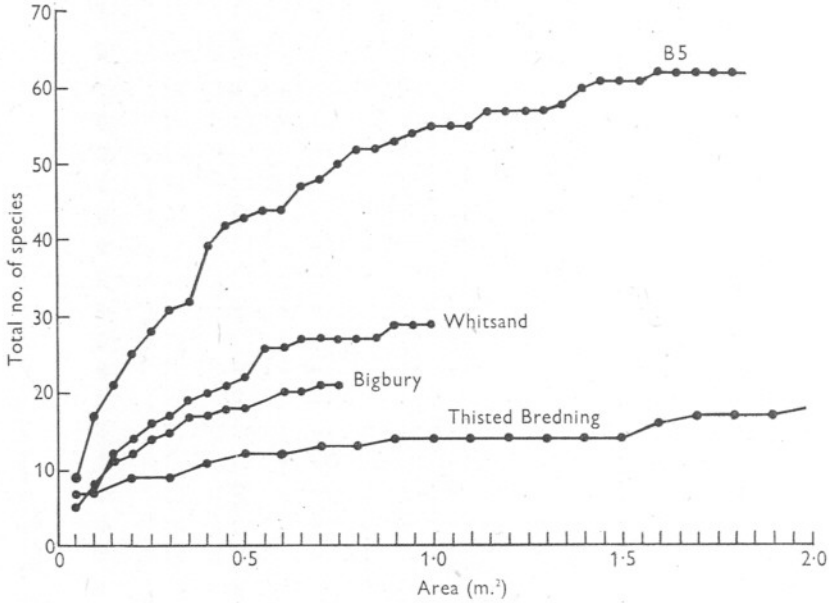

Fig. 5. Cumulative curves showing the number of species taken in successive samples of the sea-bed in four localities.

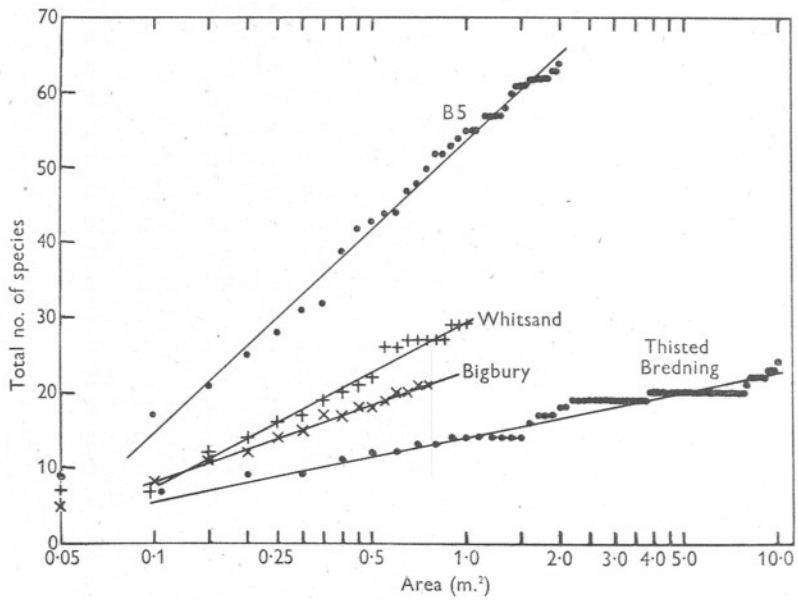

Fig. 6. The same samples as in Fig. 5 plotted with area on a log scale. Note that the samples from Thisted Bredning are each twice the area of those at the other stations.

The fauna is shown in Table VI. Most of the species occur at rather low densities, and there are no signs of gross aggregation or that the samples pass through more than one 'community'. On the other hand, there seems to be almost as much variation between the pairs of samples from one haul as between samples in successive hauls.

A statistical examination has been made of the distribution of the common species. The variance for each species has been analysed as follows, that for *Ophiura affinis* being chosen as an example:

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio	Probability
Successive hauls	37·4	19	1·97	1·64	0·05–0·02
Pairs of samples	34·9	19	1·84	1·53	—
Residual	0·1	1	0·10	0·08	—
Total	72·4	39	1·86	1·55	0·02–0·01

The variance between successive hauls is a measure of the large-scale patchiness of the fauna, there being perhaps 100 or 200 yards distance between each haul. The variance between the pairs of samples taken in each haul is a measure of distribution on a small scale, and there is a residual variance due to any differential sampling by the two scoops. Since no record was made of which of each pair of samples came from either scoop, this residual variance should not be significant. Analyses for the more frequent species to be compared against the expected random variance given by the Poisson distribution are given in Table VII.

As might be expected the variance between hauls tends to be greater, and therefore more frequently significant, than that between the pairs of samples from a single haul. An exception is *Magelona papillicornis* where the variance between pairs of samples is significant, but that between hauls is not.

A measure of the degree of patchiness is given by Fisher's 'coefficient of dispersion' (used by Holme, 1950*b*), which equals the Variance Ratio. This tends to unity in a randomly distributed population, to less than unity in an evenly distributed population, and to more than unity if there is aggregation. Treating the forty samples separately, the coefficients have been calculated for the same species (Table VII). Although significantly high for six species, the coefficients do not indicate any great degree of aggregation. *Edwardsia*, *Ampelisca tenuicornis*, *Thyasira*, and *Cultellus* appear to have a random distribution.

On this ground the fauna is sufficiently well dispersed for repeatable results to be obtained; but closer inshore the grounds are so patchy that this is not possible.

Seasonal changes

Most of the collections were made in the summer months, and no account has been taken of any seasonal variation in the fauna.

Blegvad (1925) found a seasonal variation in the *Macoma* and *Abra* communities of the enclosed Danish Fjords, but in more open waters the variation was less marked. Cold winters seem to play a large part in reducing populations in shallow water. Steven (1930), in a grab survey of an offshore area at Plymouth, found no marked seasonal changes in the fauna, and a recent paper by Jones (1952) confirms this view from observations made off the Cumberland coast, in the Irish Sea.

TABLE VII. COEFFICIENTS OF DISPERSION AND ANALYSIS OF VARIANCE OF CERTAIN SPECIES FROM B 5 (SEE TABLE VI)

Significant coefficients of dispersion are in bold type (test of significance $1 \pm 0{\cdot}4586$). Only significant probabilities are given.

	Coefficient of dispersion	Probability			
		Hauls	Scoops	Residual	Total
Edwardsia sp.	1·060	..	..	..	..
Lumbriconereis sp.	**1·888**	..	0·01–0·001	0·02–0·01	<0·001
Magelona papillicornis	**1·852**	..	<0·001	..	<0·001
Magelona cincta	1·273	0·05–0·02	..	..	..
Ampelisca tenuicornis	1·112	..	..	..	..
Bathyporeia tenuipes	**1·692**	0·02–0·01	..	..	0·01–0·001
Thyasira flexuosa	1·000	..	..	..	..
Dosinia lupinus	**1·513**	<0·001	..	..	0·05–0·02
Cultellus pellucidus	0·872	..	..	..	..
Amphiura filiformis	**1·926**	0·01–0·001	0·05–0·02	..	<0·001
Ophiura affinis	**1·547**	0·05–0·02	..	..	0·02–0·01

Some seasonal changes must occur coinciding with the settling and growth of young, but the larger benthic invertebrates are usually considered to have a life-span of several years and fluctuations in total population density are thus reduced.

Most of the populations studied were composed of large numbers of species, few of which occurred in abundance, and this diversity should minimize the effects of seasonal and annual fluctuations.

Sieving

The samples were washed through a gauze sieve of 2·2 mm. aperture on board ship, and the sievings kept for examination in the laboratory. The use of a sieve to separate the fauna from the sediment necessarily results in a lower size limit to the animals collected. With animals of a definite size and shape, e.g. Mollusca, the proportion of animals passing through the sieve is dependent on the size of the individuals, but many worms actively crawl through the meshes and quite large individuals may be lost during a prolonged

sieving. It is advisable to wash out the contents of the sieve into a jar as often as possible to minimize such losses.

While a finer meshed sieve would undoubtedly retain many more small amphipods, worms, etc., the 2·2 mm. sieve was used because of the high percentage of coarse particles at some stations which soon clog a finer sieve.

TABLE VIII. NUMBERS AND WEIGHTS OF ANIMALS AT C4 (7 NOVEMBER 1950) AFTER WASHING SAMPLE THROUGH FIRST THE 2·2 MM. AND THEN THE 1·2 MM. SIEVE

(Numbers in brackets indicate doubtful identity.)

	2·2 mm.		1·2 mm.	
	No.	Dry weight (g.)	No.	Dry weight (g.)
Hydroid	+	0·003	..	..
Anemone	1	0·083	..	..
Polychaeta	6	0·085	+	0·31
Diastylis laevis	..	..	(5)	
Isopoda	..	..	1	
Ampelisca tenuicornis	..	..	5	
Ampelisca diadema	..	..	1	
Bathyporeia tenuipes	..	..	6	0·002
Harpinia antennaria	..	..	2	
Photis longicaudata	1	< 0·001	..	
Pycnogonida	..	..	4	
Nucula turgida	..	..	8	
Thyasira flexuosa	4	0·022	7	
Dosinia lupinus	2	0·003	(1)	
Venus striatula	1	0·001	..	
Mysia undata	2	0·16	..	
Abra alba	1	0·006	..	0·022
Abra prismatica	1	0·022	(7)	
Gari fervensis	..	..	3	
Cultellus pellucidus	1	0·042	1	
Hiatella arctica	1	0·001	..	
Thracia sp.	4	0·011	10	
Cellaria sp.	+	0·005	..	..
Amphiura filiformis	1	0·001	7	
Ophiura affinis	1	0·001	(1)	0·001
Echinocyamus pusillus	..	..	1	
Cucumaria elongata	1	0·073	..	..
Total		0·52		0·34

The same mesh was employed at all stations to ensure a uniformity in sampling technique. Petersen & Boysen Jensen (1911) used a sieve of 1·5 mm. aperture for sieving deposits consisting mainly of fine particles, and where the soil is fine no coarser mesh should be employed.

The use of a series of sieves, such as the *Challenger* series, has not been found practicable, as the smaller meshed sieves are liable to clog and overflow, and some of the smaller animals are thereby lost.

On 7 November 1950 station C4 was sampled a second time. The sample, which was of fine sand, was sieved through first a 2·2 mm. and then a 1·2 mm.

sieve. The fauna taken in each sieve is shown in Table VIII. In this sample the coarse sieve failed to retain a high proportion of the individuals. Of the total dry weight, nearly two-fifths passed the 2·2 mm. sieve and was retained by the finer mesh. At most stations, however, the *relative* losses are probably not as high as this. In the twenty stations worked, 64·4% of the total dry weight was made up of fairly large individuals, each weighing more than 0·2 g. dry (p. 38), whereas at this station there were no individuals of this size. The previous samples taken at C4 had given a total of 5·7 g. per $\frac{1}{2}$ m.^{2} (p. 35), and the second series has an exceptionally low dry weight. If we take the losses at each station through using the 2·2 mm. sieve as about 0·4–0·5 g., or say 10% of the total taken, a reasonable correction can be made.

The animals lost through the 2·2 mm. sieve correspond to the 'small macrobenthos' of Mare (1942). In the Rame mud a mile or two north of A2 she found that small polychaetes, etc., retained on the 1 mm. sieve represented about 30·6% of the total (fresh) weight of macrobenthos. Polychaetes are abundant in the Rame mud, and a smaller percentage would therefore be expected for other grounds.

Biomass estimations

Any estimate of the productivity of a community must start with an assessment of the 'standing crop', that is, the quantity of living tissue present at any one time. Owing to the very different sizes of species and individuals, an estimate of their numerical density is insufficient, and comparisons are best made in terms of weight.

The biomass may be expressed either as: (i) the 'fresh weight', i.e. the weight of fresh tissue after surface moisture has been blotted off, the weight of mollusc shells may or may not be included; (ii) the dry weight, shells usually being excluded from the total.

In this investigation the dry weight of the specimens has been determined, and is here defined as the weight of the residue obtained after evaporation at 110° C. to a (more or less) constant weight, surface moisture having previously been removed. The weight of all substances soluble in dilute hydrochloric acid has been excluded from the total. The dry weight thus includes chitinous or similar non-calcareous skeletal structures, inorganic salts in the blood and tissues, and gut contents (except in *Echinocardium*), but excludes all calcareous structures.

The animals were usually preserved in formalin on board ship, but were transferred to and stored in 70% alcohol within a day or two. The 'alcohol weight' was determined from preserved specimens by blotting off surface moisture with filter-paper, and weighing as quickly as possible to the nearest centigram.

During preservation certain constituents of the tissues dissolve out into the alcohol. To find the relation of dry weight to alcohol weight two or three

specimens of certain species were preserved in 70% alcohol for at least a week. The preserved animals were then dried at 110° C. overnight and to the dry weight was added that of the residue obtained from evaporating the alcohol.

The dry weight has been calculated as a percentage of the alcohol weight for a number of species (Table IX). For the rest a value of 27·72% has been taken, being the mean of determinations for species not having a calcareous skeleton. It was more convenient to measure the size rather than the weight

TABLE IX. DRY WEIGHT EXPRESSED AS A PERCENTAGE OF ALCOHOL WEIGHT

(Species marked with an asterisk were treated with acid before the dry weight was determined. Figures in italics are assumed or approximate percentages. The figure given for Amphipoda is derived from Molander (1928), and allows for matter dissolved out by the alcohol. It appears to be too low, but in any case the 'biomass' of the amphipod population is extremely small.)

COELENTERATA	
Hydroids and anemones	*27·72*
*Alcyonium**	*10·00*
POLYCHAETA	
Chaetopterus	25·24
All other spp.	*27·72*
CRUSTACEA	
Amphipoda etc.	4·12
*Callianassa** (and other Macrura)	19·83
*Gonoplax** (and other Brachyura)	24·20
MOLLUSCA	
Cyprina islandica	28·14
Solecurtus chamasolen	31·05
Tellina crassa	25·74
All other spp.	*27·72*
POLYZOA	
*Cellaria**	3·43
ECHINODERMATA	
*Ophiothrix fragilis**	14·56
*Amphiura** (and other Ophiuroidea)	5·54
*Echinocardium** (and *Echinocyamus*)	2·29
*Cucumaria**	14·66
*Leptosynapta** (and *Labidoplax*)	24·16
CEPHALOCHORDATA	
Amphioxus	28·41

of some species, and by a series of determinations of dry weight a factor was obtained for converting a size measurement directly into terms of dry weight.

(The conversion factors from alcohol to dry weight differ from those given by Petersen & Boysen Jensen (1911, p. 52). They apparently added the weight of the material, obtained from evaporating the alcohol, to the total *after* applying their conversion factor. In the present investigations the quantity dissolved in the alcohol is allowed for in the conversion factor.)

A number of observations were made to assess the validity of these techniques:

(i) It was found that after about a week most of the soluble substances had dissolved out into the alcohol. A change to fresh alcohol did not greatly increase the total amount dissolved out.

(ii) Provided that a reasonable quantity of preservative was used, the amount of dry matter dissolved out of the tissues was not dependent on the relative volumes of the animals and the spirit.

(iii) About half as much again was dissolved out if 96% alcohol was used in preservation.

(iv) Weight of gut contents. Where a species contains much sand in the gut, a considerable error might be introduced in dry-weight determinations. A number of alcohol-preserved animals were weighed before and after ignition. The residue after ignition is ash, together with any sand from the gut. The weights after ignition, expressed as percentages of the alcohol weight, were:

Chaetopterus variopedatus	(Polychaeta)	1·09%
Solecurtus chamasolen	(Lamellibranchia)	1·00%
Abra alba	(Lamellibranchia)	7·43%
Ophelia bicornis	(Polychaeta)	23·93%

Miscellaneous small worms from bottom-sampler hauls: 2·23%.

Taking ash as 1% of the alcohol weight (i.e. assuming that the *Chaetopterus* and *Solecurtus* residue was entirely 'organic ash'), the rest is sand. *Ophelia* is a deposit-feeding worm in which the gut can be seen to be full of sand, and represents a maximum figure. *Abra* feeds on detritus collected from the soil surface, and might be expected to take in some sand with the food. *Chaetopterus* and *Solecurtus* both draw their food from material in suspension just off the bottom, and so probably take in very little sand under normal conditions.

An estimate that the *alcohol* weights include 5% of sand in the guts of animals seems a reasonable overall estimate.

(v) Calcareous skeletons. The shells of molluscs were removed before weighing, but with other forms it was necessary to determine an alcohol weight which included the skeleton. To determine the dry weight, the skeleton was then dissolved by placing the animal in dilute hydrochloric acid, the mixture being filtered, washed with distilled water, dried and weighed.

Treatment with dilute acid did not appear to release into solution appreciable quantities of non-calcareous substances within the tissues. Treatment of a non-calcareous organism (the polychaete *Nereis*) with acid did not alter the alcohol weight/dry weight ratio, but it was thought that where an animal had calcareous spicules embedded in the tissues, some loss might result from the mechanical break-up of cells due to formation of bubbles of carbon dioxide.

For non-calcareous animals there is a fairly constant proportion of the total dry weight found in solution in the alcohol. If acid treatment after determination of the alcohol weight resulted in the loss of material into the acid solution, a resultant change in the ratio of the weight of dried animal tissue to total dry weight would occur. In six species which were not acid treated, the percentages of the total dry weight found in the dried preserved animal were: *Nereis diversicolor*, 59·04; *Amphioxus lanceolatus*, 68·33; *Solecurtus chamasolen*, 64·78; *Tellina crassa*, 51·13; *Chaetopterus variopedatus*, 56·53; *Cyprina islandica*, 73·69; Mean: 62·25. In four species in which the skeleton was dissolved in acid, the percentages were: *Ophiothrix fragilis*, 50·06; *Callianassa subterranea*, 58·28; *Gonoplax rhomboides*, 54·90; *Cucumaria elongata*, 69·55; Mean: 58·20. There is therefore no evidence of any considerable loss due to acid treatment.

In *Echinocardium cordatum* the alcohol weight is made up largely of both calcium carbonate in the skeleton and sand in the gut, plus a certain amount of sea water inside the test. After acid treatment, as above, the residue was ignited and the percentage of sand calculated. Allowance for the sand content of the gut has been made in the factor given in Table IX.

Nomenclature

Nomenclature is that of the *Plymouth Marine Fauna* (Marine Biological Association, 1931), except for the Mollusca, which are named as in Winckworth's (1932) list of British marine Mollusca. The following species are not in the *Plymouth Marine Fauna*:

POLYCHAETA

Sigalion mathildae Audouin & M.-Edwards* (see Fauvel, 1923).
Magelona cincta (Ehlers) (see Mare, 1942, p. 542).

AMPHIPODA

Ampelisca diadema (A. Costa) (see Chevreux & Fage, 1925).
Bathyporeia elegans Watkin (see Watkin, 1938).
B. tenuipes Meinert (see Watkin, 1938).
Urothoë grimaldii Chevreux var. *poseidonis* Reibisch (see Chevreux & Fage, 1925, p. 100 as var. *inermis*).
Urothoë elegans Bate (see Chevreux & Fage, 1925).
Leucothoë lilljeborgi Boeck (see Chevreux & Fage, 1925).
Leucothoë sp. (the undescribed species mentioned in Spooner, 1950, p. 249).

LAMELLIBRANCHIA

*Cochlodesma praetenue** (Montagu) (see Forbes & Hanley, 1853).

ENTEROPNEUSTA

Glossobalanus sarniensis (Koehler)* (see Koehler, 1886).

Species marked with an asterisk (*) are first records for the Plymouth area.

DETAILS OF STATIONS AND COLLECTIONS

Station A 1

50° 19′ 40″ N., 4° 14′ 45″ W. Clean sand (Table I, Fig. 2). Depth *c.* 15 m. (at all stations the depth is given from soundings on the Admiralty Chart of the area). 18. x. 50. Area: 0·5 m.². Sieve: 2·2 mm. *Ship anchored.*

This station is slightly out of line with the other stations to avoid a 'dumping ground'. The fauna (Table X) is similar to that given in Table V, taken at a position about half a mile distant.

The bottom in this part of Whitsand Bay is very patchy, areas of rock or gravel occurring in places. The small polychaete *Magelona papillicornis* is common, and some specimens were probably lost through the sieve. *M. cincta*, a rather larger worm, occurs here and at several other stations. It was first recorded from Plymouth by Mare (1942); it is probably not new to the

area, and may have been confused with *M. papillicornis* in earlier records (D. P. Wilson, personal communication).

The total dry weight at this station is 1·5 g./$\frac{1}{2}$ m.^{2}, which is lower than for most stations in the survey. There were no large individuals apart from five specimens of *Echinocardium*.

TABLE X. STATION A1. NUMBERS AND DRY WEIGHTS PER $\frac{1}{2}$ M.^{2}

(Numbers in brackets are approximate. *j*: young individuals.)

	No.	Weight		No.	Weight
Nemertinea indet.	(2)	0·50	*Portunus* sp.	1*j*	0·025
Sigalion mathildae	2		*Natica poliana*	2*j*	0·004
Aphroditidae	1		*Philine* sp.	1*j*	0·007
Phyllodocidae	1*j*		*Nucula turgida*	1*j*	0·001
Nephthys sp.	2		*Dosinia lupinus*	3*j*	0·004
Glycera sp.	1		*Tellina fabula*	5	0·037
Magelona papillicornis	(65)		*Ensis ensis*	1*j*	0·013
Magelona cincta	1		*Ensis arcuatus*	1*j*	0·011
Spionidae	1		*Mactra corallina*	1	0·069
Polychaeta indet.	1		*Cellaria* sp.	+	< 0·001
Diastylis laevis	1	< 0·001	*Ophiura affinis*	1	0·002
Ampelisca brevicornis	1		*Echinocardium cordatum*	5	0·87
Gastrosaccus sanctus	2		Total dry weight		1·5

TABLE XI. STATION A2. NUMBERS AND DRY WEIGHTS PER $\frac{1}{2}$ M.^{2}

(Where it has been necessary to assume the weight of an individual, either because it was badly fragmented or because only part of it was taken, the figures are italicized.)

	No.	Weight		No.	Weight
Hydroid	+	0·092	*Alpheus ruber*	2	0·93
Anemones	2	0·73	*Callianassa subterranea*	1	0·025
Eunice harassi	1	0·99	*Portunus depurator*	1	0·42
Maldanidae	1		*Chlamys* sp.	1*j*	0·001
Melinna palmata	(120)		*Cucumaria elongata*	2	0·67
Polychaeta indet.	1		*Leptosynapta inhaerens*	2	*0·97*
Phyllochaetopterus tubes	(4)	..			
Scalpellum scalpellum	1	0·011	Total dry weight		4·8

Station A2

50° 17′ N., 4° 15′ W. Very fine muddy sand (Table I, Fig. 2). Depth: 46 m. 26. vi. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm. Ship drifting at this and at all subsequent stations.

This station on the southern edge of the 'Rame mud' is characterized by the abundance of *Melinna palmata*, and the presence of *Alpheus ruber*, *Callianassa subterranea*, *Cucumaria elongata* and *Leptosynapta inhaerens*, which together constitute a large proportion of the total dry weight (Table XI). Lamellibranchs are scarce in this deposit, the only species commonly taken in dredgings being *Solecurtus chamasolen*.

The station is somewhat to the south of stations worked in the Rame mud by Ford (1923) and Mare (1942). The latter estimated a total of over 100 g./m.^{2} of fresh tissue in the Rame deposit (see p. 43).

Station A3

50° 15′ N., 4° 15′ W. Hauls nos. 1, 2 and 6 on clean medium-grade sand, hauls 3–5 on mixed muddy sand and gravel. Depth: 51 m. 26. vi. 50. Area: 0·6 m.^{2}. Sieve: 2·2 mm.

The sediment at this station is very patchy, two distinct grades of deposit being taken in the sampler. The first is a medium-grade sand with less than

1% over 2 mm., and 1·5% silt; the second a muddy gravel with 28·9% over 2 mm. and 7% silt (Table I and Fig. 2). The fauna (Table XII) in the different hauls probably varied somewhat, but is characteristic of the muddy sand grounds inside the Eddystone, where typical species are: the polychaetes *Nephthys* sp., *Eunice harassi*, *Hyalinoecia bilineata*, *Lumbriconereis* sp., burrowing prawns *Upogebia deltaura* and *U. stellata*, the lamellibranchs *Dosinia lupinus*, *Cultellus pellucidus* and *Solecurtus chamasolen*, and the heart-urchin *Echinocardium cordatum*. The polyzoan *Cellaria* occurs in clumps and was quite abundant in some samples. Three species of *Cellaria* are recorded in the *Plymouth Marine Fauna* (Marine Biological Association, 1931), but specific identifications were not made in this survey.

Polychaeta make up about half the total dry weight. *Cellaria* contributes only 0·5 g. to the total; its fresh weight being largely composed of calcium carbonate in the skeleton. The total dry weight, 4·0 g./$\frac{1}{2}$ m.^{2}, is rather below the average for the area.

TABLE XII. STATION A3. NUMBERS AND WEIGHTS PER 0·6 M.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·40	*Phyllochaetopterus* tubes	4	..
Anemone	1	0·53	*Owenia* tube	1	..
Edwardsia sp.	2*j*	0·083	*Ampelisca spinipes*	4	0·011
Nemertinea indet.	1		*Ampelisca tenuicornis*	2	
Nephthys sp.	3		*Upogebia stellata*	1	0·34
Glycera sp.	2		*Modiolus phaseolinus*	1	0·007
Eunice harassi	1		*Dosinia lupinus*	1*j*	0·001
Nematonereis unicornis	1		*Cultellus pellucidus*	2	0·050
Hyalinoecia bilineata	1		*Cellaria* sp..	+	0·51
Lumbriconereis sp.	(22)	2·0	*Amphiura filiformis*	1	0·014
Maldanidae	1		*Amphipholis squamata*	3	0·003
Capitellidae	5		*Ascidiella aspersa*	1	0·007
?*Jasmineira elegans*	2		Total dry weight (0·6 m.^{2})		4·0
Terebellidae	1				
Polychaeta indet.	4				

Station A4

50° 13′ N., 4° 15′ W. Muddy sand with small stones and shell fragments. Depth: 55 m. 18. x. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

As at A3, there was considerable variation in the grade of deposit brought up in successive hauls. Two types of sediment were taken, their grades corresponding closely to those at A3 (Table 1 and Fig. 2).

The fauna (Table XIII) is similar to that at the previous station. Polychaete worms are common and make up over a quarter of the total weight. Three specimens of *Upogebia deltaura* were taken, whereas *U. stellata* was taken at A3.

The total dry weight is 6·9 g., largely made up of Polychaeta, *Upogebia*, and the crab *Portunus depurator*.

Station A5

50° 11′ N., 4° 16′ 15″ W. (Eddystone bearing 150° T., 0·5 miles.) Shell gravel (Table I, Fig. 2). Depth: *c.* 42 m. 21. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

This station is situated on the Eddystone shell gravel where dredgings are frequently made for *Amphioxus*. This position was worked to avoid a possibly rocky ground on the east side of the reef, and since an area was selected where the fauna was known to be quite rich a slight bias is given to the results as a whole, since all but one of the other stations were selected at definite intervals irrespective of the possible nature of the ground.

Since the position is so close to the Eddystone Lighthouse, it is possible to obtain an accurate position fix, but the ship soon drifts off the ground, and while making hauls with the bottom-sampler it was necessary to steam back to the original position after two or three hauls.

TABLE XIII. STATION A4. NUMBERS AND WEIGHTS PER 0·5 M.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·16	*Galathea* sp.	3*j*	0·019
Anemone	1	0·049	*Upogebia deltaura*	1, 2*j*	1·6
Edwardsia sp.	3*j*	0·11	*Portunus depurator*	1	2·0
Glycera sp.	7	1·9	Pycnogonida	1	<0·001
Hyalinoecia bilineata	22		*Cylichna cylindracea*	4	0·011
Onuphis conchylega	4		*Lepton squamosum*	1	0·003
Lumbriconereis sp.	4		*Phacoides borealis*	1	0·040
?*Owenia* sp.	(16)		?*Cardium echinatum*	1*j*	0·010
Pectinaria sp.	3*j*		*Solecurtus chamasolen*	1	0·075
Maldanidae	1		*Cellaria* sp.	+	0·046
Terebellidae	2		*Alcyonidium* sp.	6	0·43
Sabellidae	1		*Ophiura affinis*	1	0·002
Polychaeta indet.	+		*Echinocyamus pusillus*	2	<0·001
Phyllochaetopterus tubes	+	..	*Labidoplax digitata*	1	*0·48*
'Prawn'	1*j*	0·006	Total dry weight		6·9

TABLE XIV. STATION A5. NUMBERS AND WEIGHTS PER 0·5 M.^{2}

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Porifera indet.	2*j*	<0·001	*Venus fasciata*	1	0·078
Hydroid	+	0·014	*Tellina crassa*	1	0·89
Aphroditidae	1	0·33	*Gari tellinella*	1	0·055
Glycera sp.	1		*Solecurtus scopula*	1	1·5
Petta pusilla	1		?*Lutraria* sp. (siphons)	1	5·5
Terebellidae	1		*Cellaria* sp.	+	0·012
Polychaeta indet.	3		*Ophiothrix fragilis*	7	1·4
Phascolosoma sp.	1		*Echinocyamus pusillus*	5	0·003
Ampelisca spinipes	1	0·004	*Amphioxus lanceolatus*	7	0·35
Conilera cylindracea	4	0·030	(Egg case of skate)	1	..
Ebalia tuberosa	1	0·13			
Cardium scabrum	1*j*	0·011	Total dry weight		10·3

Smith (1932) has studied the fauna and deposits around the Eddystone in some detail. The species characteristic of the shell-gravel are species of the 'SpVf' (*Spatangus purpureus-Venus fasciata*) communities of Ford (1923).

The siphons of a large lamellibranch, probably *Lutraria*, were taken (Table XIV). Ford records specimens of *L. magna* from shell-gravel in Plymouth Sound, so these may be of the same species. The animal was judged to be adult, and was assumed to have an alcohol weight of 20 g. (5·5 g. dry).

Polychaete worms form only a small fraction of the total dry weight, which is largely made up of moderate-sized lamellibranchs, and the *Lutraria*. *Ophiothrix* seems to be patchily distributed on this ground.

For stations A6 and A7 see pp. 32–33.

Station B1

50° 18′ N., 4° 10′ W. Shell gravel with coal and clinker. Depth: 31 m. 26. vi. 50. Area: 0·1 m.². Sieve: 2·2 mm.

Three hauls were made, one of which brought up a sample of gravel (Table I); the other two were evidently on a rocky bottom.

A single specimen of *Tellina crassa* was taken with a dry weight of 0·64 g. This is equivalent to 3·2 g./$\frac{1}{2}$ m.².

TABLE XV. STATION B2. NUMBERS AND WEIGHTS PER $\frac{1}{2}$ M.²

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·078	*Phyllochaetopterus* tubes	+	..
Alcyonium sp.	1	0·018	*Owenia* tubes	10	..
Nemertinea indet.	1		*Ampelisca spinipes*	1	0·005
Glycera sp.	2		*Callianassa subterranea*	1*j*	0·013
Marphysa belli	1		*Upogebia deltaura*	2	3·5
Cirratulidae	1		*Mysia undata*	1	0·057
Notomastus latericeus	2	1·4	*Abra alba*	1	0·018
Amphicteis gunneri	10		*Solecurtus chamasolen*	1, 10*j**	1·1
?*Melinna* sp.	11		*Cellaria* sp.	+	0·098
Maldanidae	2		*Cucumaria elongata*	1	0·081
Myxicola infundibulum	1		Total dry weight		6·4

* One small specimen in a $\frac{1}{10}$ subsample.

Station B2

50° 16′ N., 4° 10′ W. Fine muddy sand with small stones. Depth: 48 m. 18. v. 50. Area: 0·5 m.². Sieve: 2·2 mm. A marker float was achored close to the position of the first haul, and the total drift during five hauls was found to be about 150 yards (137 m.).

The percentage of coarse material was high (Table I), 27% being over 2 mm., and there was a fairly high percentage of silt (8·6%) which resulted in the presence of such species as *Notomastus*, *Myxicola*, *Solecurtus chamasolen* and *Cucumaria elongata* (Table XV). Two species of burrowing prawn, *Callianassa subterranea* and *Upogebia deltaura*, were taken.

The total weight of 6·4 g. is largely made up of two specimens of *Upogebia* and one large and several small *Solecurtus*.

Station B3

50° 14′ N., 4° 10′ W. Muddy sand. Depth: 53 m. 18. v. 50. Area: 0·5 m.². Sieve: 1·2 mm.

The soil was finer and less muddy (4·4% silt) than at B2 (Table I). Some of the same species occurred (Table XVI) (*Abra alba*, *Solecurtus chamasolen*), but the difference in soil is reflected in the presence of species characteristic of slightly muddy sand: *Cultellus*, *Echinocardium* and *Labidoplax*.

The deposit was sieved through a finer sieve than usual, and this retained a greater number of polychaete worms and small Crustacea. They appear to have little effect on the total biomass, however, the dry weight for polychaetes

being of the same order as at similar stations where the 2·2 mm. sieve was employed (A3, A4 and B2), and the weight of the small Crustacea is negligible.

The large sabellarian worm *Pallasia murata* contributes about a quarter to the weight of polychaetes. Much of the total dry weight is made up of large individuals—*Solecurtus*, *Echinocardium* and *Labidoplax*.

For stations B4–B7 see pp. 29–36.

Station C1

50° 17′ N., 4° 5′ W. Fine gravel of shell fragments and small stones. Depth: 37 m. 7. xi. 50. Area: 0·5 m.². Sieve 2·2 mm.

The soil at this station was rather variable, some hauls being rather muddy and others quite clean (Table I).

The fauna is rather poor (Table XVII), the total dry weight being only 1·3 g.

Station C2

50° 15′ N., 4° 5′ W. Muddy sand with a few stones (Table I). Depth: 46 m. 7. xi. 50. Area: 0·5 m.². Sieve: 2·2 mm.

The fauna (Table XVIII) is typical of muddy sand, but is rather poor. The occurrence of *Lepton squamosum*, a small lamellibranch commensal with *Upogebia*, is of interest.

Apart from *Labidoplax*, there are no individuals of any size, and the total dry weight is only 2·5 g.

Station C3

50° 13′ N., 4° 5′ W. Muddy sand with a few stones (Table I). Depth: 46 m. 7. xi. 50. Area: 0·5 m.². Sieve: 2·2 mm.

The bottom at this station is evidently rocky with pockets of sediment. A single core sample (Table II) showed no sediment overlying the rock. The fauna (Table XIX) is rather poor, except for fifteen specimens of *Ophiothrix*, which were all or nearly all taken in a single haul of the sampler.

The total dry weight, 4·5 g., is rather below the average.

Fauna of sandy grounds around the Eddystone

At the stations so far described there is much variation in both grades of deposit and fauna, but at certain stations farther offshore, to the south and east of the Eddystone, there is a marked uniformity in grade of soil and fauna.

As already shown (pp. 14–19), the fauna in successive hauls at B5 does not vary greatly, being little more patchy than would be expected from a random distribution. The same uniformity extends over a much larger area, a very similar fauna being found at stations A6, A7, B4, B5, B6, B7, C4, C5 and C6.

TABLE XVI. STATION B3. NUMBERS AND WEIGHTS PER ½ M.²

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·13	*Diastylis laevis*	1	
Aphroditidae	3		*Ampelisca diadema*	2	
Nephthys sp.	(8)		*Ampelisca tenuicornis*	4	0·002
Eunice harassi	2		*Maera othonis*	2	
Lumbriconereis sp.	2, 8*j*		*Photis longicaudata*	2	
Scoloplos armiger	1		*Abra alba*	1*j*	0·001
Magelona cincta	1	1·4	*Solecurtus chamasolen*	3	2·4
Cirratulidae	6		*Cultellus pellucidus*	2*j*	0·003
Ammotrypane aulogaster	1		*Cellaria* sp.	+	0·51
Pallasia murata	1		Polyzoa indet.	+	0·009
Polychaeta and Nemertinea indet.	(40)		*Acrocnida brachiata*	1	0·067
Phascolion strombi	2		*Echinocardium cordatum*	2	*0·57*
Chaetopterus tube	1	..	*Thyone raphanus*	1	0·14
Phyllochaetopterus tubes	+	..	*Labidoplax digitata*	1	*0·48*
Scalpellum scalpellum	3*j*	0·007	Total dry weight		5·7

TABLE XVII. STATION C1. NUMBERS AND WEIGHTS PER ½ M.²

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Nephthys sp.	1		*Upogebia deltaura*	2	0·16
Glycera sp.	3		*Chlamys opercularis*	2	0·49
Hyalinoecia bilineata	19	0·56	*Cellaria* sp.	+	0·004
Nerine sp.	1		*Echinocyamus pusillus*	22	0·011
Polychaeta indet.	(60)		*Thyone fusus*	(10)*	0·073
Ampelisca spinipes	3	0·003			
Maera othonis	1		Total dry weight		1·3

* One specimen in a $\frac{1}{10}$ subsample.

TABLE XVIII. STATION C2. NUMBERS AND WEIGHTS PER ½ M.²

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Hydroid	+	0·014	*Galathea* sp.	1*j*	0·004
Edwardsia sp.	1*j*	0·014	*Upogebia deltaura*	1*j*	0·047
Anemone indet.	1	0·20	*Lepton squamosum*	1	0·001
Nephthys sp.	(8)		*Venus ovata*	3*j*	0·004
Glycera sp.	1		*Venerupis rhomboides*	1	0·14
Lumbriconereis sp.	6		*Cultellus pellucidus*	3	0·062
Cirratulidae	2		*Cellaria* sp.	+	0·001
?*Owenia* sp.	(6)	1·3	*Alcyonidium* sp.	1*j*	0·057
Pallasia murata	1		*Amphiura filiformis*	1	0·008
Amphicteis gunneri	1		*Cucumaria elongata*	1	0·11
Polychaeta indet.	(20)		*Labidoplax digitata*	1	*0·48*
Ampelisca spinipes	2	0·009			
Maera othonis	1		Total dry weight		2·5

TABLE XIX. STATION C3. NUMBERS AND WEIGHTS PER ½ M.²

(For further details see Tables X and XI.)

	No.	Weight		No.	Weight
Anemone	1	1·1	*Processa* sp.	1	0·13
Nephthys sp.	1		Burrowing prawn*	1*j*	
?*Owenia* sp.	(4)	0·68	*Dosinia lupinus*	1	0·043
Polychaeta indet.	15		*Tellina donacina*	1	0·032
Ampelisca spinipes	1	0·004	*Ophiothrix fragilis*	15	2·4
Maera othonis	1*j*		*Polycarpa pomaria*	1	0·064
Ebalia cranchi	2	0·088	Total dry weight		4·5

* Damaged specimen of *Upogebia* or *Callianassa*.

The fauna corresponds to that described by Ford (1923) as the EcVg (*b*) community, i.e. a community characterized by *Echinocardium cordatum*, *Venus striatula* (=*gallina*) and *Abra prismatica*.

The fauna taken at each station is shown in Tables XX–XXII. No one species occurs in any great numbers, but even comparatively sparse species occur regularly at most or all of the stations.

The commoner species were:

Edwardsia sp.	*Thyasira flexuosa*
Nephthys sp.	*Phacoides borealis*
Glycera sp.	*Cardium echinatum*
Lumbriconereis sp.	*Dosinia lupinus*
Magelona papillicornis	*Abra prismatica*
M. cincta	*Cultellus pellucidus*
Pectinaria koreni	*Cellaria* sp.
Chaetopterus variopedatus	*Amphiura filiformis*
Phyllochaetopterus sp.	*Ophiura affinis*
Ampelisca tenuicornis	*Echinocardium cordatum*
Nucula turgida	*Labidoplax digitata*

Venus striatula, although a 'characteristic' species in the community described by Ford, occurs only at two out of the nine stations. Its distribution is sufficiently restricted to set the stamp on the community which it inhabits, and its continued use as a characteristic species therefore seems justified. The writer has nowhere in the Plymouth area found it in any abundance.

The occurrence of *Glossobalanus sarniensis* at one station is of interest—it has also been taken at B 5 (Table VI), and has been subsequently taken in a new dredge (see Forster, 1953) by Mr G. R. Forster. In the *Plymouth Marine Fauna* (Marine Biological Association, 1931) the only Enteropneusta recorded are tornaria larvae of *Balanoglossus*, which are sometimes taken in plankton-nettings at Plymouth. The tornaria larvae are presumably larvae of *Glossobalanus* from these grounds. The adult was not taken in the survey of Allen (1899), probably because the dredges used did not dig sufficiently deeply. The same species has also been taken at Salcombe by Dr D. P. Wilson (see Burdon-Jones, 1953).

Details of the stations are given below:

Station A 6

50° 9′ N., 4° 15′ W. Depth: 64 m. Clean sand. 21. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

Station A 7

50° 7′ N., 4′ 15′ W. Depth: 70 m. Clean sand. 21. vii. 50. Area: 0·5 m.^{2}. Sieve: 2·2 mm.

Station B 4

50° 12′ N., 4° 10′ W. Depth: 55 m. Slightly muddy fine sand. 18. v. 50. Area: 0·5 m.^{2}. Sieve: 1·2 mm.

TABLE XX. NUMBER AND WEIGHTS PER ½ M.²

(For further details see Tables X and XI.)

Station	A6		A7		B4	
	No.	Weight	No.	Weight	No.	Weight
Hydroid	+	<0·001	+	0·19	+	0·36
Edwardsia sp.	10*j*	0·19	1*j*	0·024	1*j*	0·014
Anemone indet.	..	..	1	0·12	1	0·18
Porifera	..	..	..	..	1*j*	<0·001
Nemertinea	1		1		3	
Aphroditidae	..		..		3	
Oxydromus sp.	..		..		2	
Hesionidae	..		..		1	
Nereidae	..		1		..	
Nephthys sp.	1		4		(11)	
Glycera sp.	..		1		2	
Lumbriconereis sp.	5		4		16	
Goniada sp.	..		..		1	
?*Staurocephalus rudolphi*	..	0·42	..	1·3	1	1·3
Scoloplos armiger	..		..		2	
Magelona cincta	3		..		3	
Cirratulidae	..		..		9	
Capitellidae	..		1		16	
Maldanidae	1		(3)		(6)	
Pectinaria koreni	..		2*j*		..	
Ampharetidae	..		..		1	
Sabellidae	1		1		..	
Polychaeta indet.	(4)		(6)		20	
Chaetopterus variopedatus	..	..	1	*0·50*	3*	..
Phyllochaetopterus tubes	1	..	2	..	1	..
Diastylis laevis	..	..	..	..	2	
Ampelisca tenuicornis	..	..	1		3	0·001
Bathyporeia tenuipes	..	..	..	0·001	1	
Pseudoprotella phasma	..	..	1			
Galathea sp.	..	..	1	0·25	..	..
Ebalia cranchi	..	..	..	..	1	0·010
Eulima sp.	..	..	..	..	1	0·001
Nucula turgida	1, 2*j*	0·032	1	0·025	..	..
Nucula nucleus	..	..	..	..	2, 5*j*	0·050
Thyasira flexuosa	3	0·018	7	0·065	..	..
Phacoides borealis	1	0·061	1	0·086	2, 1*j*	0·11
?*Montacuta ferruginosa*	..	..	..	..	1	0·001
?*Cardium echinatum*	1*j*	0·001	1*j*	0·003	..	..
Dosinia lupinus	1*j*	0·001	1*j*	0·006	3, 2*j*	0·45
Venus striatula	1, 1*j*	0·051	..	..	..	..
Abra alba	..	..	..	..	1, 1*j*	0·004
Abra prismatica	1*j*	0·001	1	0·022	1	0·011
Solecurtus chamasolen	..	..	..	..	2	1·9
Cultellus pellucidus	7, 13*j*	0·30	1, 8*j*	0·058	2, 4*j*	0·024
Hiatella arctica	..	..	2	0·042	..	..
Cellaria sp.	..	..	+	0·002	+	0·91
Polyzoa indet.	..	..	..	..	+	0·036
Amphiura filiformis	2	0·012	..	..	1	0·001
Ophiura affinis	3	0·005	3	0·003	..	..
Echinocyamus pusillus	..	..	..	..	4	0·002
Echinocardium cordatum	1	*0·21*	2, 2*j*	0·41	3	1·1
Labidoplax digitata	1	*0·48*	..	..	1	*0·48*
Leptosynapta inhaerens	..	..	1	*0·48*	..	..
Glossobalanus sarniensis	1	*0·55*	..	..	..	..
Total dry weight		2·3		3·6		6·9

* Empty tubes.

TABLE XXI. NUMBERS AND WEIGHTS PER ½ M.²

(For further details see Tables X and XI.)

Station	B5		B6		B7	
	No.	Weight	No.	Weight	No.	Weight
Hydroid	+	0·026	+	0·92	+	0·45
Edwardsia sp.	7*j*	0·097	9*j*	0·13	3*j*	0·042
Cerianthus lloydi	1	0·015	..	..	..	..
Anemone indet.	..	..	1*j*	0·007	..	..
Nemertinea	2		1		2	
Sigalion mathildae	..		1		..	
Aphroditidae indet.	1		2		..	
Phyllodoce sp.	..		2*j*		..	
Nereidae	..		1		..	
Nephthys sp.	3		3		3	
Glycera sp.	1		..		1	
Eunice harassi	..		1		..	
Lumbriconereis sp.	11	1·3	8	2·0	2	0·46
Magelona papillicornis	..		..		1	
Magelona cincta	3		2		..	
Capitellidae	5		1		..	
Maldanidae	(5)		2		1	
Owenia sp.	1*j*		..		..	
Pectinaria koreni	..		1		†1	
Terebellidae	..		3		..	
Polychaeta indet.	5		9		3	
Chaetopterus variopedatus	1, 1*j*	*1·2*	2	*2·3*	..	..
Phyllochaetopterus tubes	+	..	+	..	+	..
Scalpellum scalpellum	1*j*	0·006	1*j*	0·001	..	..
Ampelisca tenuicornis	5	0·002	1	<0·001	..	..
Galathea sp.	..	..	3*j*	0·018	..	..
Eupagurus sp.	1*j*	0·005	..	..	..	..
Eurynome aspera	..	..	1*j*	0·14	..	..
Nucula nucleus	1	0·003	2	0·028	..	..
Nucula turgida	3	0·008	2	0·010	1	0·003
Chlamys sp.	..	..	..	..	3*j*	0·004
Thyasira flexuosa	..	..	2	0·018	3	0·022
Phacoides borealis	3, 1*j*	0·16	..	..	3	0·18
Montacuta ferruginosa	..	..	..	..	1	0·004
Cyprina islandica	1*j*	0·003	..	..	1	7·6
Cardium echinatum	1	3·6	..	..	..	..
?*Cardium echinatum**	4*j*	0·018	..	..	3*j*	0·008
Dosinia lupinus	1	0·094	1	0·42	1	0·46
Tellina donacina	..	..	1*j*	0·003	..	..
?*Abra prismatica*	..	..	2	0·022	..	..
Cultellus pellucidus	1, 7*j*	0·025	5, 7*j*	0·16	5	0·019
Hiatella arctica	..	..	..	..	1*j*	0·003
Cochlodesma praetenue	..	..	1	0·017	1*j*	0·003
Cellaria sp.	+	0·012	+	0·037	..	..
Polyzoa indet.	+	0·024	+	0·025	..	..
Ophiothrix fragilis	1	0·003	2	0·24	..	..
Amphiura filiformis	3	0·004	..	..	..	..
Ophiura affinis	1	0·002	1	0·002	3	0·002
Echinocyamus pusillus	..	..	..	..	1	0·001
Echinocardium cordatum	2	*0·55*	..	..	2	*0·55*
Cucumaria lactea	..	..	5	0·070	..	..
Labidoplax digitata	2	*0·97*	1	*0·48*	2	*0·97*
Total dry weight		8·1		7·0		10·8

* Small specimens, which could not be identified with certainty.
† Identity doubtful.

TABLE XXII. NUMBERS AND WEIGHTS PER ½ M.²

(For further details see Tables X and XI.)

Station	C4		C5		C6	
	No.	Weight	No.	Weight	No.	Weight
Hydroid	..	..	+	0·040	+	0·66
Edwardsia sp.	1*j*	0·014	1*j*	0·019	3*j*	0·042
Cerianthus lloydi	..	..	..		1	0·23
Anemone indet.	1	0·36	2		..	..
Nemertinea	..	..	3		2	
Sthenelais limicola	1		..		..	
Aphroditidae	2		..		3	
Nereis sp.	..		..		3*j*	
Nephthys sp.	2		2		5	
Glycera sp.	3		5		2	
Lumbriconereis sp.	3	1·2	9	1·7	18	2·0
Nerine sp.	..		..		1	
Magelona papillicornis	..		2		2	
Magelona cincta	1		5		12	
Maldanidae	..		..		1	
?*Owenia* sp.	..		..		1	
Pectinaria koreni	2*j*		?3*j*		?7*j*	
Polychaeta indet.	3		5		13	
Chaetopterus variopedatus	..	..	..	..	2	*2·3*
Phyllochaetopterus tubes	..	..	..	..	+	..
Scalpellum scalpellum	..	..	..	..	5	0·083
Leucothoë sp.*	..	..	..	..	1	
Aorid	..	..	..	..	1	0·001
Pseudoprotella phasma	..	..	..	..	1	
?*Porcellana longicornis*	..	..	..	..	1	0·015
Corystes cassivelaunus	1	1·5	1*j*	0·036	1*j*	0·042
Cylichna sp.	1	0·006	..	..	..	..
Nucula turgida	4	0·047	6	0·024	4	0·051
Thyasira flexuosa	8	0·043	9	0·050	5	0·044
Phacoides borealis	..	..	?1*j*	0·010	1	0·025
Cardium echinatum	..	..	1	4·0	..	..
?*Cardium echinatum*†	..	..	1*j*	0·010	..	..
Dosinia lupinus	1*j*	0·013	1	0·083	2*j*	0·017
Venus striatula	1*j*	0·001	..	..	..	..
Abra alba	1	0·011	1	0·003	1	0·050
Abra prismatica	1*j*		..	..	1, 3*j*	0·032
Gari fervensis	..	..	..	..	3	1·36
Solecurtus chamasolen	1	0·47	..	..	..	..
Cultellus pellucidus	14	0·11	14	0·11	23	0·096
Ensis ensis	1	0·73	..	..	..	..
?*Thracia pubescens*	..	..	..	..	1*j*	0·014
Cellaria sp.	+	0·001	+	0·010	+	0·010
Ophiothrix fragilis	..	..	..	..	1	0·051
Acrocnida brachiata	..	..	2	0·021	1	0·018
Ophiura affinis	2	0·001	3	0·002	1	0·001
Echinocyamus pusillus	..	..	..	..	2	< 0·001
Echinocardium cordatum	4, 3*j*	*1·2*	2	*0·57*	3, 1*j*	*1·2*
Echinocardium sp.†	..	..	1*j*		4*j*	
Labidoplax digitata	..	..	1	*0·48*	1	*0·48*
Ascidiella aspersa	..	..	..	..	1*j*	0·007
Aphya pellucida‡	..	..	..	..	1	..
Total dry weight		5·7		7·2		8·8

* See p. 25.
† Small specimens, which could not be identified with certainty.
‡ The weight of this fish is not included.
A query (?) before the number of individuals indicates uncertain identity.

Station B5

50° 10′ N., 4° 10′ W. Depth: 60 m. Clean sand. 3. vii. 50. Area: 0·5 m.². Sieve: 2·2 mm.

N.B. This is distinct from the series of hauls made at this station on 5. xii. 50, described on pp. 14–19.

Station B6

50° 8′ N., 4° 10′ W. Depth: 62 m. Clean sand. 3. vii. 50. Area: 0·5 m.². Sieve: 2·2 mm.

Station B7

50° 6′ N., 4° 10′ W. Depth: 68 m. Clean sand. 3. vii. 50. Area: 0·5 m.². Sieve: 2·2 mm.

Station C4

50° 11′ N., 4° 5′ W. Depth: 57 m. Clean sand. 27. vii. 50. Area: 0·5 m.². Sieve: 2·2 mm.

N.B. This is distinct from the hauls made at this station on 7. xi. 50, described on pp. 21–22.

Station C5

50° 9′ N., 4° 5′ W. Depth: 62 m. 27. vii. 50. Area: 0·5 m.². Sieve: 2·2 mm.

Station C6

50° 7′ N., 4° 5′ W. Depth: 66 m. 27. vii. 50. Area: 0·5 m.². Sieve: 2·2 mm.

The total dry weight on these grounds ranges from 2·3 g. (A6) to 10·8 g. (B7) per $\frac{1}{2}$ m.². The mean figure for the nine stations is 6·7 g. The great variability in the catch from one station to the next is due to the occurrence of large lamellibranchs, *Cyprina* and *Cardium echinatum* in particular, which are present in some samples and not in others.

Dry weight at B5

The 0·5 m.² sample of 3 July 1950, from a 2·2 mm. sieve, gave a figure of 8·1 g./$\frac{1}{2}$ m.². The 2 m.² sample of 5 December 1950, from a 1·2 mm. sieve, gave 11·6 g./$\frac{1}{2}$ m.², as set out in Table XXIII. The difference is partly accounted for by the use of a different sieve, which resulted in a doubling of the weight of Polychaeta (other than *Chaetopterus*) taken with the finer meshed sieve; but for other groups the differences were not so great. Since the total weight was made up mostly of a few large individuals, the discrepancy is partly attributable also to sampling errors.

If we ignore the weights of the Polychaeta (other than *Chaetopterus*), and of large specimens of *Cardium echinatum* and *Cyprina islandica*, the totals become:

$\frac{1}{2}$ m.² sample of 3. vii. 50: 3·2 g./$\frac{1}{2}$ m.²,
2 m.² sample of 5. xii. 50: 3·3 g./$\frac{1}{2}$ m.²,

which is a reasonably good agreement.

TABLE XXIII. STATION B5, 5 DECEMBER 1950. DRY WEIGHTS PER 2 M.^{2}

For some species the weights obtained for each series of ten samples is also given (samples: 1–10/11–20/21–30/31–40.)
Assumed weights are in italics.

		Weight
Hydroid		0·42
Virgularia mirabilis		0·004
Cerianthus lloydi		0·047
Edwardsia sp.		0·25
Chaetopterus variopedatus	—/*1·1*/*1·1*/*1·1*	*3·3*
Other Polychaeta, Gephyrea	1·8/2·1/3·6/1·9	9·4
Amphipoda		*0·012*
Other Crustacea		0·37
Acteon tornatilis	0·001/0·001/—/—	0·002
Cylichna cylindracea	—/0·001/—/0·001	0·002
Nucula nucleus	0·001/—/—/—	0·001
Nucula turgida	0·013/0·006/0·008/0·037	0·064
Musculus marmoratus	—/0·001/—/—	0·001
Chlamys sp.*	—/0·006/—/0·11	0·116
Thyasira flexuosa	0·019/0·007/0·011/0·008	0·045
*Phacoides borealis**	0·39/0·087/0·26/0·26	0·997
Montacuta ferruginosa	0·007/—/0·006/—	0·013
Cyprina islandica	9·7/—/*9·7*/—	*19·4*
Cardium echinatum	0·015/—/—/4·2	4·215
Dosinia lupinus	0·94/0·047/0·49/0·19	1·667
Venus ovata	0·001/—/—/—	0·001
Venus striatula	0·004/0·11/0·52/—	0·634
Venerupis rhomboides	0·006/0·007/—/—	0·013
Abra alba	—/—/0·014/—	0·014
Abra prismatica	0·011/0·024/0·026/0·014	0·075
Gari fervensis	0·001/0·001/0·040/—	0·042
Cultellus pellucidus	0·080/0·043/0·097/0·11	0·330
Hiatella arctica	0·006/0·008/—/—	0·014
Cochlodesma praetenue	0·010/—/0·003/0·003	0·016
Lyonsia norwegica	—/—/0·003/—	0·003
Cellaria sp.		0·066
Ophiuroidea		0·13
Echinocyamus pusillus		< 0·001
Echinocardium cordatum		1·2
Labidoplax digitata		*1·6*
Tunicata		0·63
Glossobalanus sarniensis		*1·1*
Total		46·2

* The following corrections are necessary when comparing with Table VI: *Phacoides borealis*, 8, *not* 9, specimens in samples 1–10; 8, *not* 10, in samples 21–30 (when the shells were opened for weighing three were found to be empty). *Chlamys* sp. A single specimen, not recorded in Table VI, was found in hauls 31–40.

SUMMARY OF BIOMASS DATA

For all stations, the mean dry weight of animal tissue is 11·2 g./m.^{2}, which is equivalent to about 55 g. fresh weight. While this figure is sufficient for comparative purposes, there are a number of corrections to be applied if an absolute figure is required. Apart from any errors due to the small size of the area sampled, the following sources of error are present:

(i) Animals missed by the instrument, either because they live too deeply in the deposit, or are able to evade the sampler. Since the magnitude of the error is unknown, no correction can be applied.

(ii) Loss of small animals through the sieve. It is believed that the use of

a 1·0 or 1·2 mm. sieve, the usual mesh in such investigations, would increase the total dry weight taken by about 10% (p. 22).

(iii) Weight of sand in the guts, estimated as 5% of the alcohol weight (p. 24). Taking 27·72 as the mean percentage dry weight relative to the alcohol weight (p. 23), the corrected percentage becomes 22·72, i.e. the dry weight figure should be reduced by about 18%.

Applying corrections for sieving errors and gut content, a figure of about 10 g./m.^{2} dry weight is reached.

As already emphasized, much of the total dry weight is made up of large individuals, having a relatively low metabolic rate, and therefore of less importance from the aspect of productivity than their weight would suggest. The percentage of individuals, each weighing over 0·2 g. dry (= *c.* 1 g. fresh weight), is given below:

Station	Percentage over 0·2 g.	Station	Percentage over 0·2 g.
A1	0	B5	77·6
A2	76·9	B6	45·4
A3	22·2	B7	88·8
A4	58·5	C1	37·4
A5	90·1	C2	20·0
A6	53·2	C3	23·6
A7	46·0	C4	74·3
B1	100	C5	70·1
B2	72·6	C6	62·8
B3	60·2		
B4	50·0	Total	64·4

An analysis has been made of the percentages of the total weight made up by the different groups of the animal kingdom, both for all twenty stations and for the nine stations on the sandy grounds outside the Eddystone. These show the preponderance of Polychaeta, Lamellibranchia and Echinodermata; the only other groups occurring in significant quantities being Coelenterata and Malacostraca (the percentages are shown graphically in Fig. 7):

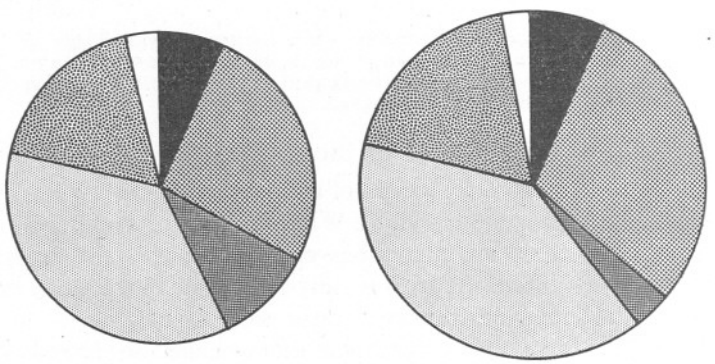

Fig. 7. Percentage composition by weight of the fauna. Left: all stations in the 1950 survey; right: stations on the Eddystone sandy grounds only. The area of the circles is proportional to the total biomass. ■, Coelenterata; ▒, Polychaeta etc.; ▓, Crustacea Malacostraca; ▒, Lamellibranchia; ▒, Echinodermata; □, others.

	All stations	Eddystone sandy grounds
Coelenterata	7·00	6·84
Polychaeta and Nemertinea	25·79	29·49
Eumalacostraca	0·16	0·16
Malacostraca	10·35	3·38
Gastropoda	0·02	0·01
Lamellibranchia	35·46	39·10
Polyzoa	2·46	1·76
Echinodermata	17·88	18·33
Protochordata	0·88	0·93

The only major difference is in the low figure for Malacostraca on the Eddystone grounds, attributable to the scarcity of the burrowing prawns *Upogebia* and *Callianassa*.

Changes in the Bottom Fauna

Allen's survey

Allen's (1899) survey of the bottom fauna in the neighbourhood of the Eddystone was largely based on dredge hauls. His grounds I–III and VI correspond with the Eddystone sandy grounds described in this paper. His species lists are mainly made up of members of the epi-fauna, among which hydroids figure prominently, but there is no reason to suppose that any major qualitative changes have occurred over the past fifty years.

One species, the scaphopod mollusc *Dentalium entalis*, is now conspicuous by its absence. Allen records this species alive from all fine sand grounds, with the exception of haul 104 on ground II, where the absence of this species was noteworthy. In the past few years no living *Dentalium* has been taken at Plymouth, although empty shells are very common. Specimens have recently been taken, however, in dredge and grab hauls in the mouth of the Channel, south of Land's End.

Ford's survey

The survey by Ford (1923) provides data on the density of the bottom fauna in 1922–23 for many positions off Plymouth. Any comparison with the 1950 results is complicated, however, by: (i) Ford used the $\frac{1}{10}$ m.^{2} Petersen grab, which has been shown (pp. 11–12) to sample less efficiently than the scoop-sampler used in the 1950 survey. (ii) The patchy nature of the inshore grounds and the fact that the same positions were not revisited in 1950 makes any exact comparison impossible. Ford did not sample the sandy grounds outside the Eddystone. (iii) Any comparison can only be in terms of numbers of animals, irrespective of their size or weight, since Ford gives no such data. (iv) Ford used the finest sieve in the *Challenger* series, which has openings of about 1·2 mm., whereas a 2·2 mm. sieve was used in 1950. (v) The samples described by Ford were selected to illustrate the different types of community to be found, and some of the less productive samples were omitted. For the same reason stations tend to be aggregated in the richer areas.

Mr Ford has kindly lent me his original notes, which include records of samples not published in his paper; all his stations within the area covered in 1950 are used in the comparisons which follow. To eliminate any bias due to aggregation of Ford's stations, those from certain areas have been averaged and scaled down so as to contribute only $\frac{1}{2}$ m.^{2} to the total. The samples, positions of which are shown in Fig. 1 (p. 4), are summated thus.

	Sampled (m.^{2})	Reduced to (m.^{2})
Whitsand Bay	4·8	0·5
Rame mud	3·7	0·5
Eddystone	3·1	0·5
Mewstone	2·1	0·5
Other samples	6·2	6·2
Total		8·2

For comparison, the following 1950 stations have been taken: A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, C1, C2, C3, C4. The total area is 7 m.^{2}, and figures for both surveys have been converted to densities per 10 m.^{2}. (Two small divergencies in the 1950 survey have been ignored—these are the use of a 1·2 mm. sieve at B3 and B4 and the sampling of 0·6 m.^{2} instead of 0·5 m.^{2} at A3.)

A comparison of the lamellibranch faunas is given in Table XXIV. Before considering this in detail, allowance must be made for the different sieves used. The 2·2 mm. sieve used in 1950 had square apertures of *c.* 3 mm. diagonal, so that a shell having a breadth of less than 3 mm. may pass through end-on.

It is difficult to estimate the percentage losses due to use of the coarser sieve. Station B 5 was sampled on two occasions: on the first (Table XXI) an area of 0·5 m.^{2} was sieved through the 2·2 mm. sieve, on the second (Table VI) an area of 2 m.^{2} was sieved through a 1·2 mm. mesh. The results indicate that the coarser sieve retained over half (57·5 %) of the numbers retained by the 1·2 mm. sieve. The figure for the razor-shell, *Cultellus*, is 80 %.

At C 4 (Table VIII) the sample of 7 November 1950 was passed through first the 2·2 mm. and then the 1·2 mm. sieve. Only 32 % of the total was found on the coarser sieve.

If the samples from A 1, where a 2·2 mm. mesh was used, and from the nearby station in Whitsand Bay (1·2 mm. mesh used) are compared, a figure of 51 % is obtained as the percentage retained by the coarser sieve.

It is not claimed that any of the above comparisons have any great validity, but if we take 50% as the correction to be made, no great error should result.

Ford (1925) gives some data on the growth of lamellibranchs, from which it follows that spat of the larger species very soon grow sufficiently large to be retained by a 2·2 mm. mesh. *Abra alba*, for which species most data is available, has a shell breadth

equal to *c.* 72% of its length. Individuals up to about 4 mm. would therefore pass a sieve of this mesh. Of a total of 7391 individuals measured from samples taken through the year in Bigbury Bay (east of Plymouth), only 9·16% were in the 4 mm. length group or below. The percentage varied from nil to 90% according to the season.

Spisula elliptica spat grew from 3·44 to 6·18 mm. length in 20 days in July and *Lutraria lutraria* increased from 3 to 12 mm. in 6 weeks. Individuals of *Cultellus* spatted in June 1922, grew to 15–20 mm. in length by the autumn of the same year. Since the shell breadth of *Cultellus* is some 20% of its length, it follows that individuals up to 15 mm. length might pass the sieve. But an analysis of the lengths of shells taken in the 2·2 mm. sieve in 1950 shows that many below this length were retained. Of 116 specimens taken 29 (25%) were 15 mm. or over, 74 (63·8%) were between 10 and 15 mm., and 13 (11·2%) were less than 10 mm. There is no reason to suppose, therefore, that losses of *Cultellus* were exceptionally high.

Where small species such as *Nucula* and *Corbula* are concerned losses through the coarse sieve may be considerable.

The sampling capabilities of the Petersen grab and the scoop-sampler vary with the nature of the sediment, and therefore the relative degree of penetration by the two samplers and also with the depth of individuals in the sediment. On the whole, older individuals (particularly of lamellibranchs) tend to be deeper down in the sediment. Data given in Table III, also in Holme (1949), suggest that the Petersen grab takes half or less than half as many individuals per unit area as does the scoop-sampler.

It therefore follows that two corrections must be applied to the data in Table II. The 1950 results must be doubled to allow for sieving error and the 1922–23 results doubled to allow for individuals missed by the Petersen grab. The *ratios* of the two sets of sample can therefore be taken direct from the table.

Of the differences in total population numbers shown in Table XXIV, the greater part is due to two species, *Abra alba* and *Cultellus*.

Taking the number of individuals of *Abra* which would have been lost had the coarse sieve been used as 9%, the population decline over the period is seen to be to 3·6% of its original value. There is reason to believe that *Abra* was exceptionally abundant at the time of Ford's survey; indeed Ford (1925, pp. 545–7) predicted that the dense bed in Bigbury Bay would soon become relatively barren owing to lack of replacement stock. The writer has been unable to locate any dense beds of *Abra* during the last few years.

The figures for *Cultellus* show a decline from 188·1 to 50·0. Sieving losses have already been shown not to be as high as might be imagined, and it seems fairly certain that a decline in population density, of the order of 75%, had occurred. The apparent decline in the population of *Nucula turgida* may be due to sieving losses in the 1950 survey (see above).

The differences in numbers of other species shown in Table XXIV cannot be considered significant, although they may indicate trends which can be confirmed at a later date. The totals for all species except *Cultellus* and

Table XXIV. Comparison of Lamellibranch Faunas in 1922–23 and 1950. Numbers per 10 m.^{2}

(The identity of the *Lutraria* taken in 1950 is uncertain. Some other doubtful identifications are included in the 1950 results, e.g. small specimens of ? *Cardium echinatum*)

	1922–23	1950
Nucula nucleus	23·2	11·4
Nucula hanleyi	1·9	..
Nucula turgida	56·6	11·4
Glycimeris glycimeris	13·9	..
Modiolus phaseolinus	..	1·4
Musculus marmoratus	1·2	..
Chlamys opercularis	0·3	4·3
Lima loscombi	0·8	..
Thyasira flexuosa	4·2	11·4
Myrtea spinifera	4·2	..
Phacoides borealis	..	11·4
Diplodonta rotundata	1·2	..
Lepton squamosum	..	2·8
Montacuta ferruginosa	0·3	1·4
Mysella bidentata	1·2	..
Cyprina islandica	1·2	1·4
Cardium echinatum	14·8	8·6
Cardium ovale	0·5	..
Cardium scabrum	0·4	1·4
Cardium crassum	0·5	..
Dosinia lupinus	4·9	17·1
Gafrarium minimum	11·8	..
Callista chione	0·8	..
Venus casina	1·2	..
Venus ovata	14·6	4·3
Venus fasciata	16·8	1·4
Venus striatula	12·1	1·4
Venerupis rhomboides	6·7	1·4
Mysia undata	..	1·4
Donax vittatus	2·2	..
Tellina pygmaea	2·6	..
Tellina fabula	..	7·1
Tellina donacina	..	1·4
Tellina crassa	2·8	8·6
Abra alba	107·8	7·1
Abra nitida	0·7	..
Abra prismatica	37·8	2·8
Gari fervensis	1·2	..
Gari tellinella	4·8	1·4
Solecurtus scopula	0·2	1·4
Solecurtus chamasolen	1·5	25·7
Cultellus pellucidus	188·1	50·0
Ensis ensis	5·5	2·8
Ensis arcuatus	..	1·4
Mactra corallina	3·7	1·4
Spisula elliptica	5·7	..
Spisula subtruncata	0·5	..
Lutraria lutraria	9·9	1·4
Corbula gibba	15·2	..
Thracia villosiuscula	0·4	..
Thracia convexa	0·2	..
Total	576·1	206·4

Abra alba are 280 for 1922–23 and 149 for 1950. The decline in population numbers appears therefore to be of the order of 50%.

Figures for the brittle-star *Ophiothrix fragilis* show an increase in numbers over the period. The species usually occurs in dense and localized beds (Vevers, 1951) that this difference may well be fortuitous. Most of the *Ophiothrix* obtained in 1950 were in one or two individual hauls at A5 and C3.

The population of the heart-urchin *Echinocardium cordatum* was 150 in 1922–23 and 18 in 1950. This species must quickly reach a size which does not pass the sieves, so that the decline in numbers is probably greater than the figures would indicate. Many young individuals were taken in the earlier survey.

While there can be little doubt that there has been a decline in the *numbers* of certain invertebrates, it is not possible to indicate what changes have occurred in the biomass, since no information is available on the exact sizes of individuals taken in Ford's survey.

Mare's survey

Mare (1942) gives a figure of 75 g./m.^{2} fresh weight for the larger macrobenthos in the Rame mud in 1939. At A2, a mile or two to the south, there was *c.* 50 g./m.^{2} in 1950, and the average for all 1950 stations was *c.* 55 g. (p. 37). It is possible that there had been a slight decline since 1939, but the differences might also be due to sampling errors.

Discussion

The biomass figures given in this paper are a measure of the standing-crop of the macrofauna on the sea-bed off Plymouth during 1950. They are intended to provide basic data for following changes in the bottom-fauna in the future.

Before any attempt can be made to correlate the density of the fauna with long-term hydrographic or other changes it will be necessary to know the extent to which the populations fluctuate under constant conditions. Few data, apart from that given by Ford (1925) for some lamellibranchs are available for the Plymouth area.

It is well known that marine populations fluctuate from time to time. Petersen (1918), for example, gives figures showing changes in the bottom-fauna of two Danish fjords over a number of years. While seasonal changes do occur, particularly in shallow water, these are far outweighed by the year to year changes.

Ursin (1952) records considerable changes in the fauna of the Dogger Bank since the survey by Davis (1923). Between 1922 and 1951 numbers of *Spisula subtruncata* and *Mactra corallina* showed a marked decline, while other species had increased in numbers. Birkett (1953), however, considers that these changes may be partly due to seasonal variations in populations and to differences in sampling gear.

Major fluctuations appear to be due to the success or otherwise of spat-fall in each year. It follows that these fluctuations are mainly influenced by the suitability of conditions for the growth and development of pelagic larvae. Thorson (1950) considers that survival during larval life is affected mainly by the availability of suitable food and also by the effects of predators. Species with a long larval life tended to show greater fluctuations in their adult populations than species with a shorter larval life. This is due to the greater risks resulting from a long pelagic life which might in some years result in scarcely any larvae reaching the settling stage. Thorson (1950, p. 11) states that over 70% of marine invertebrates have pelagic larvae with a relatively long larval life. He believes that the mortality at the settlement stage is not as great as might be thought since it is now known that many species can postpone settlement until they drift over a suitable substratum (Wilson, 1952).

Many instances could be quoted where population fluctuations appear to be associated with good and bad settlement years. Among these are Ford (1925) and Stephen (1931, 1932) for lamellibranch populations.

Great variations may occur in the density of species where soils of varying grade occur within the same area. In the stations worked in 1950 the dry weight ranged from 2·6 g. (St. C1) to 21·4 g. (St. B7)/m.^{2}. Jones (1951, p. 139) records even greater variations of weight off the Isle of Man. The biomass is not necessarily related to the quantity of organic matter in a deposit, but seems rather to be related to the suitability of the deposit as a habitat for particular species. For example shell-gravel of a particular grade, as at A5, is favourable for the development of a rich fauna of lamellibranchs and *Amphioxus*, but if the shell gravel is of a slightly different grade it no longer provides a suitable habitat and is relatively barren.

Davis (1923) and Orton (1937) have emphasized the importance of currents and eddies respectively in the dispersal and settlement of larvae. These may result in the patchy settlement of larvae over a relatively uniform deposit.

The fact that very high population densities are found in certain localized areas suggests that the space requirements of individuals do not normally limit density. Thus Davis (1923) records up to 8250 small *Spisula subtruncata*/m.^{2} on the Dogger Bank, and Stephen (1928) records populations of *Tellina tenuis* on the shore of up to 7588/m.^{2}.

Although benthic populations vary greatly in time and place, their density within an area must clearly bear some relation to the fertility of the overlying water mass. The food of most species during pelagic larval life and of suspension feeders in the adult population is the plankton, and since fluctuations in phyto- and zoo-plankton are closely linked with the supply of nutrient salts, it follows that the density of benthic species must similarly be affected.

Raymont (1947, 1949, 1950) has shown that the addition of fertilizers to an enclosed sea-loch and to an arm of an open sea-loch resulted in a marked increase in the overall density of the bottom fauna. In open sea areas no com-

parable data are available, but it should be appreciated that changes in the fertility of the water do not necessarily affect all species in the same way. Thus an increase in fertility results not only in a greater supply of food for both larvae and adults but also in an increase in the number of predators. Raymont's results suggest, however, that an increase in bottom population will result in spite of the larger number of predators.

At Plymouth there has been a decline in the fertility of the area since about 1931, associated with the replacement of water characterized by *Sagitta elegans* by water characterized by *S. setosa*. This decline is measurable in terms of diminished phosphate and other nutrients in the water and by a decline in the number of young fish and of other organisms in the plankton. (The changes are summarized in Kemp, 1938, and Russell, 1939). The Plymouth herring fishery has died out, and Cooper (1948) has shown a decline in landings of the spur-dog (*Squalus acanthias*) associated with the fall in phosphate content of the water. In the present paper evidence has been given (pp. 39–43) of a probable decline in the numbers of lamellibranchs on the sea-bed since 1922–23.

Wilson (1951) has recently shown that other factors besides a decrease in the nutrient salt content of the water may be responsible for a decline in the density of the fauna. He has shown that water from off Plymouth is less favourable for the development of the larvae of certain benthic invertebrates compared with water taken to the westward in the Celtic Sea, which is characterized by *Sagitta elegans*.

It is natural that changes in the bottom-fauna should take place more slowly than in the plankton owing to the much longer generation time of the larger benthic invertebrates. Raymont (1947, 1949, 1950) found a delay of 2 or 3 years after the commencement of fertilization before anything like a maximal development of bottom fauna was obtained, and a similar lag of a year or so in the decline of the population after addition of fertilisers had ceased.

Many benthic species, particularly lamellibranchs, are believed to have a life span of many years, and the effects of a decline in fertility of the water might only become fully operative after a number of years when the adult spawning stock began to decline in numbers. Young of the lamellibranch *Cyprina islandica* appeared to be rare or absent in 1950 on grounds where the adult was quite common. While it is true that for a long-lived species the annual rate of replacement need only be small, is it possible that the 1950 population of adult *Cyprina* was spatted under more favourable conditions 20 years or more previously?

Apart from changes in the nature of the water masses, there is some evidence of a northward spread of warmer water species due to a small increase in temperature over the last half century. Spooner (1950) records certain species of amphipod which appear to have spread northward into the Plymouth area in recent years.

The uniformity of the fauna of the sandy grounds outside the Eddystone suggests that this would be the most favourable area for the study of long-term fluctuations in the benthic population. The fauna can be sampled more accurately than on the patchy grounds inside the Eddystone. The large number of species on this ground, none of which occurs in any great abundance, should tend to lessen year to year changes in the overall population density due to fluctuations in spatfall of individual species.

The only dense beds of invertebrates in the area seem to be the *Ophiothrix* beds recorded by Vevers (1951), so it seems unlikely that pelagic larvae of other invertebrates occur in dense aggregations and so give rise to localized spat falls, as on the Dogger Bank (Davis, 1923). The absence of eddy currents on offshore grounds should minimize the chance of local settlements associated with swirls as suggested by Orton (1937). In this connexion it is interesting to note that dense beds of Mollusca found off the Devon coast have all been in inshore areas where eddy currents may occur—these are the *Abra alba* bed in Bigbury Bay (Ford, 1923, 1925), the *Spisula solida* bed in Start Bay (Ford, 1925; Holme, 1950*a*) and the *Turritella communis* bed in Teignmouth Bay (Holme, 1950*a*).

Summary

A survey has been made of the biomass of the macrobenthos at twenty stations in the English Channel off Plymouth. The object was to provide a basis for following long-term fluctuations in the fauna.

A brief survey of physical conditions in the area is given, and a grade-analysis of the soil at each station has been made.

Core-samples show that the sediment is shallow in many places and rock has been taken at 36 cm. or less below the surface at a number of stations.

Samples totalling $\frac{1}{2}$ m.^{2} were taken at each station with a modified 'scoop-sampler', covering $\frac{1}{10}$ m.^{2}, which is briefly described.

Sources of error in sampling are considered in detail. Some species may evade the sampler and others live too deep in the sediment to be taken. A comparison against a Petersen grab and a new 'suction-corer' show that the scoop-sampler does take a reasonably quantitative sample.

The number of species taken in successive hauls, when plotted against the log of the area sampled, approaches a straight-line relationship similar to that obtained by Williams (1950).

A statistical analysis is made of a series of samples taken at one station, and the variance between the two samples in each haul and between successive hauls calculated. At this particular station the fauna in successive samples is shown to be fairly random.

A sieve of 2·2 mm. mesh was employed. Compared with a finer sieve losses in terms of *numbers* may be quite large, but the total weight taken is little affected.

The methods used in dry-weight determinations are described in detail.

The fauna and dry weight at each station are listed. On grounds inside the Eddystone both fauna and deposits are patchy, but at nine stations further offshore a fairly constant fauna was taken.

The mean dry weight for all stations is 11·2 g. or *c.* 55 g. fresh weight/m.^{2}.

Much of the total weight is made up of large individuals. 64·4% is composed of animals weighing more than 0·2 g. dry (= *c.* 1·0 g. fresh weight).

The composition of the fauna in terms of different groups of animals is given—the greatest weights are Lamellibranchia (35%), Polychaeta (26%) and Echinodermata (18%).

A brief comparison is made with Allen's (1899) dredge survey. The only change noticed has been in the disappearance of *Dentalium entalis* from the outside grounds.

A comparison with the grab survey by Ford (1923) seems to indicate an appreciable decline in *numbers* of lamellibranchs compared with the 1922–23 figure. A considerable decline has occurred in populations of *Abra alba*, *Cultellus pellucidus* and *Echinocardium cordatum*.

The biomass figures obtained by Mare (1942) for the Rame mud do not indicate any considerable decline in the fauna between 1939 and 1950.

Fluctuations in benthic populations are briefly discussed, and it is emphasized that more data are required of seasonal and year to year fluctuations at Plymouth before any changes can be related to the fertility of the water-masses in the area.

The uniformity of the fauna on the outer grounds renders this area suitable for following changes in the bottom fauna.

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DEFAECATION IN RELATION TO THE SPONTANEOUS ACTIVITY CYCLES OF *ARENICOLA MARINA* L.

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(Text-figs. 1–6)

Four years ago, I showed that the behaviour of lugworms in sand could be studied by recording the water movements through their burrows, and that their activities are patterned in time, to a large extent, by inherent rhythms (Wells, 1949*a*, *b*). The following note describes a new series of experiments, in consequence of which the conclusions of the earlier papers are extended and modified in detail.

The work was done at Plymouth in July, August and September 1952. The expenses were in part defrayed by a grant from the Central Research Fund of the University of London.

Methods

Three types of apparatus were used (Fig. 1).

Type I, suitable only for the smallest worms, consists of a glass U-tube (internal diameter 25 mm.) with arms of unequal length and a short side-tube (internal diameter 5 mm.) on the longer arm. Capillary C (internal diameter about 1 mm.) is connected by rubber tubing to the side-tube. The apparatus is immersed in a tank of sea water to the level L and partly filled with muddy sand (a mixture of clean sand with a black muddy clay from Millbrook) to the height shown by stippling in the drawing. Sea water flows continually into the tank and the level L is held constant by an overflow. If a healthy worm of suitable size is allowed to enter the sand, it makes a burrow with one end in each limb of the U-tube. Such water currents as it sets up must pass through capillary C, and, because of the slight resistance of the capillary, will cause changes of water-level in the long limb of the U-tube. These changes are recorded by the following means on a slowly moving kymograph (a Casella thermo-barograph clock turning once a day). A Palmer gimbal lever (amplification $\times 6$) is connected by means of a thread and a blob of sealing-wax type adhesive to a circular cover-glass as used for microscope preparations (G). The cover-glass has a film of vaseline on the upper surface but is clean on the lower; it clings to the water surface, and is a great improvement on the paraffin floats used in my earlier work.

Types II and III differ from each other only in dimensions. Each consists of two rectangular glass plates held 22–24 mm. apart by a plasticene rim and centre-piece (cross-hatched in the drawing). The space between the plates is

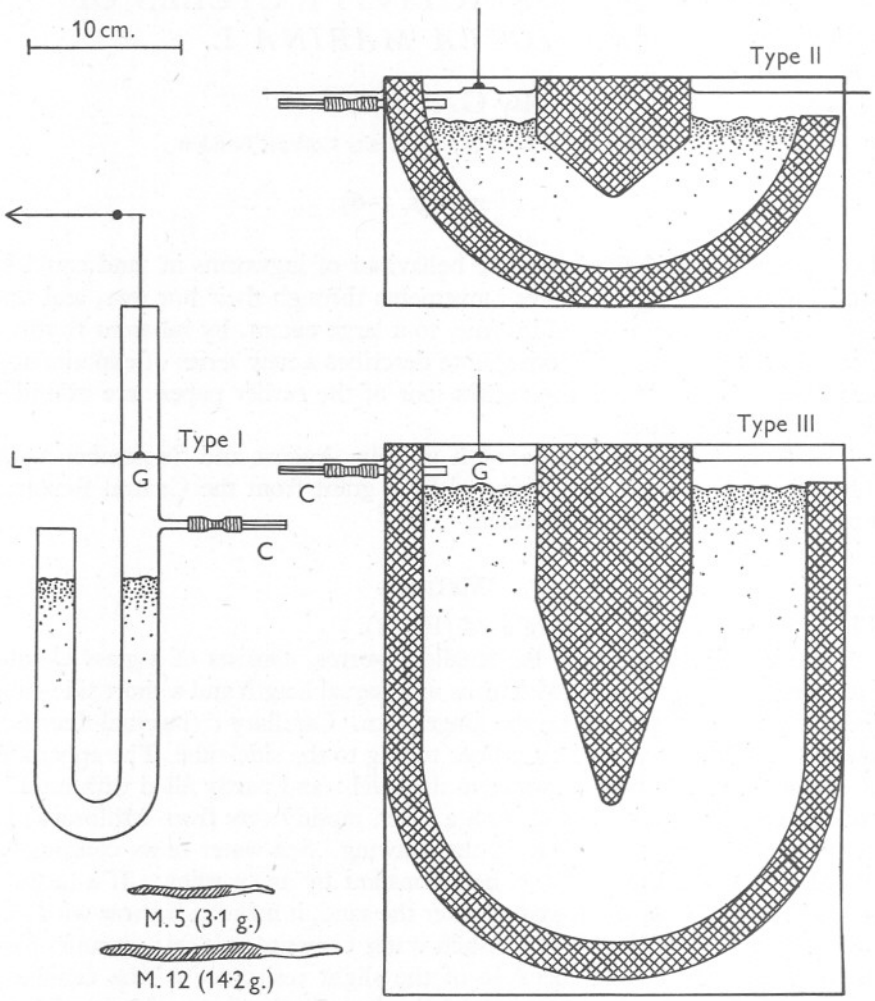

Fig. 1. Drawings of the three types of apparatus used. Outlines of two of the worms are included, to the same scale, in the lower left-hand corner.

filled with muddy sand to a maximum depth of about 100 mm. in Type II and 300 mm. in Type III. The worms burrow in the sand, and their water currents are recorded by the system already described.

The experiments were set up in groups of four. Fourteen individuals were used, the first two for 3 days only, while the apparatus was being tested and

adjusted, and the remaining twelve for from 7 to 18 days. The temperature ranged from 16·1 to 17·9° C.

The worms are generally invisible in the sand, and defaecation is almost the only activity that can be directly observed.[1] The following steps were taken to correlate the timing of defaecation with the records of water movements. (i) Two or three times a day the experiments were examined, the presence or absence of faeces was noted, and any faeces seen were destroyed with a glass rod. (ii) On several occasions the experiments were kept under continuous observation for 4–6 hr., and the time of each defaecation was marked on the tracing and noted down to the nearest minute.

Results

The worms often showed the characteristic behaviour pattern described in the following citations:

> Whenever the worms were feeding from a gradually subsiding cone and piling up castings, as they do in the field, a characteristic cyclical pattern was traced. This was marked by conspicuous diphasic excursions at intervals of about 40 min. Defaecation occurs at the summit of the first phase. By comparison with records got from worms in glass tubes, it is inferred that the first phase consists of tailward locomotion to the sand surface, and the second to headward irrigation accompanied by gentle creeping back to the feeding point. (Wells, 1949*b*, p. 477.)
>
> The pattern just described invariably accompanied feeding and defaecation. (Wells, 1949*b*, p. 471.)

These statements must be modified in two respects in the light of the new results. Firstly, the interval given as 'about 40 min.' varies considerably, one of the determining factors being the size of the worm; and secondly, defaecation may occur when the pattern is not discernible on the tracings.

Three examples of the typical pattern, taken from the new series of experiments, are seen in Fig. 2. The position of the base level (i.e. the point at which the lever settles when the capillary is removed) shows that the pattern is traced against a background of headward irrigation. At each of the conspicuous 'needles' on the tracings, the writing-point first moves towards the zero mark, as the worm ceases to irrigate and creeps backward to the surface of the sand. Then there is a very sudden further movement of the lever in the same direction (generally crossing the zero line) as a faecal cylinder shoots out. The act of expulsion takes about half a second, and the almost violent movement of the lever suggests that a powerful, piston-like swelling runs backward along the worm's body at this moment.[2] Immediately

[1] One occasionally sees the tail-tip appear at the caudal end of the burrow, move around a little as if clearing the opening, and then disappear again.

[2] Once during the earlier series of experiments I noted that the ejection of a cylinder was accompanied by a 'violent down-suck of water into the head shaft'. The fact that each 'needle' consists of two distinct parts is clearly shown in Fig. 3 of Wells 1949*b*.

after defaecation, the lever moves towards its original position as the worm creeps down to the bottom of its burrow and begins to irrigate once more. The second phase of the diphasic cycle, consisting of unusually vigorous headward irrigation, follows at once; but the extent to which this second peak stands out from the general irrigation background is variable. Sometimes, as towards the end of Fig. 2C, it is barely discernible, and it may not emerge at all.

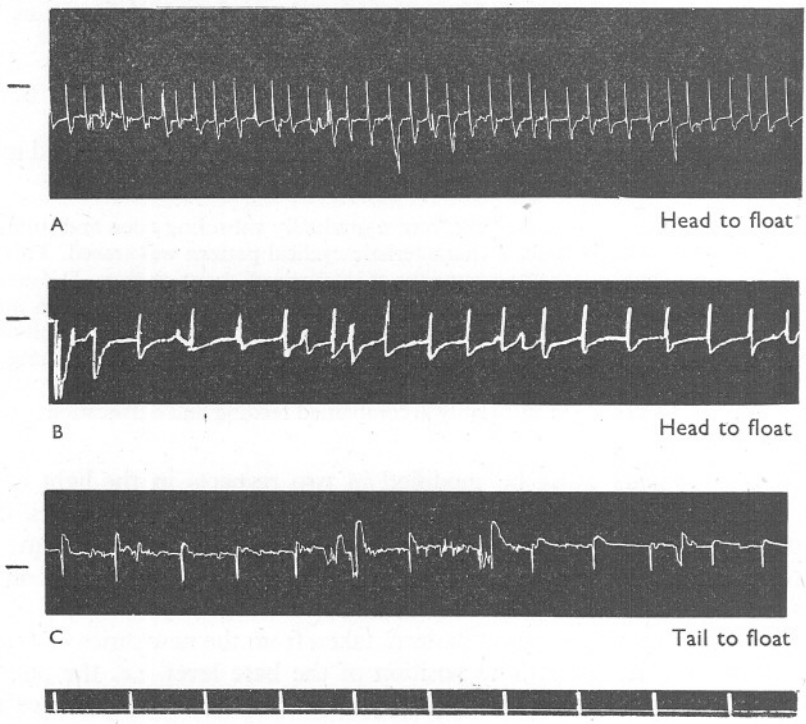

Fig. 2. Ten-hour records of frequently-defaecating worms.

A: worm M5 (3·1 g.). Apparatus Type I. Regular series of defaecations at average interval of 15 min. 20 sec.

B: worm M14 (10·1 g.). Apparatus Type III. Defaecations at average interval of 34 min. 20 sec.; occasional irregularities of the background irrigation between them.

C: worm M13 (12·1 g.). Apparatus Type III. Defaecations at intervals of about 45 min., with a rather irregular period in the middle of the record.

In all tracings: read from left to right; time signal marks once an hour; the mark to the left of each tracing is the zero-flow base level. The orientation of the worm relative to the float is printed under each extract. Wider capillaries were used for the larger worms, so a given lever excursion does not always correspond to the same flow rate.

The effect of size on the timing of the pattern is evident in Fig. 2. The experiments of 1948/9 were done on 'large' worms, unfortunately not weighed. The worms of the 1952 series ranged in weight from 2·6 to 18·6 g.

When defaecating regularly, the larger worms did so at intervals of 'about 40 min.', but the smaller gave much shorter intervals (15–20 min. for worms weighing about 3 g.).

Other types of record may be traced. In the 1948/9 experiments, the pattern of Fig. 2 appeared, accompanied by regular feeding and defaecation, during 45% of the total recorded time. The remaining records were grouped as follows: (i) cycles rather similar to those of Fig. 2 but without defaecation; (ii) periods of complete quiescence, which might last for many hours on end; (iii) periods of unexplained activity, the tracings of which were described as 'confused'. The records of the 1952 series include all of these types, and they also show a gradation of intermediate stages between the typical pattern of Fig. 2 and tracings which must be put in the 'unexplained' or 'confused' category. On several occasions, the worms defaecated frequently and quite regularly while 'confused' records were being written. The new results are reviewed in detail in the following paragraphs. Taken together, they suggest that the worms tend to settle into the pattern of Fig. 2 when conditions are favourable, and that limitation of space in the apparatus promotes 'confusion'.

Disregarding the preliminary tests, the 1952 material includes three sets of four worms each, those of the first set being the smallest and those of the third the largest.

*Set I. Worms M*3–6. (2·6–3·2 *g*)

These worms were studied in Type I apparatus. They burrowed vigorously and swiftly into the sand when placed on its surface, and their behaviour was recorded for 7 days. Extracts from the records are printed in Figs. 2A, 3 and 6C, D. The individual worms performed as follows.

M3 gave a few long spells of regular defaecation with needles like those of the first half of Fig. 3B—i.e. lacking a prominent second phase—and a few short spells (2–3 hr.) resembling Fig. 3A. Most of its tracings, however, were confused, with the defaecations few and irregularly scattered; there were several spells with pauses followed by defaecation, much as in Fig. 3C.

M4 was a consistently frequent defaecator. There were occasional short spells of irregularity, but most of its tracings were regular, like the examples in Figs. 3A and 6D. The second phase however, was not always distinct from the background irrigation.

M5 defaecated steadily for 5 days with only short spells of irregularity, its cycles being like those of Figs. 2A and 6C on some days but without a distinct second phase on others.[1] On the last 2 days the irregular periods increased in duration while the defaecation spells shortened correspondingly and finally

[1] On the 5th day, when extract 2A was taken, the worm moved nearly 10 c.c. of sand from one end of the tube to the other in 24 hr. As it defaecated about 100 times, this means a transport of 0·1 c.c. per defaecation. Weight of worm 3·1 g.; approximate length 9 cm. of which 2 cm. was tail.

ceased; and various confused patterns, including records resembling Fig. 3C, were traced.

M6 defaecated fairly frequently on most days, but gave confused records, including numerous spells of the type shown in Fig. 3C.

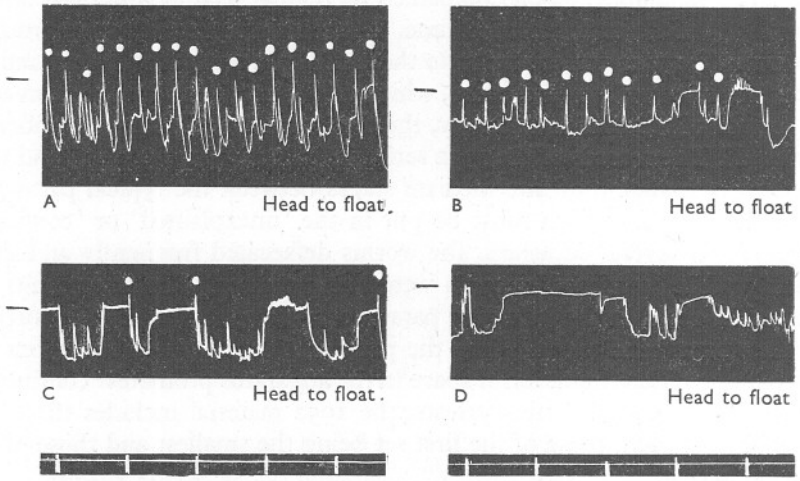

Fig. 3. Five-hour records traced by small worms in apparatus Type I. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M4 (2·6 g.). Intervals between defaecations 15, 16, 16, 15, 15, 16, 16, 18, 16, 17, 15, 18, 17, 18, 17, 18, 15 min. The record shows a pattern like that of Fig. 2A but against a more fluctuating background. There is a close correspondence between defaecation and the first peak of the diphasic excursion except that the 3rd defaecation is atypically placed; it occurs on a sharp needle sandwiched between two blunter ones.

B: worm M3 (3·0 g.). Intervals between defaecations 19, 15, 19, 14, 23, 17, 16, 16, 26, 38, 16 min. The defaecations occur on well-marked needles but there is no prominent second phase; the whole record becomes confused towards the end and defaecation ceases.

C: worm M6 (3·2 g.). Intervals between defaecations 58, 158 min. The record shows fluctuating activity alternating with periods of almost or quite complete quiescence; defaecation sometimes occurs as the worm 'wakes up' after a pause.

D: worm M3 (3·0 g.). A confused tracing with no defaecations.

Although these worms could burrow rapidly in the Type I apparatus, and often showed regularly spaced defaecations associated with typical diphasic excursions on the irrigation tracing, the results of Set II suggest that the rigid walls of the U-tubes may have been somewhat restrictive even for the small worms of Set I.

Set II. Worms M7–10 (7·0–8·0 g.)

This experiment lasted for a week. The worms were first put up in Type I apparatus, and transferred to Type II after 2 days (M7, 8) or 5 days (M9, 10).

When put into Type I, the worms began at once to make strenuous burrowing movements, but they had obvious difficulty in entering the sand.

This was doubtless because burrowing is done in the main by thrusting the sand aside, and only a small worm has room enough to do this in the U-tube. The worms of Set II could work their anterior segments into the sand, but they generally remained with the tail, or the tail and several branchiate segments, exposed at the surface. Only M 10 was seen to get itself com-

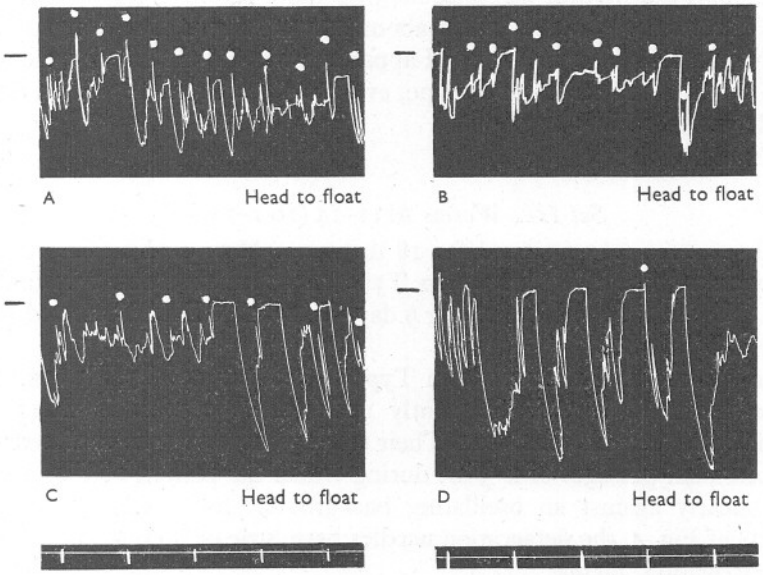

Fig. 4. Five-hour records traced by large worms in apparatus Type II. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M 7 (7·3 g.). Intervals between defaecations 26, 23, 22, 23, 24, 25, 19, 30, 31, 24, 23 min. Base level drops suddenly about 1½ hr. after start of extract, due to a change in rate of the sea-water supply to the tank in which the apparatus stood. Several of the defaecations are associated with diphasic excursions of the typical form, but many are not.

B: worm M 14 (10·1 g.). Intervals between defaecations 29, 18, 19, 19, 19, 35, 17, 36, 27, 23 min. A confused tracing, the defaecation needles bearing no relation to the general irrigation pattern.

C: worm M 12 (14·2 g.). Intervals between defaecations 58, 43, 37, 41, 56, 36 min. There is again no relation between the defaecation needles and the general irrigation pattern.

D: worm M 7 (7·3 g.). A confused record, with one defaecation only. The pattern resembles that of Fig. 3 C.

pletely into the sand; this happened on two occasions, but each time its tail soon appeared again. Nevertheless, they all defaecated repeatedly and must therefore have fed. The tracings were of small amplitude, probably because part of the irrigation water returned along the limb of the U-tube where the worms were lying, and they were very confused in pattern.

When transferred to Type II, the worms burrowed rapidly out of sight. Only M7 defaecated frequently under the new conditions, and its records were never of the simple cyclic pattern of Fig. 2. Extracts are given in Figs. 4A, D and 6B. The other worms defaecated rather seldom and gave confused tracings, e.g. Fig. 6A. Short spells of the curious rapidly-swinging rhythm seen between the reversals in Fig. 6A were shown by all these three worms, and the rhythm was once kept up quite regularly for about 6 hr. by M9. The worms of Set II were about 15 cm. long at full elongation, and the space available in the Type II apparatus was insufficient for the setting up of a burrow of the normal shape, even though they were able to descend rapidly into the sand.

*Set III. Worms M*11–14 (10·1–18·6 *g.*)

This experiment continued for 18 days, but M11 died on the 12th day. The worms were first put up in Type II apparatus, and transferred to Type III after 4 days (M11, 12) or 9 days (M13, 14). They burrowed rapidly down in both types.

The behaviour of the worms in Type II is illustrated by Fig. 4B, C. All of the worms defaecated frequently under these conditions, M13 being especially regular and consistent. Their tracings, however, are confused; there are occasional passages of 2–3 hr. during which the pattern of Fig. 2 is seen fairly clearly against an oscillating background, but generally, as in the extracts of Fig. 4, the defaecation needles have little or no relation to the rest of the irrigation tracing.

The behaviour in Type III is shown in Figs. 2B, C and 5. The case of M11 is exceptional. This worm became quiet after burrowing down in Type III and registered practically no water currents for 3 days; then it gave a rhythmic pattern without defaecation for about 12 hr. (Fig. 5C); then it suddenly became very active, tracing a graph like the usual recovery after a period of oxygen lack (as Wells, 1949*a*, fig. 6) followed by some hours of vigorous but confused activity; then its irrigation became less and less vigorous, until it died on the 12th day. The remaining worms often gave confused spells, sometimes with occasional defaecations. They also traced many long periods in which the defaecations came regularly and were associated with diphasic excursions of the usual kind (Fig. 2B, C), though this was sometimes written against a rather fluctuating background (Fig. 5B). A comparison of the records before and after the transfer leaves little room for doubt that the more spacious conditions of Type III favour the appearance of the regular pattern of Fig. 2.

Three rest periods, of 1–1½ days' duration, during which there was little or no irrigation, were shown by M12; these alternated with active periods. The pattern traced by M13 in Fig. 5A was not seen in any other record. It was

presumably caused by a regular periodic reversal of the direction of travel of the irrigation waves along the worm's body.

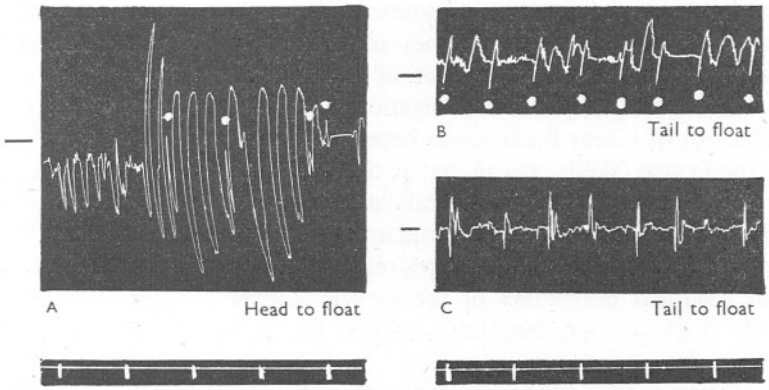

Fig. 5. Five-hour records traced by large worms in apparatus Type III. The experiments were watched and the moment of each defaecation was marked on the tracing as a spot.

A: worm M 13 (12·8 g.). Intervals between defaecations 52, 74, 18 min. The record shows an unique irrigation pattern, with periodic reversal of the direction of flow.

B: worm M 14 (10·1 g.). Intervals between defaecations 43, 39, 42, 36, 33, 37, 46 min. The defaecations occur on diphasic excursions of the typical form, against a fluctuating background.

C: worm M 11 (18·6 g.). The record shows periodic outbursts of irrigation without defaecation.

Discussion

The relation between the irrigation patterns and defaecation may be summarized as follows.

Fasting worms in glass tubes often settle down, for many hours at a stretch, to a pattern consisting of periods of rest alternating with outbursts of activity (the *i–d* cycles, in the terminology of Wells & Albrecht, 1951). Each outburst typically consists of three phases—backward locomotion, headward irrigation with headward locomotion, tailward irrigation—but their form is variable in detail; the third phase, in particular, is often dropped out altogether. They sometimes appear with almost clockwork regularity, but their timing can vary, either after a period of oxygen lack or without obvious external cause. They apparently depend on a spontaneously discharging pacemaker whose activity can be controlled according to the circumstances, much as the beat of a vertebrate heart is spontaneous although its pattern and frequency can be regulated from without (Wells, 1949*a*; Wells & Albrecht, 1951).

Worms in sand, feeding and defaecating regularly, often show essentially similar cycles, except that they now appear against a continuous but often rather fluctuating background of headward irrigation (Fig. 2). Sometimes the worms in sand show a similar pattern when they are not passing food

through the gut; and then, as in glass tubes, they generally rest between the outbursts (Figs. 5C, 6B).

Other spontaneous components may enter into the lugworm's behaviour. A second characteristic pattern of intermittent rhythmic activity originates in the oesophageal wall; it determines movements of the anterior end and proboscis accompanied by inhibition of the irrigation current (the *f* cycle of Wells & Albrecht, 1951). The irrigation records got from worms in sand sometimes show minor fluctuations between the *i–d* outbursts which may be due to the *f* cycle (Wells, 1949*b*, fig. 4; this paper, Fig. 2C). Additional types of periodic behaviour are occasionally indicated (Fig. 5A), but many of the tracings are confused, both in sand and in glass tubes, and it is often impossible to decide whether particular passages represent the appearance of something new, or modified derivatives of the *f* cycle (centre portion of Fig. 6A) or *i–d* cycle (Figs. 3C, 4D, beginning of Fig. 6A; compare Wells & Albrecht, 1951, Fig. 4).

Defaecation is frequently coupled with great regularity to the *i–d* cycles, with one defaecation on the first phase of each outburst (Fig. 2). In favourable circumstances, and given plenty of space, the worms tend to settle into this routine for many hours on end, and there is little doubt that they spend much of their time in this way in the field. However, as we have seen, it is not a necessary condition for the appearance of the *i–d* cycles that the worms should feed and defaecate; neither is it a necessary condition for defaecation that a regular irrigation pattern of this type should be traced.

The advantages of basing the organization in time of the lugworm's activities on spontaneous pacemaker systems, rather than on *ad hoc* reflexes to its various needs as they arise, were discussed elsewhere (Wells, 1949*a*, *b*). The *i–d* cycle is well suited to serve as a means of integration when the worm is living in an established burrow of the typical form. It brings the worm alternately to the place of feeding and the place of defaecation; it can be modified according to the composition of the water and the worm's respiratory needs; under low-tide conditions, it can be adapted for aerial respiration. Some method of coupling defaecation to the *i–d* cycle is therefore to be expected. On the other hand, the coupling should not be too rigid, for circumstances might arise in which the *i–d* pattern would be inappropriate. The worms of Set II fed and defaecated regularly in the Type I apparatus though unable to burrow wholly into the sand; this indicates a flexibility of behaviour which may sometimes be of service under natural conditions.

The mechanism of coupling appears to involve more than one factor. It is suggestive that defaecation often occurs as the worms 'wake up' suddenly and begin to irrigate in a headward direction after a period of rest (Figs. 3C, 4D). Similarly, in a typical *i–d* outburst, it is at the momentary pause at the top of the first phase, just before the onset of headward irrigation, that defaecation takes place (Fig. 2). The headward irrigation cannot be regarded as a conse-

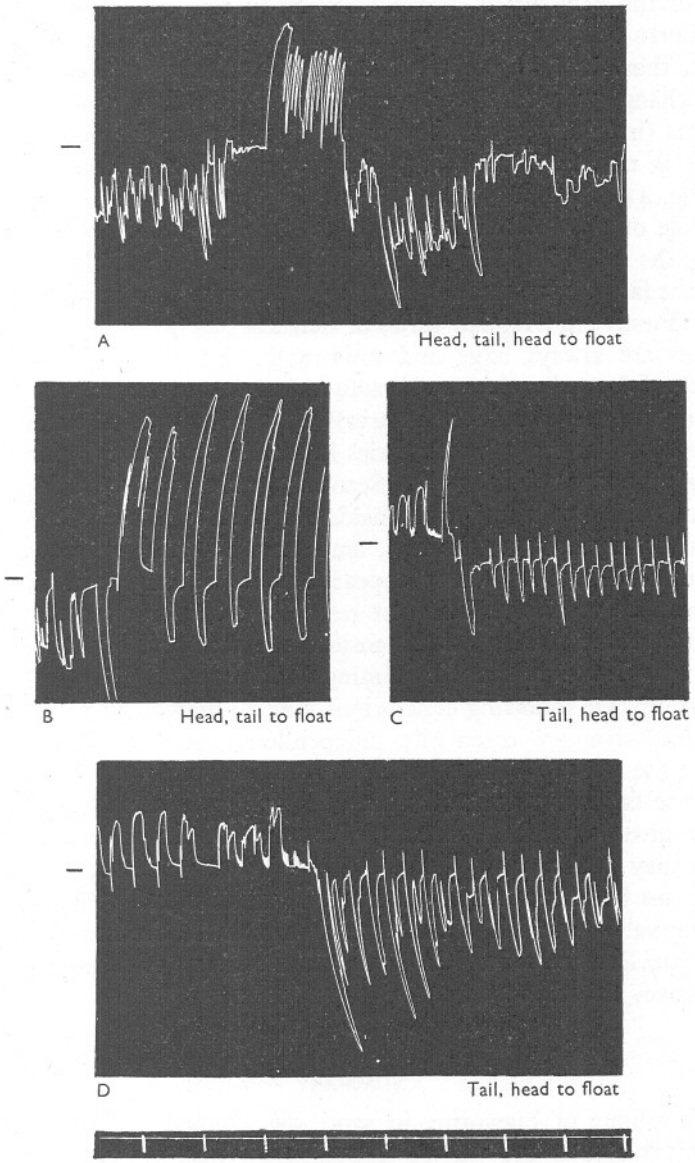

Fig. 6. Five- and nine-hour records to show reversal of the worms in their burrows.

A: worm M8 (8·0 g.). Apparatus Type II. A very confused tracing, with no defaecations. The worm reverses itself twice, with about 1¼ hr. between reversals.

B: worm M7 (7·3 g.). Apparatus Type II. Reversal is followed by a regularly spaced series of irrigation outbursts without defaecation.

C: worm M5 (3·1 g.). Apparatus Type I. Reversal is followed by a regular series of defaecations with diphasic excursions of the usual form.

D: worm M4 (2·6 g.). Apparatus Type I. Defaecation occurs four times at the beginning, and frequently after reversal.

quence of the defaecation, because resting worms can 'wake up', and the *i–d* outburst can assume its typical form, in the absence of defaecation. Possibly, therefore, the onset of headward irrigation is preceded by some internal change which acts as a stimulus for defaecation, provided that other conditions (including a certain loading of faeces in the gut) are satisfied. But this is not the whole story, for the worms may defaecate at other times (Fig. 4B, C).

The role of rectal distension could perhaps be studied by collecting and weighing the individual faecal cylinders. Rough observations on the dimensions of the faeces were made during the present work, from which two points emerge. Firstly, in a regular series of defaecations (as in Fig. 2 or Fig. 3A), the faeces are always large and uniform in size. This suggests that the frequency of the *i–d* cycles can be adjusted to fit in with the rate of feeding, and the slight variations in the intervals between successive outbursts which appear even in the most regular series in sand may be due to this cause (see details in legends to Figs. 3–5). Secondly, when faeces are less regularly produced, their size may vary considerably. Thus, three of the cylinders produced during Fig. 3B (the first, tenth and eleventh) were conspicuously smaller than the rest, and these appeared after the longest intervals (27, 26 and 38 min. respectively). The last result is rather unexpected, and shows that factors other than rectal distension and a particular inflexion of the irrigation graph can influence the timing of defaecation.

To sum up, the following conclusions may be drawn. (i) Defaecation and the *i–d* outbursts are essentially independent, cyclically repeated events. (ii) When the worm is living under favourable conditions in an established burrow, the familiar pattern in which defaecation is closely coupled to the *i–d* cycle gives a workable means of integrating its activities. (iii) The coupling may perhaps be brought about by two factors, a tendency to defaecate just before the onset of headward irrigation and a 'fine adjustment' of the intervals between *i–d* outbursts to match the feeding rate. (iv) The factors controlling irrigation behaviour and the timing of defaecation in other circumstances are still obscure.

Summary

The behaviour of lugworms in sand was studied by recording kymographically the water movements through their burrows and observing the timing of defaecation. The worms ranged in weight from 2·6 to 18·6 g. Three types of apparatus were used, differing in the amount of space allowed to the worms.

The worms often traced a characteristic pattern described elsewhere, consisting of regularly spaced diphasic excursions of the irrigation graph with a defaecation on the apex of each first phase. The interval between

excursions was about 15–20 min. for worms weighing about 3 g. and increased with size to about 40 min.

The worms sometimes traced similar periodic bursts of irrigation activity without defaecation.

Many of the tracings were confused in pattern. The worms often defaecated—sometimes sporadically, sometimes at regular intervals—in the absence of the characteristic diphasic excursions. Restriction of a worm to a small space appears to favour the production of confused tracings.

The relation between the irrigation patterns and the timing of defaecation is discussed.

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LUMINESCENCE IN POLYNOID WORMS

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(Plates I and II and Text-figs. 1–24)

Luminescence is well known in polychaete worms and occurs sporadically in unrelated species having different modes of life. Families containing luminescent species are Aphroditidae, Tomopteridae, Syllidae, Alciopidae, Chaetopteridae, Cirratulidae and Terebellidae. Among Aphroditidae luminescent forms are confined to subfamily Polynoinae,[1] and the response is limited to the dorsal scales which are a diagnostic feature of these animals. Bonhomme (1942) has recently demonstrated in polynoid worms that light production is intracellular. This contrasts with certain other polychaetes such as *Chaetopterus*, in which luminescence is extracellular and is due to the discharge of a luminescent secretion. The regulation of luminescence in *Chaetopterus* has recently been investigated in some detail (Hasama, 1941; Nicol, 1952*b*); and I considered that a study of the physiology of intracellular luminescence in polynoids would form an interesting comparison with that work (see Harvey, 1940, 1952, for general reviews of animal luminescence).

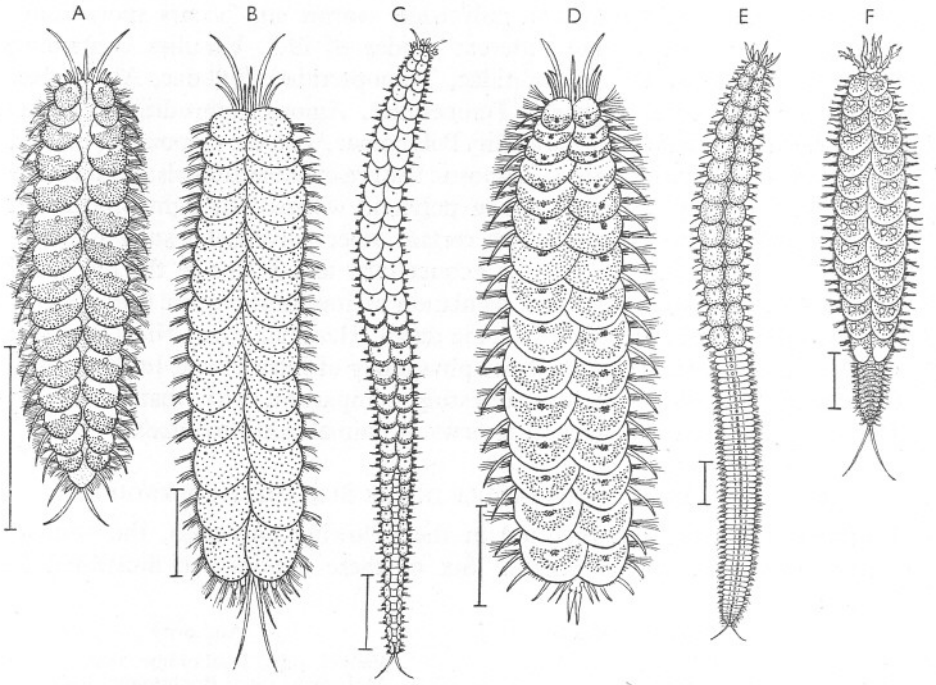
Distribution of Luminescence in the Subfamily Polynoinae

Luminescence has been reported in the following polynoids, the nomenclature based on Fauvel (1923). Six of these species are illustrated in Text-fig. 1.

Luminescent species	Authority
Harmothoë impar (Johnston)	Fauvel, 1923; Bonhomme, 1942
H. impar (=*Evarne impar*)	McIntosh, 1900; Bonhomme, 1942
H. longisetis (Grube)	Fauvel, 1923
H. imbricata (L.)	McIntosh, 1872, 1900
H. imbricata (=*Polynoë cirrata*)	Kallenbach, 1883
H. spinifera Ehlers (=*Polynoë torquata*)	Jourdan, 1885
H. spinifera (=*H. torquata*)	Darboux, 1899
H. lunulata (Delle Chiaje)	McIntosh, 1877, 1900; Fauvel, 1923
H. lunulata (=*Polynoë lunulata*)	Bonhomme, 1942
H. aspera (Hansen) (=*Polynoë aspera*)	Khvorostànsky, 1892
Lagisca extenuata (Grube)	Darboux, 1899
Gattyana cirrosa (Pallas)	McIntosh, 1900
Lepidasthenia stylolepis Willey	Lloyd, 1907
Eunoë nodosa Sars (sp. ?)	McIntosh, 1872
Polynoë scolopendrina Savigny	McIntosh, 1872, 1900
Malmgrenia castanea McIntosh	Original
Acholoë astericola (Delle Chiaje)	Darboux, 1899; McIntosh, 1900; Kutschera, 1909; Dahlgren, 1916; Fauvel, 1923; Bonhomme, 1942
Lepidonotus squamatus (L.) (=*Polynoë squamatus*)	Dahlgren, 1916

[1] McIntosh (1900) and Hartman (1938*a*, *b*) treat this subgroup as a separate family.

This list is certainly not final. It is also important to record species of polynoids that do not luminesce, in order to restrict the field of further search and investigation. Contrary to Dahlgren (see preceding list), the two species of *Lepidonotus*, *L. clava* (Montagu) and *L. squamatus* (L.) are not luminescent. This is noted by Jourdan (1885) and Bonhomme (1942) and I can confirm the observations of these two authors. *Halosydna gelatinosa* M. Sars and *Lepidasthenia argus* Hodgson are two other non-luminescent species.



Text-fig. 1. Luminescent species of polynoids, drawn from life. A, *Malmgrenia castanea*; B, *Gattyana cirrosa*; C, *Acholoë astericola*; D, *Harmothoë lunulata*; E, *Polynoë scolopendrina*; F, *Lagisca extenuata*. Scale 5 mm.

A number of authors have merely recorded luminescent genera, viz. *Polynoë*, *Harmothoë* and *Lepidonotus*. With repeated changes in nomenclature these observations are only of general value.

Histology and Innervation of the Luminescent Tissue

There is general agreement that luminescence in polynoids is confined to the scales which cover all or part of the dorsal surface, according to the species. These structures are disk-shaped, and are attached to the body above the parapodium by a relatively thin stalk or elytrophore. Fauvel (1923) notes

that the elytra are inserted on segments 2, 4, 5, 7, 9, ..., 23, 26, and subsequently on each successive third segment; segments without elytra have an elongated dorsal cirrus.

General Structure of the Elytrum

An elytrum is a thin disk covered externally by a cuticular layer which may be smooth or adorned on its superior surface with rugosities and papillae. Underneath the cuticle is a unicellular epidermis (or hypodermis) which is continuous except over the insertion of the stalk. Frequently the scale bears a pigment pattern which is due to the aggregation of dark granules in the epidermal cells.

Extending vertically between the upper and lower surfaces of the scale is a strut-work of rather fine fibres of somewhat indeterminate nature. They are patently extracellular structures, presumably of connective tissue nature; their staining affinities are poor, but they are coloured by eosin. Since they are not concerned with luminescence it was unnecessary to analyse them further in this investigation. The meshwork of spaces among the fibres communicates with the general body cavity through the stalk. Well-developed muscles are present in the elytrophore, some of them inserted on the rim forming the junction between stalk and elytrum, but none extend into the body of the elytrum (Pls. I and II) (Darboux, 1889; Haswell, 1882; Jourdan, 1885; Pflugfelder, 1933).

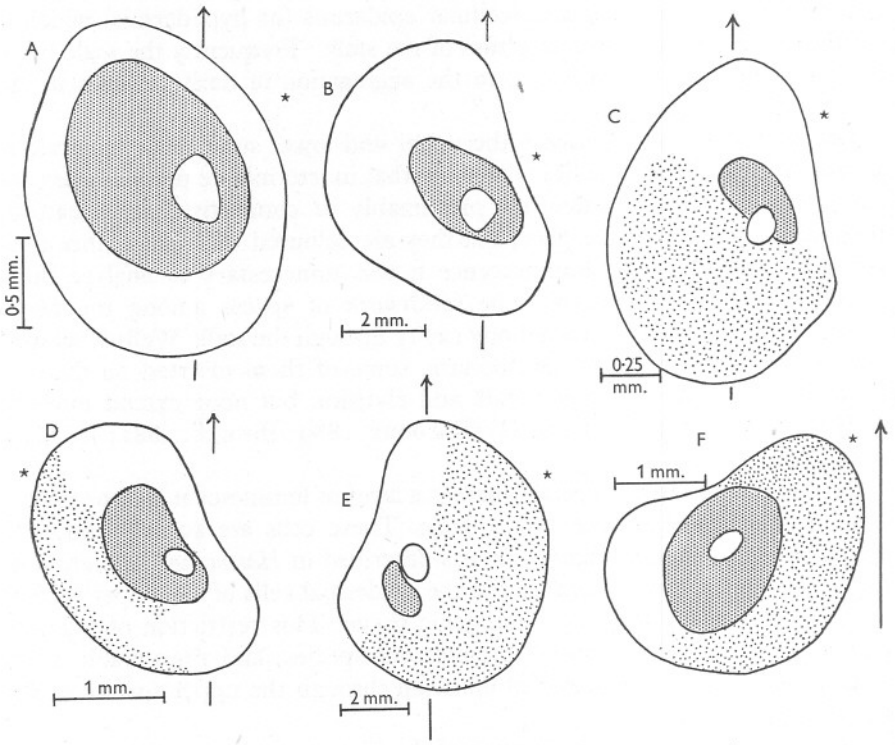
In the light-producing species there is a layer of luminescent cells or photocytes on the lower surface of the scale. These cells are actually modified epidermal cells. Bonhomme (1942) has described in *Harmothoë lunulata* how the density of pigment is reduced in the epidermal cells of the upper surface over the region occupied by photogenic tissue. This restriction of pigment pattern is a general feature of luminescent species, and presumably is an adaptation which allows maximal emission through the upper surface of the scale (Text-fig. 2).

Innervation of the Elytrum

An easily recognizable nerve trunk ascends in the wall of the elytrophore, and on reaching the elytrum forms a well-demarcated nodal point from which nerves radiate out in all directions towards the periphery of the scale. These nerves divide, subdivide, and become attenuated towards their termination (Pl. I, fig. 1). Kallenbach (1883) refers to the node of nervous tissue above the elytrophoral stalk as a ganglion, but gives no details of the occurrence of nerve cells.

The nervous supply of the elytrum has been described and figured by Haswell (1882), Jourdan (1885), and Bonhomme (1942), to which I can add few additional observations. In favourable material (e.g. *Acholoë*, *Malmgrenia*), the nerves in the scales can be seen in the living state, but staining is necessary

to bring out the finer nerves and fibres. Many of the smaller fibres terminate in relation to the papillae on the dorsal surface, or go to sensory terminals on the exposed margin of the scale. The dorsal papillae are generally believed to represent the loci of sensory structures. In the figures of Jourdan (1885, 1887), Bonhomme (1942) and others, the papillae are drawn with a central canal through which runs a fibre that is connected with an underlying group of ganglionic cells (Darboux, 1899; Haswell, 1882; Panceri, 1874).



Text-fig. 2. Outline drawings of the scales of polynoids to show the extent of the luminescent area. A, *Acholoë*; B, *Gattyana*; C, *Malmgrenia*; D, *Harmothoë*; E, *Lagisca*; F, *Polynoë*. Magnification as shown. The central clear area is the region of attachment of the elytrophore. The luminescent area is marked with mechanical shading. Pigmented areas are dotted. Direction cephalad is indicated by the arrow, and the external margin by an asterisk.

By means of silver impregnation (Bielschowsky method) Bonhomme has shown up the elytral nerves of *Harmothoë lunulata* (=*Polynoë lunulata*). Besides the sensory fibres and peripheral sensory cells he has discovered numerous efferent fibres which leave the main nerve trunks, and pass downwards to reach the photogenic cells, or even to penetrate into them and run between the secretory granules.

I can confirm these descriptions from preparations of *Acholoë astericola* treated with various nerve-stains. The scales of this species can be stained readily with supravital methylene blue, whereas other species (*Lagisca extenuata*, *Polynoë scolopendrina*) proved refractory. Clear pictures were obtained of nerves radiating outwards from a nodal point at the edge of the stalk, and ramifying over the expanse of the scale (Pl. I, fig. 1). This is the broad picture of the nervous supply. Silver-impregnation with Bodian's activated protargol has been tried with varied success on the scales of several species (*Polynoë*, *Acholoë* and *Lagisca*). In sections so treated the nerve trunk can be traced through the wall of the elytrophore into the centre of the elytrum. Here it gives rise to a well-marked ganglion consisting of a central mass of nerve fibres about which are grouped several distinct nerve cells (Pl. II, figs. 7, 8). From this central ganglion nerve trunks proceed peripherally through the centre of the scale (Pl. II, fig. 9). In their course the nerves give off fine nerve fibres which run obliquely or vertically downwards. In fixed material they are very fine, less than 1 μ in diameter. Fairly certainly, therefore, all the photogenic cells are innervated by fine nerve fibres, and these emerge from the nerve trunks which radiate outwards from the elytrophore like the spokes of a wheel. Consequently, the nerve fibres which occur in the elytrum are of two types, viz. sensory fibres, and efferent fibres supplying the luminescent cells.

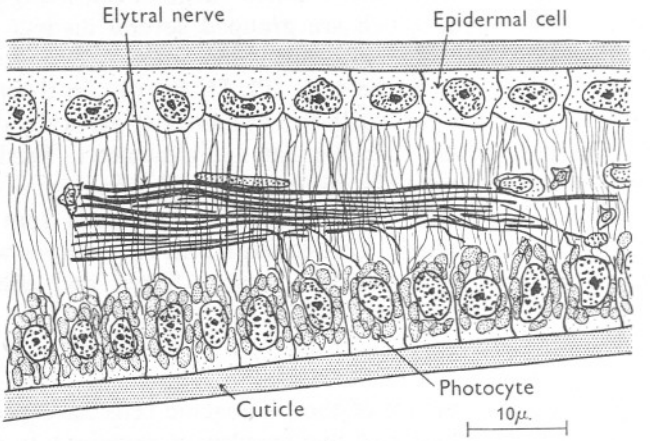
Photogenic Tissue

The disposition and structure of the photogenic cells have been debated for some time, and the solution of this problem is necessary to understand the physiological processes involved in light production in polynoids. Kutschera (1909), followed by Dahlgren (1916), believed that the luminescent cells were grouped under the dorsal papillae of the elytra. Although unable to detect an extracellular secretion they supported the view that a photogenic material was discharged to the exterior through a canal in the papilla. These papillae, are however, probably receptor structures. The true photogenic cells form a glandular layer in the lower surface of the scale of luminescent species (Harvey, 1952).

The photogenic tissue in *Harmothoë spinifera* (= *Polynoë torquata*) has been described by Jourdan (1885). He showed that it occupied the region of the scale around the elytrophore and consisted of a layer of mucus-cells which replaced the epidermis over part of the lower surface of the scale. In his figure (3) masses of large granules are shown in the photogenic epithelial cells. Recently, the photogenic cells of *H. lunulata* have been described in more detail by Bonhomme (1942), who has made confirmatory observations on *H. impar* and *Acholoë astericola*. These cells are tall, polygonal in cross-section, and contain a large oval nucleus. Proximally the cells are filled with large oval or round granules which are closely packed together; distally, the

granules decrease in size. Immediately under the cuticle the cell shows a basal cytoplasmic layer free of secretory granules and containing mitochondria. On histochemical grounds the granules were considered to be protein in nature, and were regarded as representing the luminescent material. The non-luminescent species of *Lepidonotus* lack a layer of corresponding photogenic tissue in their elytra.

I have prepared sections of the elytra of six luminescent species, namely *Acholoë astericola*, *Malmgrenia castanea*, *Harmothoë lunulata*, *Gattyana cirrosa*, *Polynoë scolopendrina* and *Lagisca extenuata*, and one non-luminous species, *Lepidasthenia argus*, and I can confirm Bonhomme's observations. The



Text-fig. 3. Drawing of a vertical section through the elytrum of *Acholoë astericola*, showing photocytes, elytral nerves and connective tissue framework.

photogenic cells in the first six species listed above form a layer of unicellular epithelium on the lower surface of the scale near the elytrophore region, and extending a greater or lesser distance peripherally, according to the species (Pls. I and II). In all species the photogenic cells are packed with coarse granules, which are acidophilic after fixation in Bouin's and Helly's fluids, and stain well with eosin and orange G, and also with azocarmine (Text-fig. 3). Some measurements are as follows:

Species	Dimensions of cell (μ)	Size of granules (μ)
Lagisca extenuata	3–4 × 8–14	2·5–3
Polynoë scolopendrina	5–6 × 8–25	2–5
Acholoë astericola	3–4 × 8–18	2–5
Malmgrenia castanea	6–8 × 8	2
Gattyana cirrosa	10–18 × 4–8	2–7
Harmothoë lunulata	6–18 × 4–5	3–5

There are considerable specific differences in the area of the scale occupied by the photogenic tissue. This is brought out in Text-fig. 2, compiled from

Bonhomme and from original observations. Drawings (Text-fig. 2) illustrate the region of the scale which luminesces in different species. Reconstructions have also been made from serial sections of whole elytra of *Acholoë* to show the area of scale occupied by photogenic tissue, and reasonably close correspondence was found to exist between the area occupied by photogenic cells and the luminescent region.

It follows that luminescence is intracellular in polynoid worms, and takes place in a layer of eosinophilic photogenic cells lying on the lower surface of the elytrum. Presumably the granules occurring in these cells are the luminescent material.

Miscellaneous Observations on Luminescence in Polynoids

Early observations on luminescence in polynoids, reviewed by Darboux (1899), do little more than record its occurrence.

Luminescence is evoked by mechanical stimulation of the animal. *Acholoë astericola*, the species which has been most investigated, is very sensitive to mechanical disturbance; and, if the container is shaken or the animal touched, a few or many scales will luminesce, depending on the strength of the stimulus. Falger (1908) notes that when an animal is touched, the luminescent response proceeds anteriorly and posteriorly over the elytra from the stimulated region (Dahlgren, 1916; Darboux, 1899). When an animal is quickly cut into two, however, only the elytra of the posterior portion lighten, while the anterior half of the animal remains dark (Harvey, 1940, 1952; Kutschera, 1909). This kind of response is apparently common to all luminescent species, and has been observed in *Lagisca extenuata* and *Malmgrenia castanea*, as well as in *Acholoë astericola*. In *Polynoë scolopendrina* the posterior half of the body is devoid of scales, and when this region is transected there is no luminescent response. When, however, the anterior half of the body is cut across, the scales posterior to the cut become luminescent.

These observations show that luminescence in polynoids is under nervous control, since the excitation proceeds along the length of the body from the stimulated region. With suitable tactile stimulation the luminescent response proceeds both forwards and backwards along the worm. Consequently, pathways exist for the transmission of excitation anteriorly and posteriorly through the nerve cord. Since a sharp cut produces luminescence in the posterior region only, these nervous pathways are apparently functionally polarized, as has been observed in *Chaetopterus* (Nicol, 1952*a*, *b*). Possibly, strong stimulation is required to overcome the resistance in anteriorly directed nervous pathways, in which resistance stands at a higher level than in pathways leading posteriorly.

A notable feature of luminescence in polynoids is that the light appears in flashes or scintillations when the animal is irritated. This has been recorded

for various species including *Harmothoë imbricata* and *H. lunulata* (Haswell, 1882; McIntosh, 1877, 1900). In *Acholoë astericola*, according to Falger (1908) and Kutschera (1909), the response lasts a few seconds and elytra light up intermittently for 20 or 30 times in quick succession.

The luminescent polynoids readily autotomize their elytra when irritated, and these separated scales continue to glow for some time when cast off from the animal. The nerve trunk is severed when a scale is cast off, and the excitation produced is sufficient, presumably, to start the scale flashing. Bonhomme observes that in the intact animal there are reflex pathways involving sensory receptors on the scale, the nerve cord, and efferent nerve fibres, all of which are concerned with mediating the luminescent response, but in the isolated scale only efferent fibres are in a position to be excited so as to lead to a response (Haswell, 1882; Jourdan, 1885; Kallenbach, 1883; McIntosh, 1900).

There are reports that the intact animal and isolated scales recover the ability to luminesce after stimulation when they are left for some time in sea water. Falger's results (1908) seem to indicate that recovery is a rather slow process.

Electrical stimulation has been employed by several workers (Bonhomme, 1942; Kutschera, 1909; Panceri, 1874). Falger (1908), using *A. astericola* found that direct current at make stimulated the animal to luminesce. By alternating the current he produced momentary flashes corresponding to each change of current, and finally, with faradic stimulation, he obtained prolonged responses in which the light appeared to flicker rapidly. These results are interesting in appearing to show that the response to a single shock is a quick flash. This kind of response should lend itself well to physiological analysis, and such a study was undertaken.

Results Obtained with Photoelectric Recording

Material and Methods

Recordings were made of luminescence in several species of polynoids which were collected in the Plymouth area: *Lagisca extenuata* (Grube), a common species on the shore, in dredgings, and among *Chaetopterus* tubes; *Harmothoë lunulata* (Delle Chiaje), commensal with *Arenicola marina* L., *Echinocardium cordatum* (Pennant), and *Leptosynapta inhaerens* (O. F. Müller); *Polynoë scolopendrina* Savigny, a commensal with *Polymnia nebulosa* (Montagu) which is most easily collected from among *Chaetopterus* tubes; *Acholoë astericola* (Delle Chiaje), a commensal living in the ambulacral groove of *Astropecten irregularis* (Pennant); *Gattyana cirrosa* (Pallas), occurring in the burrows of *Amphitrite johnstoni* Malmgren; and *Malmgrenia castanea* McIntosh, a commensal species found about the mouth region of *Spatangus purpureus* O. F. Müller (Text-fig. 1). The physiological basis of luminescence appears to be

identical in all these species, and the results are considered together. *Acholoë astericola* and *Polynoë scolopendrina* were used in most of the investigations, but sufficient records were obtained of luminescence in the other species to show that their behaviour was the same.

Each scale is an effector unit, and individual scales may be expected to show independent time courses of excitation and response. Therefore, to obtain quantitative data and to analyse the luminescent response, records must be secured from single scales. As brought out in the preceding section, the scales flash when detached from the body, and their luminescent ability thereby becomes reduced. To prevent this, and to obtain scales in a functionally fresh condition for examination, the animals were anaesthetized with an isotonic solution of magnesium chloride for 30 min. before handling them. With *Acholoë astericola*, which is very sensitive to tactile stimulation, and which luminesces strongly when an attempt is made to evict it from its host, it is best to anaesthetize starfish and worm together. This treatment narcotizes and immobilizes the worm, and the scales can be removed with fine scissors under a dissecting microscope without causing them to luminesce. Subsequently the magnesium should be washed out with several changes of sea water for an hour or more. Detached scales in sea water can be stored for a few days in a refrigerator (4–8° C.), until required for experimentation.

To record luminescence from a single scale, the scale was mounted on a pair of platinum electrodes lying in a moist chamber which was placed underneath a multiplier photocell. The arrangement is shown in Text-fig. 4. The light from the scale was focused on the photocathode of the photocell. The latter was connected through a direct coupled amplifier to a double-beam oscilloscope. Since there is much variation in the intensity of light produced by different species, it is necessary to regulate the degree of amplification, and this was achieved both by control of the amplifier, and by adjusting the voltage on the photocell by means of a potentiometer.

Electrical stimulation consisted of shocks of brief duration (capacitor discharges) delivered from an electronic stimulator which permitted independent control of frequency, voltage, and pulse duration.

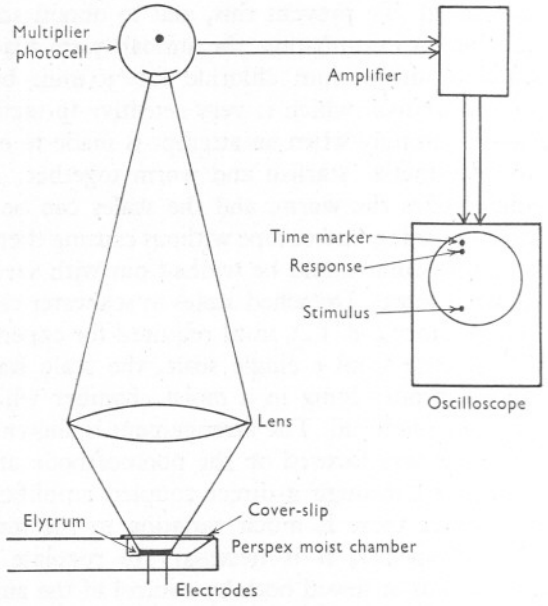
Observations

Effect of Single Shocks (Acholoë)

When an elytrum is stimulated with a single shock it gives a bright luminescent response. This frequently takes the form of a series of flashes. An example of such a response is given in Text-fig. 5. The frequency of flashing is rapid at first, often at rates of 5 or more per sec. The pattern of flashing frequently takes the following form. After a rapid initial outburst lasting about a second, the rate of flashing settles down to a steady level of about 1 per sec. which continues for around 1 min., and then gradually

begins to decline while the flashes themselves decrease in intensity. Text-fig. 10 shows a plot of flashing interval against time, taken from one of the photographic records, and bringing out certain of the features just described. In some records the rate of flashing increases instead of decreasing as the flashes die away, but this kind of response is less frequent.

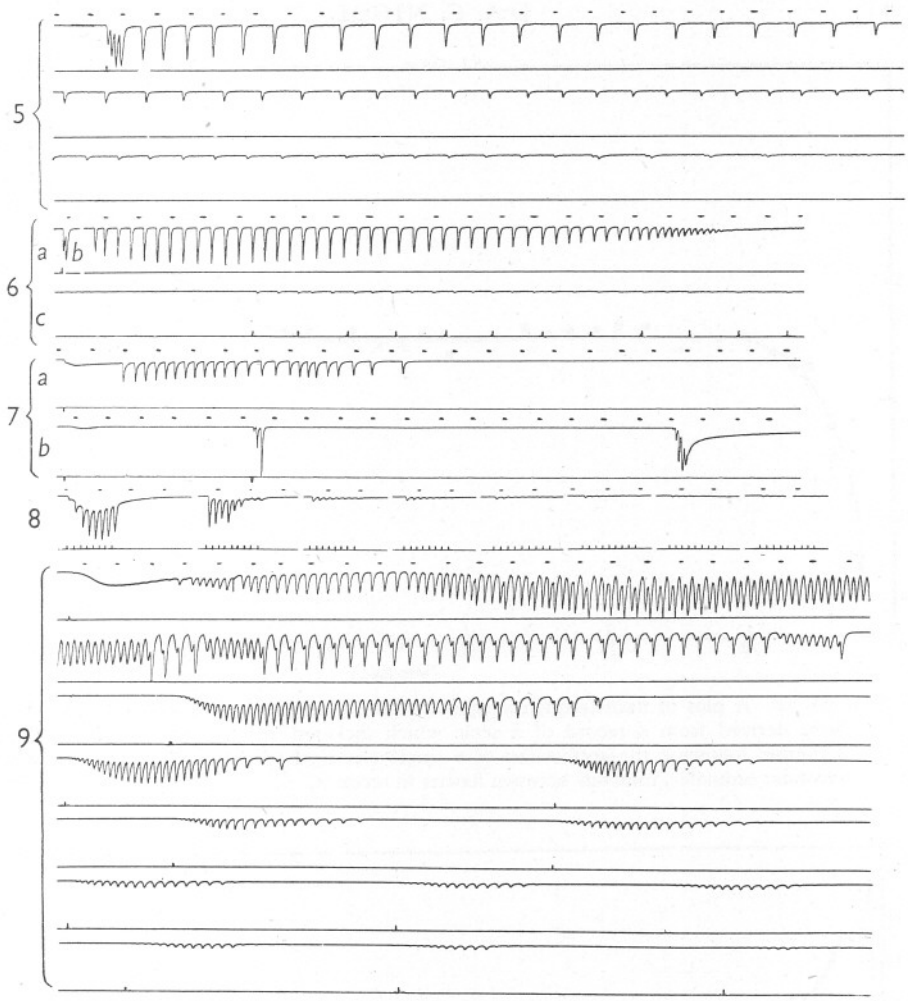
A second characteristic of this repeated flashing or flickering is seen in a falling off of intensity. Initially the intensity of the flashes is relatively high, but thereafter the height of the separate responses decreases with time. This



Text-fig. 4. Diagram of the apparatus used in recording the luminescent flashes of single elytra from polynoids. Not drawn to scale.

is shown in Text-figs. 5 and 6. When the rate of flashing is fairly rapid (intervals of less than 0·6 sec.), the light intensity does not return to zero between flashes (Text-fig. 5).

During repetitive flashing (induced by a single stimulus) the level of intensity reached by the first few flashes is often noticed to rise rapidly to a maximum, and then gradually decrease (Text-fig. 5). This effect shows in Text-fig. 8, which gives a plot of the light intensity of individual flashes against time. In the record from this elytrum the increase in intensity follows a linear course for the first three flashes, and then slowly decreases. Maximal intensity of flashes is reached in one-third of a second (third flash), and intensity of subsequent flashes falls to one-half the value of the maximal flash in 11 sec. (15 flashes). The rate of decay of intensity of subsequent



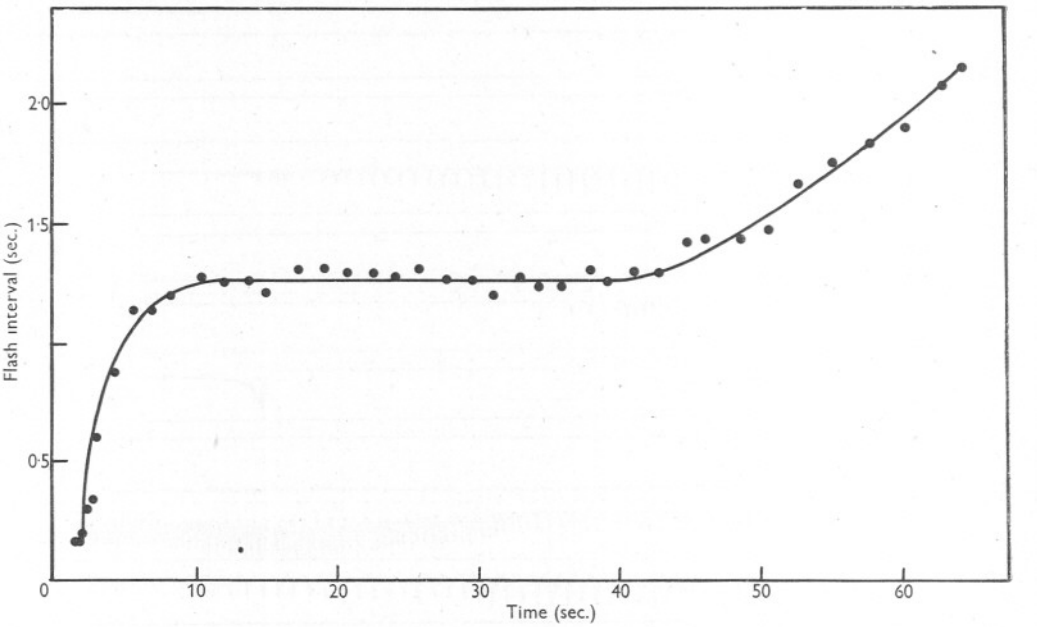
Text-fig. 5. Prolonged flashing from an elytrum of *Acholoë*, as the result of a single electrical stimulus. This is a continuous record. Time scale above, pips at 1 per sec. Deflexions downwards of the oscilloscope upper beam are records of the luminescent responses. Stimulus appears as pip on the lower line (oscillograph lower beam).

Text-fig. 6. Rhythmic flashes in an elytrum of *Acholoë* following the application of a single stimulus (*a*, *b*), and repeated stimuli (*c*). Stimulation frequency, 42 per min. in *c*. Time scale, 1 per sec. 1 sec. elapsed between records *a* and *b*; 1 sec. between *b* and *c*.

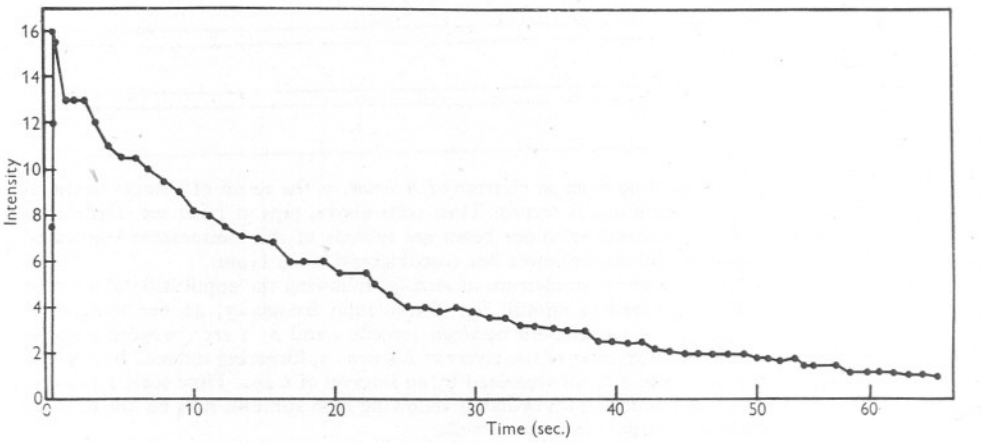
Text-fig. 7. Luminescent responses of the elytra of *Lagisca*: *a*, flickering induced by a single impulse; *b*, effect of two stimuli separated by an interval of 6 sec. Time scale 1 per sec. The small prolonged deflexion immediately following each stimulus may be due to some direct depolarization of the luminescent cells.

Text-fig. 8. Fatigue of the luminescent response of *Acholoë* as the result of successive bursts of electrical stimuli. These consisted of 7–8 shocks at a frequency of 6 per sec. Time scale 1 per sec.

Text-fig. 9. Rhythmic flashing of an elytrum of *Gattyana*. This is a continuous record showing consecutive periods of rhythmic flashing, each period of flashing being induced by a single shock. Time scale 1 per sec.



Text-fig. 10. A plot of flash-frequency against time for an elytrum of *Acholoë*. The data were derived from a record of a scale which flickered rhythmically for more than a minute following the application of a single electrical stimulus. Abscissae, time in seconds; ordinates, intervals between flashes in seconds.



Text-fig. 11. A plot of the light intensity of successive flashes in an elytrum of *Acholoë* against time. Rhythmic flashing was induced in the preparation from which this record was obtained by a single electrical shock. Light intensity in arbitrary units. Time in seconds. Each point on the curve represents the intensity of a separate flash.

flashes follows an exponential curve, and in about 1 min. is 10% of the maximal flash, and is barely measurable (Text-fig. 11). A curious feature of some records is that the flashes sometimes occur in bursts with considerable pauses in between. This is recorded in Text-fig. 6 (*Acholoë*) and in Text-fig. 7 (*Lagisca*). After a pause of as much as 10–14 sec. the scale may spontaneously start to flash once more, and continue until its powers of luminescence are exhausted.

Fatigue

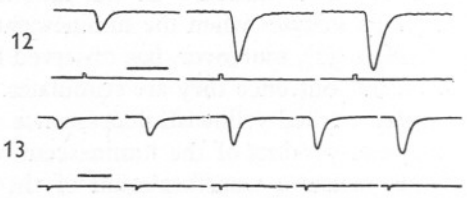
Several factors are involved in determining the magnitude of the response, and one of the most important is the onset of fatigue. After a scale has been flashing for some time the flashes gradually become weaker. A highly excitable scale may continue flashing until it is nearly or completely exhausted, when the light becomes very weak or disappears. Subsequent stimulation then fails to evoke a response. Other scales, less excitable, respond with shorter periods of flashing to single shocks or brief bursts. These scales can be fatigued by repeating the stimulation at suitable intervals, when the consecutive flashes or groups of flashes gradually decrease in amplitude (Text-figs. 8 and 9). Isolated scales which have been stimulated to exhaustion show little or no recovery of luminescent ability when left overnight in sea water.

Since the luminescent response is an intracellular process, some oxidation of a cellular constituent must occur. Bonhomme (1942) has shown that after excitation there is no apparent decrease in the amount of intracellular secretory material in the photocytes when the luminescent response has run its course. Harvey (1926, 1952), moreover, has observed that fresh elytra of *Acholoë* are non-fluorescent, but once they are stimulated and luminescence has subsided, they display marked yellowish fluorescence. It appears, therefore, that some waste or end-product of the luminescent reaction is strongly fluorescent, and by this means a transformation of the intracellular constituents concerned with luminescence can be detected.

There are suggestions by earlier workers (p. 72) that polynoids recover the ability to luminesce after a period of rest, but since no comparative measurements were made these statements are rather indefinite. My observations show that recovery is a very slow process in isolated elytra. Apparently, then, either the luminescent reaction is irreversible, or reduction of the oxidized photogenic material takes place very slowly within the photocytes. Repeated flashing, therefore, progressively exhausts the store of potentially luminescent material until the scale becomes dark, and renewal of this store is dependent upon protracted chemical transformations within the photocytes. Isolated scales, of course, are deprived of trophic supplies from the animal, and this lack may restrict the energy sources available for reconstitution of the photogenic material.

It is of interest to compare fatigue of luminescence in different forms. In glowworms and fireflies (Lampyridae), luminescence is also an intracellular phenomenon, but these animals are able to produce light for many hours and do not show fatigue. On the other hand, various marine Crustacea which possess photophores, e.g. *Nyctiphanes*, *Sergestes*, fatigue readily as the result of repeated stimulation (Terao, 1917; Vallentin & Cunningham, 1888). I have myself shown (Nicol, 1952*a*), that the luminous polychaete *Chaetopterus variopedatus* discharges a luminescent secretion into the surrounding sea water, and when it is repeatedly excited the amount of secretion, and consequently light, rapidly diminishes with each period of stimulation. These few selected observations demonstrate that there exists extensive variation in the ability of different animals to maintain sustained or recurrent luminescence at a high level. This is of first importance in considering the role of luminescence in the life of the animal, and in seeking to unravel the physiological events involved in the control of light-production in each species.

Most biochemical work on luminescent substances has been done on the photogenic secretion of *Cypridina* (see Harvey, 1952, for a review of the relevant literature). *Cypridina* luciferin has been prepared in a highly purified state and this substance is known to undergo an irreversible oxidation when it is converted into oxyluciferin, with the release of light energy, through the activity of luciferase. These exact and detailed studies afford some ground for believing that in *Chaetopterus*, and possibly in polynoids, we are also dealing with an irreversible oxidation of photogenic material.



Text-fig. 12. Single flashes in an elytrum of *Acholoë*, induced by single impulses. Stimulating shock shown on lower line. There was an interval of 3 sec. between the first and second stimuli, and 5½ sec. between the second and third. Time scale, shown by the horizontal line at the bottom left, 100 msec.

Text-fig. 13. Luminescent responses of *Polynoë scolopendrina*. This record shows consecutive single flashes, 1 per stimulus, induced by a slow rate of stimulation (1 per sec.). Time scale below, 100 msec. Note the progressive increase in the height of consecutive flashes.

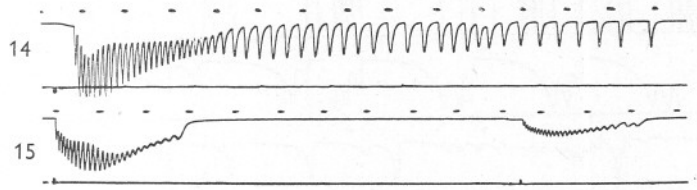
Time Characteristics of Single Flashes

Favourable records of single flashes photographed at fast camera speeds permit an analysis of the temporal relations of the luminescent response (Text-fig. 12). In *Acholoë* the luminescent response (flash) begins 18–20 msec. after the stimulus, rises to a peak in 18–23 msec. from initial deflexion, and returns to base-line (zero intensity) from maximal deflexion in 45–80 msec.

Mean values are 19 msec. for latent period and 83 msec. for total flash duration. Time for half decay from peak intensity ranges from 11 to 19 msec. The response times for *Polynoë* scales are somewhat similar. The latent period varies from 13 to 21 msec., peak intensity is reached 26–30 msec. after initial deflexion, and the response is finished 108 msec. after maximal intensity is reached. Half decay from maximal deflexion occurs in about 17 msec. (Text-fig. 13).

Comparison of the Luminescent Responses of Different Species

The luminescent responses of different species of polynoids appear to conform to the same pattern as that found in *Acholoë*. In *Lagisca extenuata* (Text-fig. 14), *Harmothoë lunulata* (Text-fig. 15), *Malmgrenia castanea* (Text-fig. 16), *Polynoë scolopendrina* (Text-fig. 17), and *Gattyana cirrosa* (Text-fig. 9), a single shock either evokes one flash or initiates flickering, according to the condition of the elytrum. Rates of rhythmic flashing in the various



Text-fig. 14. Luminescent responses of an elytrum of *Lagisca*, showing rhythmic flashing induced by a single electrical stimulus. Time scale, 1 per sec.

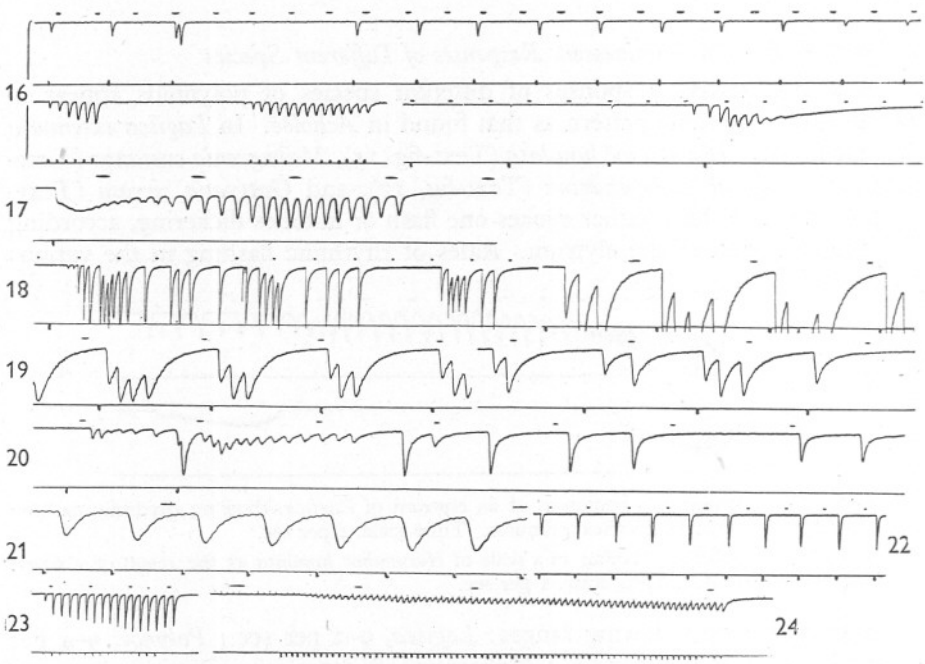
Text-fig. 15. Rhythmic flashing in a scale of *Harmothoë lunulata* as the result of a single electrical stimulus. Time scale, 1 per sec.

species show the following ranges: *Lagisca*, 9–2 per sec.; *Polynoë*, 9–4 per sec.; *Harmothoë*, 9–1 per sec.; *Malmgrenia*, 8–1 per sec.; *Gattyana*, 9–2 per sec. Rates of flashing in *Acholoë*, for which a large series of records are available, vary from 18 to 1 per sec., but usually lie below 10 per sec. In general, it may be said that the scales of all species examined begin flashing at a rate of about 9 per sec., and the rate progressively falls off to a frequency of about 1 flash per sec. Other features common to all species are the gradual build-up in intensity of the first few flashes, and, once maximal flash intensity is reached, the progressive diminution in intensity of successive flashes as fatigue sets in.

Effect of Stimulating the Nerve Cord

Most experiments have been made, for convenience, on isolated scales. To get additional information about the nature of nervous control of the luminescent response, some additional experiments were tried on *G. cirrosa* in which the nerve cord was stimulated. The preparations consisted of pieces of the body amounting to three or four segments. A single scale was left on

each piece, and the body-wall was slit longitudinally along the ventral surface on one side of the nerve cord opposite to that bearing the scale. The preparation was then mounted on a glass plate, with the elytrum downwards, above the photocell. A micromanipulator was used to place a pair of needle electrodes on the nerve cord in that segment bearing the scale. Conveniently, the entire central nervous system, brain and nerve cord, in all polynoids is



Text-fig. 16. Single flashes and repeated flashes from an elytrum of *Malmgrenia* as the result of electrical stimulation. Time scale, 1 per sec.

Text-fig. 17. Luminescent responses from a scale of *Polynoë*. Rhythmic flashing induced by a single stimulus. Time scale, 1 per sec.

Text-figs. 18–24. Luminescent responses from an elytrum of *Gattyana cirrosa* resulting from stimulation of the ventral nerve cord. Time scale in all records, 1 per sec. Fig. 18: rhythmic flashing induced by a single electrical shock. Fig. 19: rhythmic flashing in response to stimulation at a frequency of 72 per min. Fig. 20: rhythmic flashing induced by a pair of stimuli. Fig. 21: burst of shocks at a frequency of about 5 per sec. The response takes the form of a single flash for each stimulus. Fast paper. Figs. 22 and 23: responses to bursts of stimuli at 1 and 5 per sec. respectively. Note the gradual increase in intensity of successive responses. Fig. 24: burst of stimuli at 20 per sec. The rate of flashing follows that of stimulation.

easily recognizable in being coloured bright scarlet and visible through the body-wall. The colour is presumably due to haemoglobin, as in *Aphrodite* (see Fordham, 1925). Shocks of minimal strength (threshold) were used in order to restrict the stimulation to the nerve cord. The strength of shocks

required to stimulate the nerve cord was found empirically to be only a small fraction of that necessary to stimulate an isolated scale. Consequently, the possibility of a spread of the stimulus to the scale itself can be excluded in these experiments.

As in isolated scales, a single shock applied to the nerve cord usually evokes a series of rhythmic flashes (Text-fig. 18). A gradual increase in the intensity of the first few consecutive flashes appears in some of these records (Text-figs. 18 and 19), and some preparations showed considerable irregularity in the pattern of responses, both in height and timing (Text-fig. 18 and 20). The irregularity which appears in some of the *Gattyana* records suggests strongly that several neuro-effector units are present, each one consisting of a group of photocytes supplied by one neurone, and that these units may be responding at slightly different rates, and be out of phase.

Stimulation at higher frequencies is shown in Text-figs. 21 and 24. Above 1 per sec. the flashes often follow the stimuli faithfully, and successive flashes increase gradually in intensity.

The conclusion from these observations is that the luminescent responses of the elytrum are controlled by the nervous system; and that efferent pathways exist from the nerve cord to the photocytes in the elytrum. It is significant also that a single impulse from the nerve cord will cause repetitive flashing in the elytrum, possibly through the intermediation of the ganglion situated in the latter structure.

Discussion

The most striking feature of the luminescent response of polynoids is that it usually takes the form of quite rhythmic flashes. The evidence presented shows that the response is under nervous control and is normally evoked by tactile stimulation (touch or mechanical disturbance). A single electrical shock, mechanical injury to an isolated scale, and autotomy of the scale result in rhythmic flashing. Without further information there is little value in speculating on the mechanism responsible for this effect, but suggestions that would be worth further exploration are injury potentials from severed nerve fibres, rhythmic oscillations in the soma of ganglion cells supplying the photocytes, and reverberation of impulses in some system of closed neuronal arcs in the elytrum. Since electrical stimulation of the nerve cord as well as of the isolated elytrum causes rhythmic flashing, it appears that this type of response is characteristic of the normal animal, and a mechanism involving the elytral ganglion may be operating.

A second point of interest, revealed by the photographic records, is the gradual increase in intensity of the first few discrete consecutive flashes in a rhythmic response, or under repetitive stimulation. There is good evidence here for a process of facilitation taking place at the neuro-effector junctions,

and closely analogous in many respects to the corresponding phenomenon described in neuro-muscular physiology. The quick flashes, closely following nervous stimulation, and the sharp records which can be obtained are very suitable for physiological analysis, and further studies are being pursued.

The regular rhythmic flashing, often lasting for a minute or more, and leading to exhaustion of the luminescent material, may in itself have marked functional significance. A mechanism of this kind allows discontinuous light emission over a much longer period of time than would be effected by continuous emission at the same intensity, and is a more economical method of utilizing a given amount of photogenic material. It can be argued that rhythmic flashes at rates of 10 to 1 per sec. are adequate to attract and secure the attention of another animal, and thereby fulfil their intention. A flickering light also may be more effective as a photic stimulus than a continuous light; but here experimental confirmation is required.

Several authors have commented on the possible significance of luminescence in polynoids, and have suggested that it acts in the nature of a sacrifice lure (Dahlgren, 1916; Haswell, 1882; Kutschera, 1909). This viewpoint is based on the behaviour of the animal. As noted previously, only the posterior half of the animal luminesces after transection. The anterior half is capable of directed locomotory movements, whereas the posterior half shows writhing movements but retains its same relative position. It has therefore been argued that when a polynoid is attacked and part of it is seized, the luminescing posterior region holds the attention of the predator, while the anterior region has the opportunity of escaping. Dahlgren (1916) presents an illustration by Horsfall which depicts such a situation. The anterior region of a polynoid is, of course, capable of regenerating the missing segments. Active, luminescent polynoids also possess a well-developed muscular mechanism for autotomizing their scales and these are readily cast off when the animal is irritated. Such isolated scales have been observed to flash rhythmically when removed from the body, and could well serve the function of a sacrifice lure. The reaction time is also fairly rapid, the first flash occurring in about $\frac{1}{50}$th of a second after stimulation of the nerves, and this would make the mechanism quite efficient. Since the scale soon exhausts itself after a series of flashes, the mechanism is evidently designed to function only at infrequent intervals. Such a theory, of course, must remain speculative until direct evidence is adduced to substantiate it.

It is of some interest to speculate on the evolution of luminescence among polynoids. In the family Aphroditidae, luminescence is confined to the subfamily Polynoinae, and to certain genera of the latter only. Known luminescent genera are *Harmothoë*, *Lagisca*, *Polynoë*, *Acholoë*, *Eunoë* and *Malmgrenia*. Except for the species *Lepidasthenia stylolepis*, *Lepidasthenia* and *Lepidonotus* are non-luminescent. Discussing the affinities of the Polynoinae, Darboux (1899) groups together the first four genera as closely related and probably

derived from some common form, such as *Harmothoë*. Evidence suggests that they may have evolved independently of a second group including *Lepidasthenia* and *Lepidonotus*. Except for *Lepidasthenia stylolepis*, luminescence would seem to have appeared in some ancestral form common to one group of polynoids, and has been retained by its modern derivatives. This is a simpler explanation than one which assumes that luminescence has evolved on a number of separate occasions in this subfamily. Luminescence may, of course, have been lost by certain species, for example, *L. argus*.

Summary

Six species of luminescent polynoids of the Plymouth fauna have been studied, namely *Lagisca extenuata*, *Gattyana cirrosa*, *Harmothoë lunulata*, *Polynoë scolopendrina*, *Acholoë astericola* and *Malmgrenia castanea*. Their scales are luminescent, and the light is produced by granular eosinophilic photocytes, which form a unicellular layer on the lower surface of the scale. The nervous supply of the elytrum is described, and the luminescent response is shown to be under nervous control. Luminescent responses from all six species have been recorded by the use of a photomultiplier cell and oscilloscope. The normal response has been found to consist of a series of rhythmic flashes, from 9 to 1 per sec., lasting up to 1 min. Some characteristics of the luminescent responses are given, and the part they may play in the normal life of the animal is discussed.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Scale of *Acholoë* stained supravitally with methylene blue (scale, 0·5 mm.).

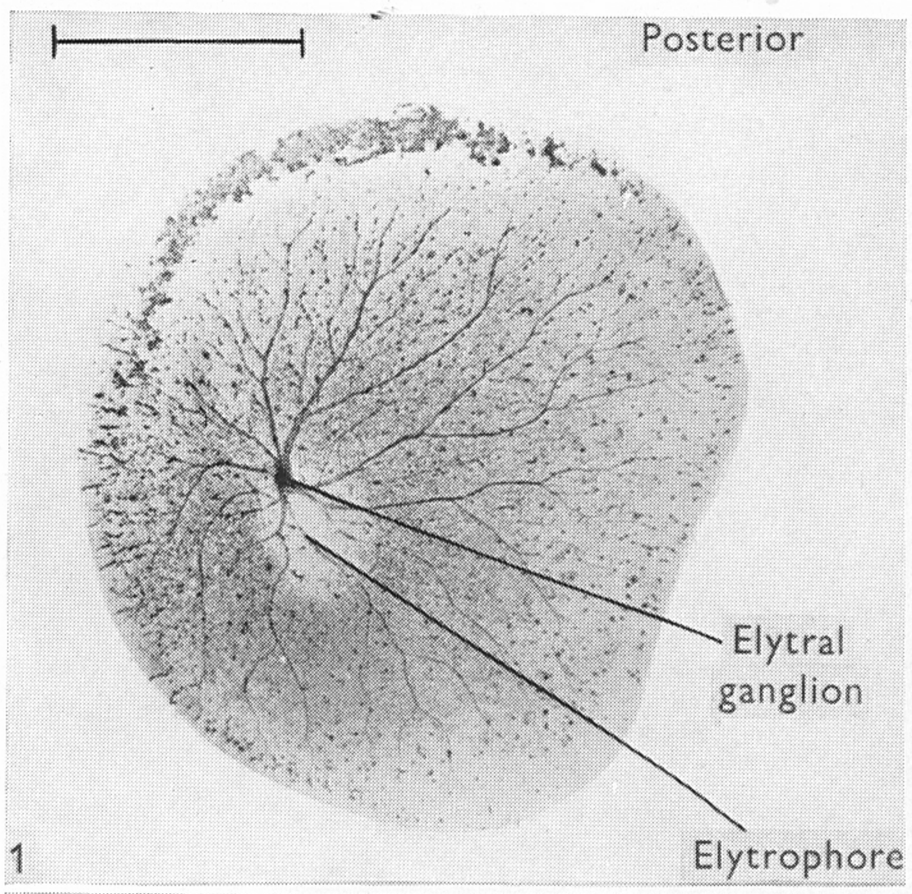
Figs. 2–6. Horizontal sections through elytra to show internal structure. Fig. 2: *Harmothoë* (25 μ). Fig. 3: *Gattyana* (10 μ). Fig. 4: *Polynoë* (25 μ). Fig. 5: *Acholoë* (20 μ). Fig. 6: *Lagisca* (50 μ). Heidenhain-azan and haematoxylin-eosin.

PLATE II

Fig. 7. Section through elytrophore and scale of *Malmgrenia* (Heidenhain-azan). Scale, 100 μ.

Fig. 8. Section through the central region of the elytrum of *Acholoë*, to show elytral ganglion and origin of an elytral nerve. (Bodian silver stain). Scale, 20 μ.

Fig. 9. Section through an elytrum of *Acholoë* to show elytral nerve and photogenic cells. (Bodian silver stain.) Scale, 20 μ.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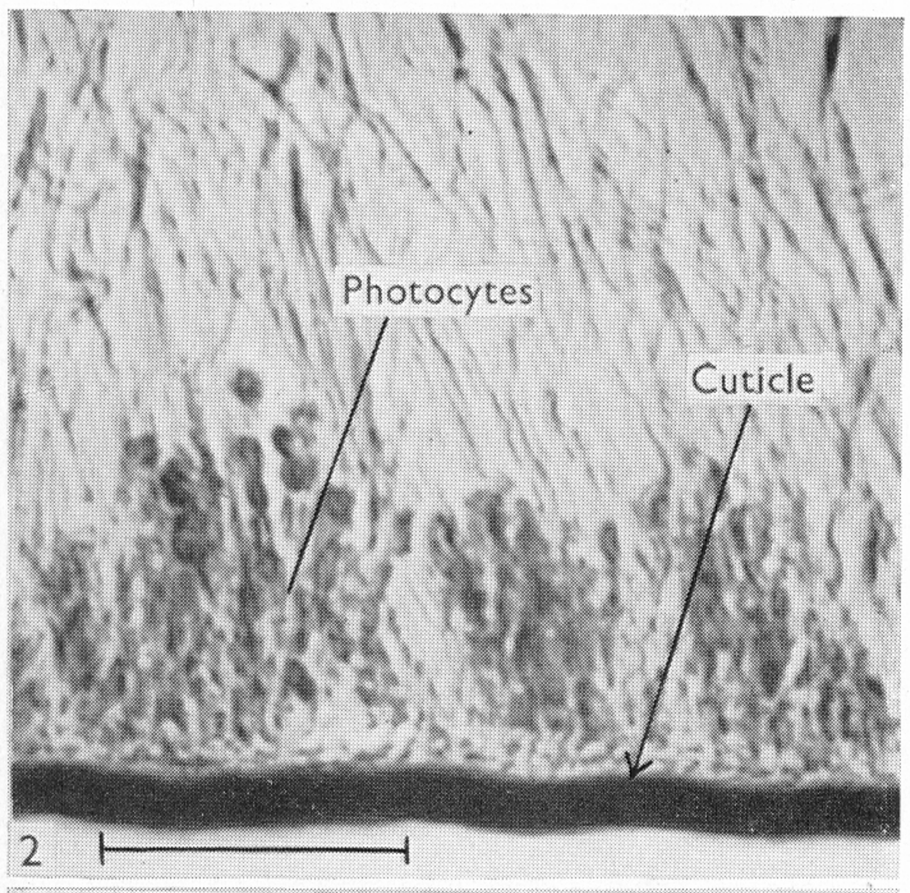
Elytral ganglion
Elytrophore
1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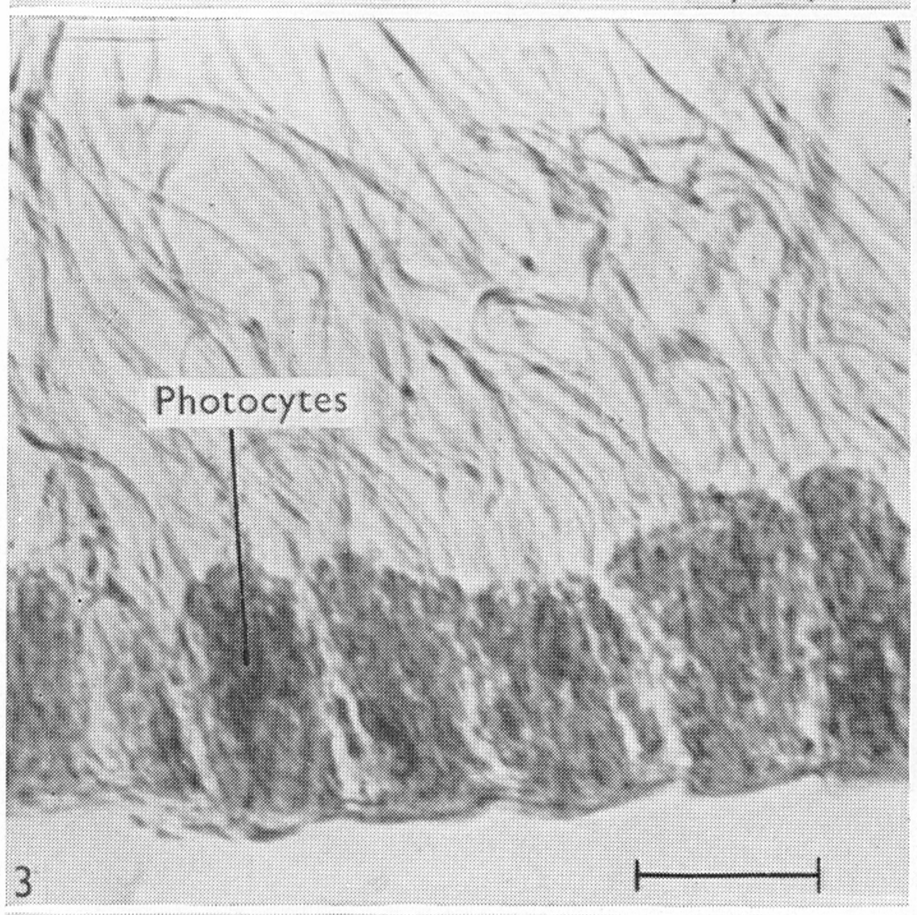
Cuticle
2


Photocytes
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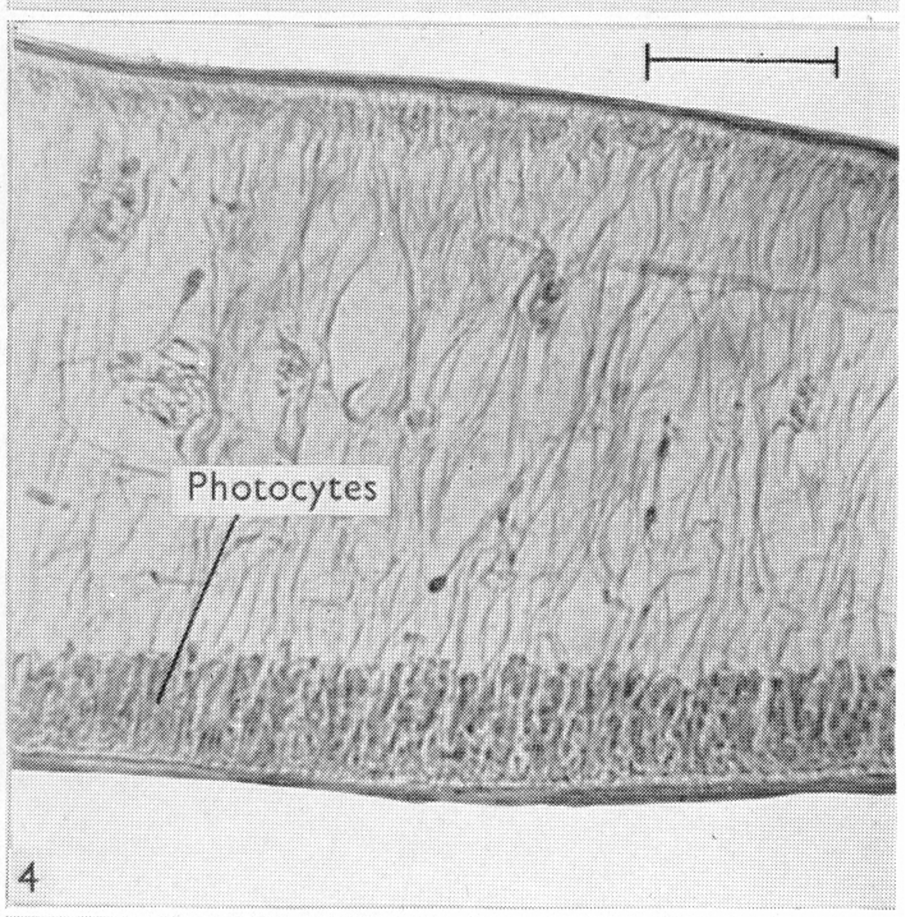
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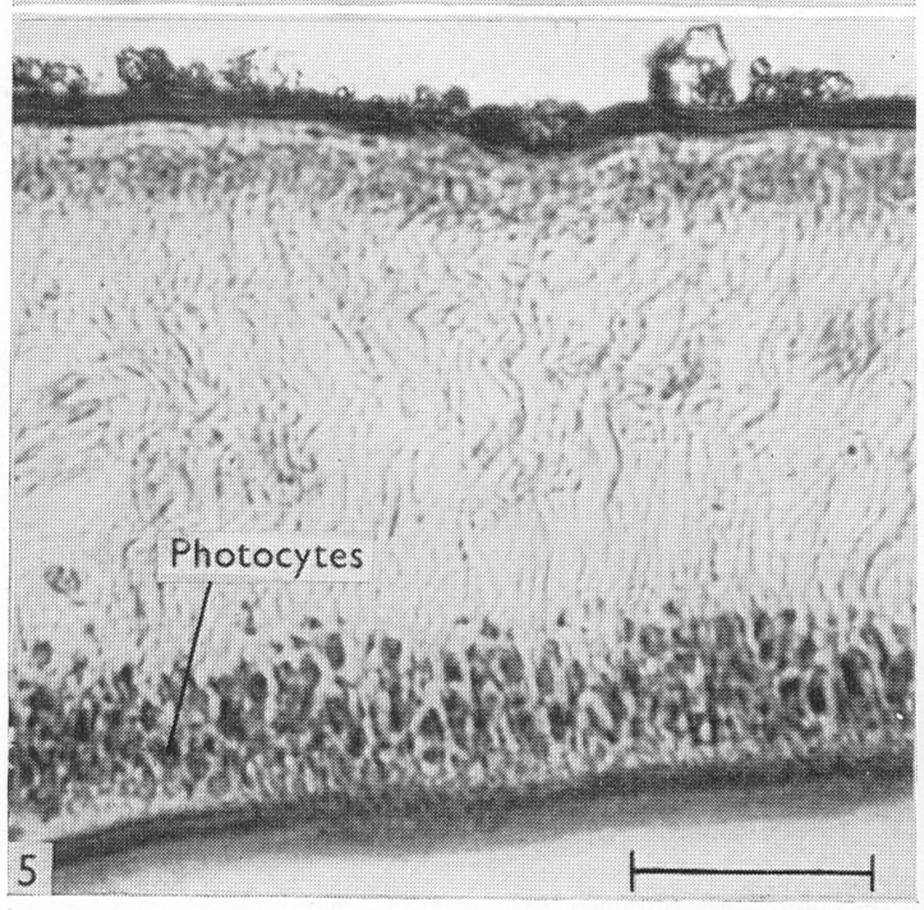
Photocytes
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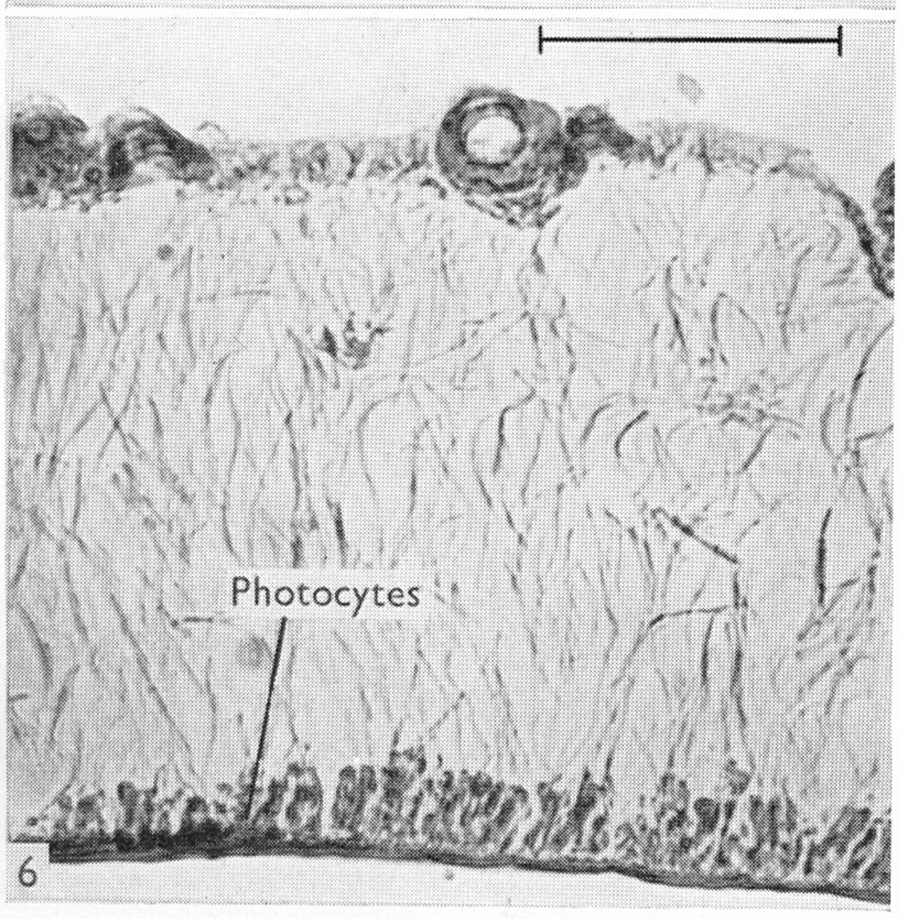
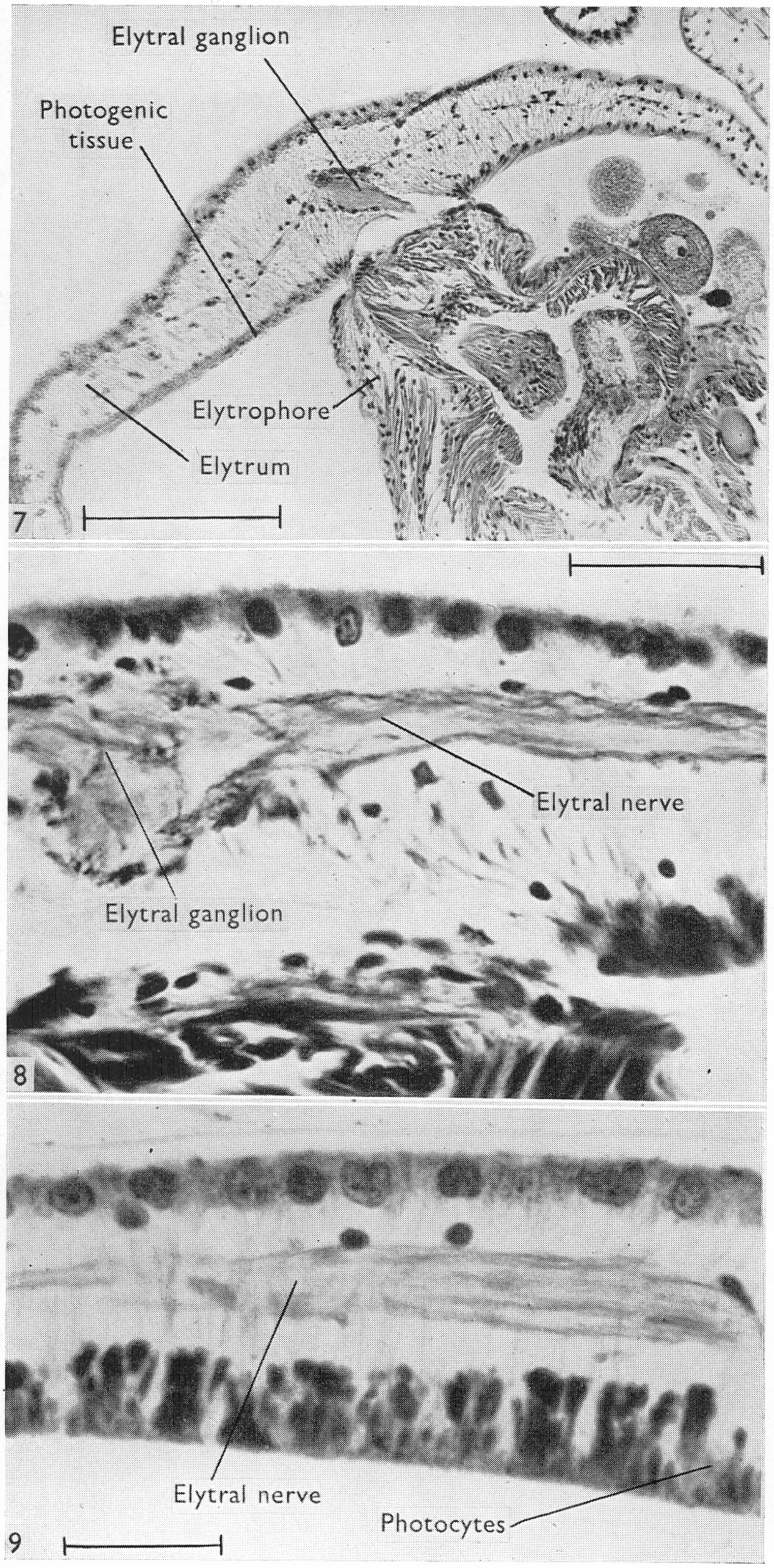

Photocytes
6

Figs. 1–6.


Elytral ganglion
Photogenic tissue
Elytrophore
Elytrum
7
Elytral nerve
Elytral ganglion
8
Elytral nerve
Photocytes
9

Figs. 7–9.

ON THE BIOLOGY OF *GLOSSUS HUMANUS* (L.) (*ISOCARDIA COR* LAM.)

By G. Owen

The Marine Station, Millport, and the Department of Zoology, University of Glasgow

(Text-figs. 1–9)

Glossus humanus (L.), the only living representative of the genus, is a large eulamellibranch living in Western European seas and the Mediterranean. Nicol (1951) has reviewed the geological history and geographical distribution of the species, but little is known of its structure and habitat. Specimens were obtained from the Clyde Sea Area by use of an Agassiz trawl in depths of over 50 m. in the channel between the islands of Arran and Bute. Work on these specimens was carried out at the Marine Station, Millport, and the Zoology Department of the University of Glasgow.

I wish to express my gratitude to Prof. C. M. Yonge, F.R.S., for his encouragement and guidance during the course of this work and also to the Director and Staff of the Millport Laboratory for assistance in many ways. I am indebted to Dr Vera Warrender for the drawing of the shell reproduced in Fig. 1. Acknowledgement is also due to the Development Commission for financial assistance.

The Shell

The shell in *G. humanus* is equivalve, inequilateral and markedly globular with the umbones spirally enrolled and directed anteriorly. Many of the generic and specific names that have been applied to this species result from the resemblance of the shell, when viewed anteriorly, to the mammalian heart (Fig. 1).

In the Lamellibranchia, the direction of growth at any region of the valve margins may be resolved into: (*a*) a radial component radiating from the umbone and acting in the plane of the generating curve; (*b*) a transverse component acting at right angles to the plane of the generating curve; (*c*) a tangential component acting tangentially to, and in the plane of, the generating curve (Owen, 1952). The radial component is always present and affects the form of both valves. In the primitive bivalve, the normal axis was probably a plano-spiral and the form of the shell the resultant of radial and transverse components. In *G. humanus*, however, the normal axis (Fig. 2, NA) is a turbinate spiral, indicating that the form of the shell is the resultant of radial (R), transverse and tangential (T) components.

In genera where the tangential component is absent (e.g. *Tellina*), the cardinal teeth radiate across the hinge plate, increasing slowly in all dimensions with growth. But in *G. humanus*, the mantle isthmus (Owen, Trueman & Yonge, 1953) is continuously displaced posteriorly due to the effect of the tangential component on the mantle edge (Fig. 2). As a consequence, the cardinal teeth of both valves are long and extend almost parallel to the sides of the hinge plate (CC, ACR and PCR). The anterior lateral teeth are much reduced or absent (Lamy, 1920) while the posterior lateral teeth (PLR) are elongate, since in this region the tangential component, by displacing the secreting surfaces posteriorly, reinforces the effect of the radial component.

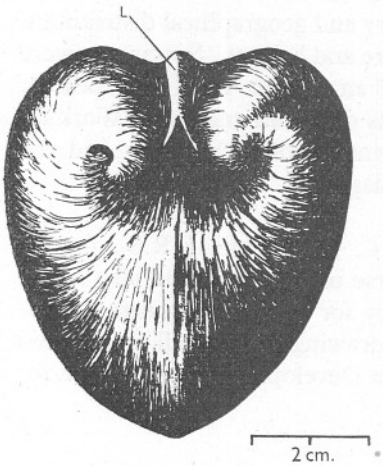


Fig. 1.

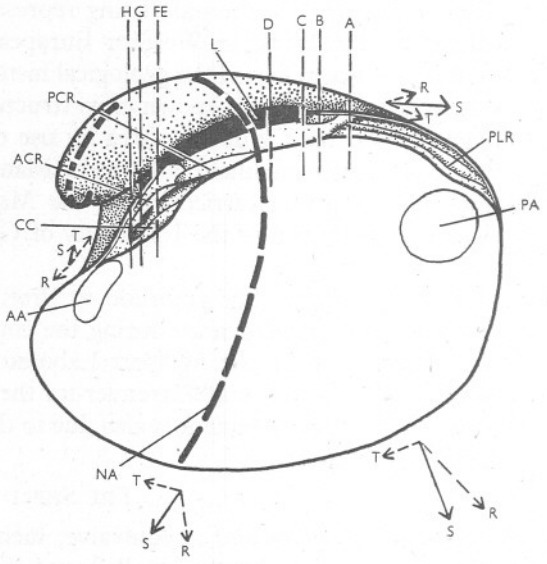


Fig. 2.

Fig. 1. *G. humanus*, the shell viewed anteriorly. L, ligament.

Fig. 2. *G. humanus*, the right valve showing the dentition of the hinge plate. AA, anterior adductor muscle scar; ACR, right anterior cardinal tooth; CC, central cardinal tooth; L, ligament; NA, normal axis; PA, posterior adductor muscle scar; PCR, right posterior cardinal tooth; PLR, right posterior lateral tooth; R, radial component; S, direction of growth at the mantle/shell margin; T, tangential component. The letters A–H refer to positions of transverse sections drawn in Fig. 3. Sections 3E and F are represented by a single line.

The description of the hinge teeth given by Jeffreys (1863) is confusing, for the dentition of the right valve is described as that of the left and vice versa.

The tangential component also affects the anterior region of the external opisthodetic ligament (Owen, 1953). When there is no tangential component affecting the form of the valves, as in *Glycymeris*, growth of the mantle/shell (Yonge, 1953) anterior to the umbones takes place anteriorly, i.e. away from

the umbones. In *Glossus humanus*, however, as shown in Fig. 2, the radial (R) and tangential (T) components anterior to the umbones are opposed. Owing to the greater magnitude of the tangential component at this region, growth of the mantle/shell margin is in a posterior direction between the umbones, causing them to be increasingly separated as growth proceeds. This separation of the umbones results in a progressive splitting of the anterior region of the ligament, the split portions extending under the spirally twisted umbones (Fig. 1, L).

Covering the external surface of the shell is a reddish brown periostracum which, except at the anterior ventral to the umbones, is produced into numerous radiating rows of small bristle-like projections ('cilia' of Forbes & Hanley, 1853). The shell is thin and remarkably light in weight, the smaller specimens being extremely fragile. Bøggild (1930) has described the detailed structure of the layers composing the shell valves. Internally the valves have a smooth porcellaneous appearance while the pallial line is poorly defined. The posterior adductor muscle scar (Fig. 2, PA) is larger than the deeper anterior scar (AA). This deeper insertion of the anterior adductor muscle is possibly due to the lower rate of growth of the anterior region of the shell. The rate of migration of the anterior adductor muscle is, therefore, considerably less than that of the posterior adductor, while the rate of deposition of the inner layers of the shell is presumably equal for both regions.

The Ligament and Mantle Isthmus

In describing the structure of the ligament in *G. humanus* the nomenclature suggested by Owen *et al.* (1953) has been used. The relationships of the mantle, ligament and valves are shown in Fig. 3 (A–H). These figures form a series taken from posterior of the ligament to a region near the umbones as shown in Fig. 2. The interpretation of the structure and formation of the ligament will be more readily understood by comparing the sections shown in Fig. 3 with the diagrammatic representation of the ligament shown in Fig. 4.

Fig. 3A represents a section through the valves and mantle between the siphons and the posterior end of the ligament. Here, as shown in Fig. 4B, the inner lobes (FI) are fused, forming a ridge of tissue lying between the middle lobes (M). The periostracum (P) is secreted from the groove between the middle and outer (O) lobes. Anteriorly (Fig. 3B), the periostracum is present as a single delicate thread and the middle lobe (M) is represented by a median ridge of tissue between the two outer lobes. This appearance in transverse section of a single periostracal thread and of a single middle lobe does *not* represent a fusion of the periostracal grooves and of the middle mantle lobes. As will be seen from Fig. 4A, the transverse section represented in Fig. 3B passes through the periostracal groove (PG) and the middle lobe (M) of the mantle edge at the depth of the posterior embayment between the

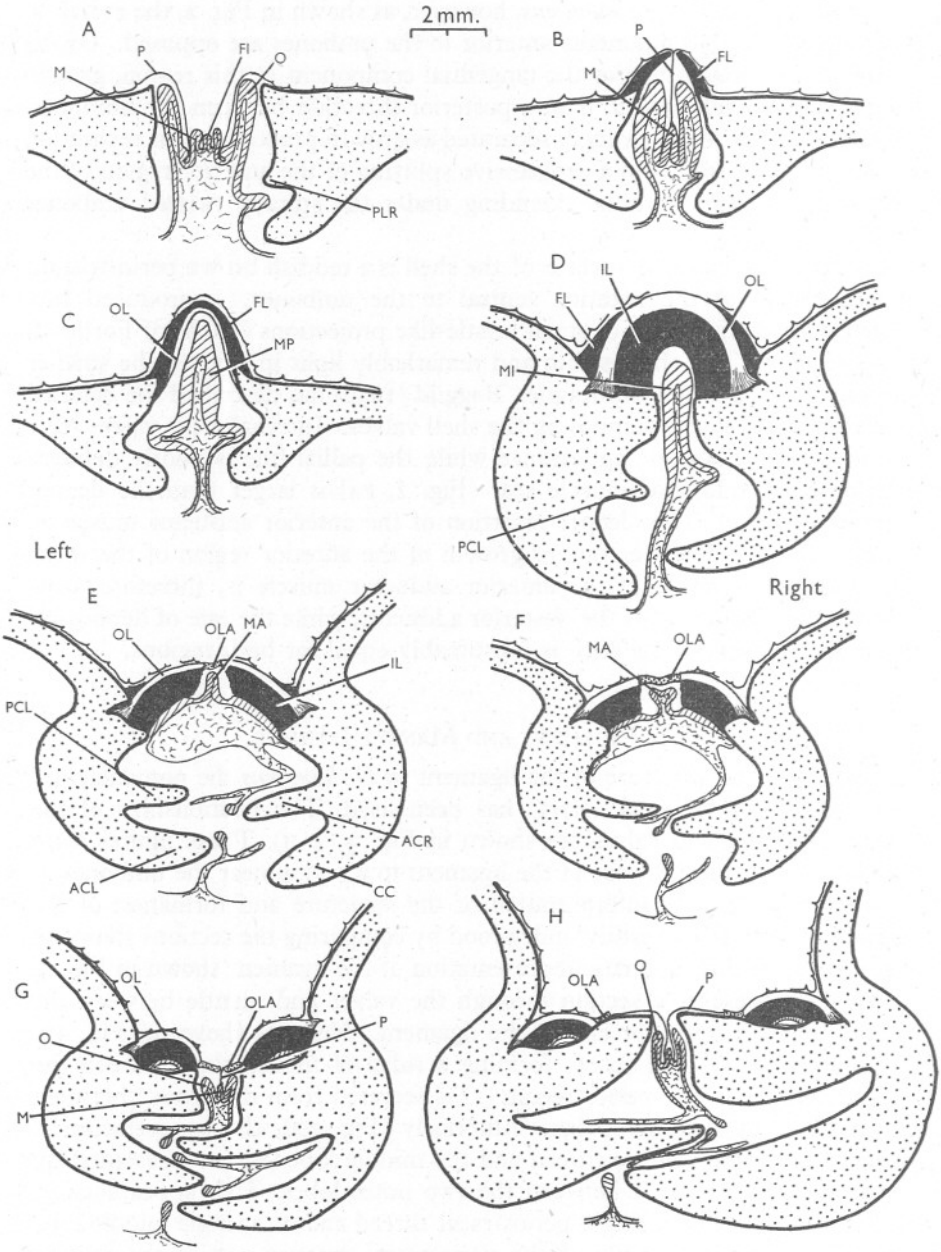


Fig. 3. *G. humanus*, diagrams representing transverse sections through the ligament, valves and mantle. The positions of the sections are shown in Fig. 2. ACL, left anterior cardinal tooth; FI, fused inner lobes; FL, fusion layer; IL, inner layer; M, middle lobe; MA, mantle edge anterior to the mantle isthmus; MI, mantle isthmus; MP, mantle edge posterior to the mantle isthmus; O, outer lobe; OL, 'outer' layer; OLA, anterior 'outer' layer; P, periostracum; PCL, left posterior cardinal tooth. Other lettering as before.

two lateral lobes of the mantle. The outer lobes secrete on each side a flange of conchyolin-like material (FL) attached laterally to the edges of the valves. Anteriorly (Fig. 3C), this secretion forms an inverted V-structure linking the two valves. This fusion layer (Owen *et al.* 1953) is secreted by the fused outer lobes posterior to the mantle isthmus (Fig. 4, FO). At the posterior end of the mantle isthmus, the mantle edge (Fig. 3C, MP) secretes additional layers of horny material (OL) on the inner surface of the fusion layer. This horny material is the 'outer' layer of the ligament (Fig. 4B). Fig. 3D represents

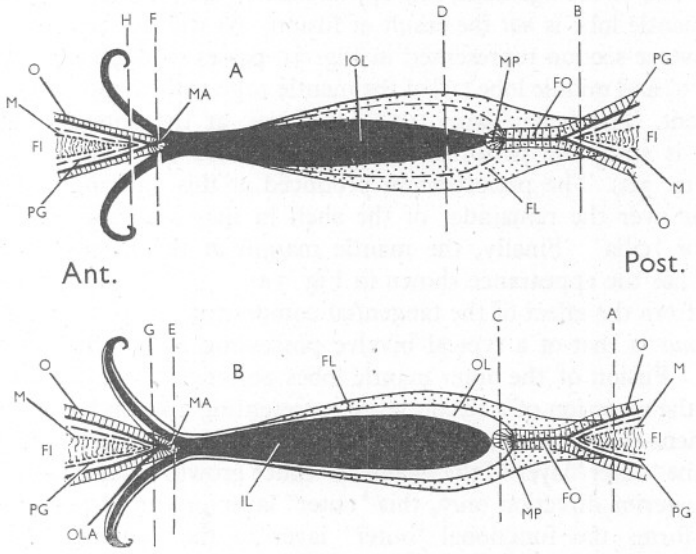


Fig. 4. *G. humanus*, diagrammatic representation of the ligament, A, viewed dorsally, B, horizontal longitudinal section. FO, fused outer lobes; IOL, 'outer' and inner layers of ligament; PG, periostracal groove. Other lettering as before. The letters A–H refer to the sections drawn in Fig. 3.

a section through the mantle isthmus (MI) and shows the bulky inner layer (IL) secreted by the epithelium of the isthmus and characteristic of the ligament of most lamellibranchs (Owen *et al.* 1953).

Although the sections shown in Fig. 3(A–D) were taken from different regions of the same specimen they could be interpreted equally well as having been taken, a constant distance posterior to the umbones, from different specimens at various stages of growth. Fig. 3A would then represent the youngest growth stage and Fig. 3D the oldest growth stage. During growth, the mantle isthmus extends posteriorly below the periostracum, fusion layer and 'outer' layer. In the region of the isthmus, the ligament therefore consists of a superficial periostracum, a fusion layer, an 'outer' layer and a bulky inner layer (Figs. 3D and 4B). The fusion layer becomes split in the mid-line above the posterior region of the inner layer (Fig. 4A).

The anterior region of the ligament in *G. humanus* is progressively split along the mid-line as growth proceeds. This split first appears in the inner layers but, as shown in Fig. 3E, soon extends to the outer layers of the ligament. A ridge of epithelium, representing the edge of the mantle anterior to the mantle isthmus (Fig. 4B, MA), secretes a layer of horny material (OLA) connecting the split portions of the 'outer' layer. Anteriorly (Fig. 3G), the periostracum (P) is present as a single thread while the middle lobe (M) is represented by a median ridge of tissue between the outer lobes (O). As at the posterior end of the ligament, this appearance of the periostracum and of the middle mantle lobe is *not* the result of fusion. As will be seen from Fig. 4B, the transverse section represented in Fig. 3G passes through the periostracal groove (PG) and middle lobe (M) of the mantle edge at the depth of the anterior embayment. The horny layer (OLA) produced at the anterior end of the ligament is split, the periostracum being attached laterally to the broken edges (Fig. 3G). The periostracum produced at this anterior region differs from that over the remainder of the shell in that it is not produced into bristles or 'cilia'. Finally, the mantle margin at the anterior end of the ligament has the appearance shown in Fig. 3A.

Apart from the effect of the tangential component upon it, the ligament in *G. humanus* is that of a typical bivalve possessing an external opisthodetic ligament. Fusion of the outer mantle lobes posterior to the mantle isthmus involves the secretion of a fusion layer representing a secondary extension of the ligament (Owen *et al.*, 1953). The mantle edge posterior to the isthmus secretes the 'outer' layer of the ligament. Since growth of the mantle isthmus is in a posterior direction only, this 'outer' layer produced posterior to the isthmus forms the functional 'outer' layer of the ligament. The horny material (OLA) or 'outer' layer secreted by the mantle edge (MA) anterior to the isthmus is progressively split as growth proceeds due to the effect of the tangential component on the mantle/shell margins. A fusion layer similar to that produced by the fused outer lobes posterior to the mantle isthmus is not produced anteriorly, there being no fusion of the outer marginal lobes in the anterior embayment of the mantle. As with the splitting of the anterior region of the ligament, this absence of fusion of the outer lobes is a result of the effect of the tangential component on the mantle margins.

The structure of the ligament in *G. humanus* is similar to that described by Trueman (1949, 1950) for *Tellina tenuis* and *Mytilus edulis*. The fusion layer is probably comparable to Trueman's layer 1*a*. In *Glossus humanus*, as in *Mytilus*, this layer becomes split in the mid-line above the posterior region of the inner layer of the ligament. There can be no doubt that the periostracum in *G. humanus* is continuous over the external surface of the ligament. Observation was aided by the presence of the characteristic bristles which serve to differentiate the periostracum from the underlying layers. Indeed, the only region of the shell not covered by periostracum appears to be the 'outer'

layer secreted by the mantle edge anterior to the isthmus. Over the posterior region of the ligament the periostracum is, however, often worn and only patches are left adhering to the outer surface.

Habit and Habitat

G. humanus has been described as an inhabitant of sand, sandy-mud and mud. The specimens from the Clyde Sea Area were found in very soft mud in company with such other typical mud-dwellers as *Brissopsis lyrifera*, *Nucula sulcata* and *Abra alba*. Occasional specimens of *Aporrhais serresiana*, a typical soft-mud dweller (Yonge, 1937) were also found. Colonies of *Perigonimus* sp. were often found growing on the shells of older specimens near the posterior margins of the valves. On one extremely large specimen obtained in an otter trawl from Loch Sween and measuring 11·0 cm long by 10·2 cm deep, there were a number of small anemones attached near the posterior margins of the valves.

The species is probably restricted to a soft muddy substratum since the inflated form of the shell and the relatively weak musculature of the foot would render it unfit for life in firmer substrates. *Glossus humanus* is a suspension feeder and in soft substrates there is danger of sinking beneath the surface with subsequent fouling of the short siphons. The light weight of the shell is probably associated with the habitat. It is interesting to compare *G. humanus* with the similar-sized bivalve *Arctica* (*Cyprina*) *islandica*, an inhabitant of coarser and firmer substrates of sand and sandy mud. In this species, the spiral angle is low and the shell much more compressed laterally. The shell is thick and heavy and the foot large and extremely powerful. Specimens of *Glossus humanus* and *Arctica islandica* were placed on sand in an aquarium tank. The specimens of *A. islandica* found little difficulty in burrowing rapidly into the sand until their siphons were flush with the surface. One specimen of *Glossus humanus*, after an interval of 12 hr., did succeed in covering approximately a third of the shell; the others remained more or less on the surface of the sand. The most critical feature restricting the animal to a soft type of substrate may well be the poor elastic properties of the hinge ligament. According to information received personally from E. R. Trueman, the opening thrust of the ligament of *Arctica islandica* is about eight times that of *Glossus humanus*. Occasional small specimens of *G. humanus* were found in the Clyde Sea Area in somewhat firmer muds than those off the coast of Arran but medium or large specimens were never obtained from these areas. This could be explained by the fact that the elastic properties of the ligament decrease with increase in size of the animal. In young specimens, cutting of the muscle attachments causes the valves to gape, but in older specimens the ligament appears to produce no opening thrust, the valves remaining closed when the muscles are cut.

Reports on vertical distribution are confused. Jeffreys (1876) records the fry and very young as ranging from depths of 50 to 1785 fathoms (91–3265 m.) in the North Atlantic and from 40 to 1456 fathoms (73–2663 m.) in the Mediterranean. Nicol (1951) points out that Jeffreys misidentified species of *Kelliella* for the young of *Glossus humanus* and that the latter probably range from 5 to 150 m. The specimens from the Clyde Sea Area were obtained from depths of more than 50 m.

The foot of *G. humanus* possesses a well-developed byssus gland and groove but threads were observed only in the younger specimens. The foot is capable of considerable extension (Fig. 5, F) but appears not to be much used in the larger forms. The siphons are short and surrounded by a common sheath of some ninety or more tentacles; the inhalant siphon is surrounded by a second inner sheath of approximately fifty tentacles. The siphons are flecked with reddish brown and opaque white areas, and their musculature appears to be more or less identical with those of *Aloidis* as described by Yonge (1946). The animal is extremely sensitive to any form of vibration. Specimens placed in a jar withdrew their siphons when a hand was placed lightly on the bench on which the jar stood. This sensitivity is further evidence that *Glossus humanus* normally inhabits quiet waters.

The Mantle

The mantle edge consists of the usual three lobes, the innermost being fused except in the regions of the pedal gape and the siphons (Fig. 5). The mantle is thin and delicate, the inner epithelium being ciliated and concerned with the elimination of waste material from the mantle cavity. The general trend of particles affected by this ciliation is from anterior to posterior toward the inhalant siphon. The main tract is from a region near the labial palps, ventrally and posteriorly to the region of fusion of the inner mantle lobes at the posterior end of the pedal gape (PG), and then posteriorly to the inhalant siphon (I). An interesting and apparently unique feature is that the pseudo-faeces do not collect in a single mass at the base of the inhalant siphon but accumulate laterally on the side walls of the siphon (PS).

Mucus is supplied by glands distributed among the cells of the inner epithelial layer of the mantle. In addition, there are, in the connective tissue immediately beneath the epithelial layer, numerous spaces filled with a granular material. These spaces extend in a broad band dorsal to the ventral ciliated tract of the mantle from a region near the anterior adductor muscle to the posteriorly situated siphons. The contents are discharged on to the inner mantle surface through narrow pores extending between the epithelial cells. The nature of this substance is somewhat obscure. It gives a positive reaction with Schiff's leucofuchsin after oxidation with periodic acid. This indicates a polysaccharide component. Staining for glycogen with full controls gives

negative results, as does the Toluidine Blue metachromatic reaction for chondroitin and mucoitin sulphuric acids. Alcian Blue 8GS (Steedman, 1950)—a mucin stain—fails to stain it. Nevertheless, it seems probably to be some form of mucin-like substance, using the term mucin in a physical sense.

Organs in the Mantle Cavity

Both anterior and posterior adductor muscles are well developed, the anterior being about half the size of the posterior (Fig. 5). The pedal muscles are delicate with the anterior somewhat stouter than the posterior (PR). The labial palps are well developed and function in the usual manner (LP).

The ctenidia are heterorhabdic and deeply plicate with the inner demibranchs somewhat deeper than the outer (Fig. 5). There is an extensive supra-axial region (SA) and both inner (ID) and outer (OD) demibranchs curve inward over the visceral mass. Cuticular fusion of the type described by Atkins (1937*b*) connects the inner lamellae of the inner demibranchs of the two ctenidia, the inner lamellae of the inner demibranchs and the visceral mass, and the outer lamellae of the outer demibranchs and the mantle. Where the visceral mass passes between the two inner demibranchs posteriorly, there is a short region where the inner lamellae are not fused to the visceral mass or to one another. As in *Aloidis* (Yonge, 1946), a direct connexion can be established between the inhalant chamber below and the exhalant chamber above.

As shown in Fig. 5, material is carried by the frontal cilia to the free edges on both sides of the inner demibranch, on the outer surface of the outer demibranch, and on the ventral half of the inner surface of the outer demibranch. A narrow marginal groove is present at the free edges of both the inner and outer demibranchs. The frontal cilia of the dorsal region of the inner lamella of the outer demibranch convey material to the ctenidial axis where there is a strong anteriorly directed current. Material from the inner marginal groove passes directly between the palps to the mouth while material from the outer marginal groove and the axis reaches the mouth by way of the distal oral groove.

As noted by Ridewood (1903), the plications of the gill are not evenly spaced but are arranged in pairs. The inter-lamellar septa are alternately high and low so that, in the dorsal regions of the demibranchs, one principal filament is associated with an inter-lamellar septa while the next is not. Lateral cilia are well developed and produce a powerful inhalant current. Large eu-latero-frontal cilia, 35 μ in length, guard the inter-filamentar spaces, and there are smaller pro-latero-frontal cilia between them and the frontal cilia. The ciliation is similar on all filaments including the principal filaments.

Despite the deep plication of the gills, the walls of the marginal grooves are not scalloped since the plicae, as in *Solecurtus*, *Cardium* and *Lutraria* (Atkins, 1937*a*), tend to smooth out toward the free margins. Examination of the free

margins of the gill under the microscope show what appear to be clear peg-like processes, some 10 μ long, projecting from the filaments on either side of the marginal groove. Staining with Heidenhain's haematoxylin indicates that they are made up of tufts of cilia comparable to the 'sensory' tufts of cilia occurring on the frontal surfaces of the filaments of the gills in a number of species (Atkins, 1936). In *Glossus humanus*, they are largely restricted to the free margins of the posterior regions of the gills.

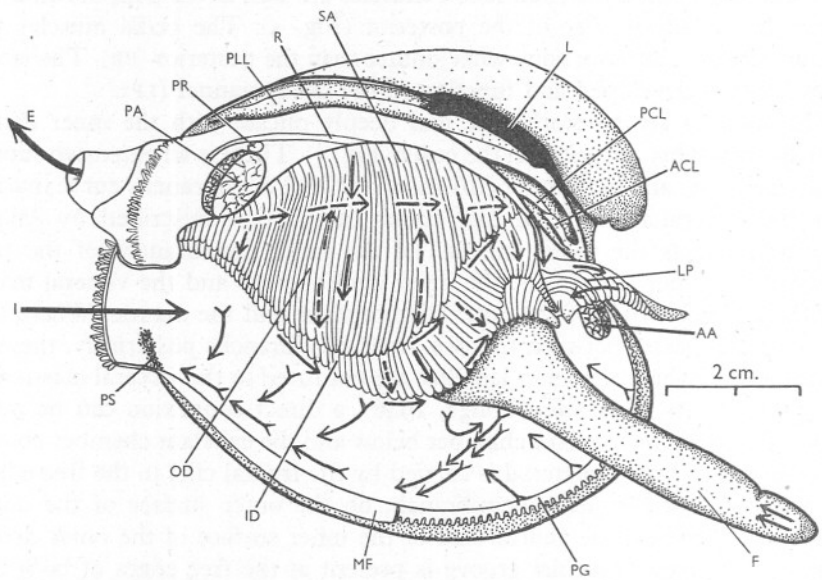


Fig. 5. *G. humanus*, organs of the mantle cavity exposed after removal of the right shell valve and mantle lobe. F, foot; ID, inner demibranch; LP, labial palp; MF, region of fusion of inner marginal lobes; OD, outer demibranch; PG, pedal gape; PLL, left posterior lateral tooth; PR, posterior retractor muscle; PS, pseudofaeces; R, rectum; SA, supra-axial region. Large arrows indicate inhalant (I) and exhalant (E) currents; small arrows, ciliary currents on exposed surfaces; broken arrows, currents on inner surfaces of demibranchs; feathered arrows, rejection currents. Other lettering as before.

Despite the muddy type of substrate, guard cilia (Atkins, 1937*a*) are not present at the edges of the marginal grooves. As in many lamellibranchs, coarse cirrus-like cilia, 40–50 μ long, occur along the free margins of both the inner and outer demibranchs. Similar cilia occur scattered over the frontal surfaces of the filaments and on the surface of the ctenidial axis. Those present on the filaments attain a length of some 70 μ while those of the axis reach the surprising length of 130 μ. Presumably, as suggested by Yonge (1946), they are concerned with the movement of large particles and masses.

Atkins (1937*a*) has emphasized the part played by the gills of most lamellibranchs in the sorting of material. The narrow marginal grooves in *G. humanus* ensure that only small particles are conveyed anteriorly toward the palps and mouth. Large particles and masses fail to enter the marginal grooves and soon

fall off the gill edge on to the rejectory tracts of the mantle surface. The paired arrangement of the plicae may also help in the rejection of material. Contraction of the gill musculature results in the apposition of adjacent plicae; presumably this would happen if the inhalant current contained a considerable amount of suspended matter. With the gill in this condition, particles removed from the water tend to concentrate on the almost flat portions of the lamellae between the pairs of closely approximated plicae; they travel ventrally toward the free edges. The presentation of these 'concentrated' masses of particles to the narrow marginal grooves increases the possibility of their rejection on to the ciliated surface of the mantle.

The gills of *G. humanus*, however, cannot be regarded as adapted for dealing with water containing large amounts of sediment. The deep plication may be considered primarily as a means of increasing the collecting surfaces of the ctenidia; it will also increase the power of the inhalant current. Thus, despite the muddy nature of the substrate to which the species is almost certainly restricted, the gills in *G. humanus* appear to be adapted for dealing with large volumes of water containing relatively small quantities of suspended material. Such conditions are likely to occur in water where there is little or no disturbance of the bottom deposits.

The Alimentary Canal

The mouth, situated between the paired palps, leads into the oesophagus which is compressed dorso-ventrally and richly supplied with mucous glands. It enters the antero-dorsal region of the stomach, which lies in the dorsal region of the visceral mass and is enclosed antero-laterally and ventrally by the digestive diverticula. Leaving the stomach posteriorly and ventrally is the combined style sac and mid-gut, partially separated from one another by the major and minor typhlosoles. The style sac is curved and extends ventrally to the junction of the foot and visceral mass. Within the visceral mass, and anterior to the style sac, the mid-gut is thrown into three loops. The hind-gut ascends dorsally, posterior to the style sac, to penetrate the pericardium and pass through the ventricle of the heart. A well-developed aortic bulb extends posteriorly from the ventricle to the posterior adductor muscle. The hind-gut lies within this aortic bulb attached to the mid-dorsal wall. Finally, the hind-gut passes over the posterior adductor to open at the anus near the exhalant siphon.

The Morphology of the Stomach

The external form of the stomach in *G. humanus* is shown in Fig. 6. Dissections were carried out on living specimens and the course of the ciliary currents followed by means of fine carborundum powder and carmine. No matter how carefully the stomach was opened, some part of the internal anatomy was invariably lost or distorted. The drawing of the stomach is

therefore the result of observations on a number of specimens, the stomachs having been opened by a variety of incisions (Fig. 7). As far as possible the nomenclature introduced by Graham (1949) has been used. It is proposed, however, as suggested by Purchon (1953), to describe the 'right half' and 'left half' of the caecum as the 'right caecum' and 'left caecum' respectively.

The stomach in *G. humanus* may be conveniently divided into three regions; a globular posterior region, an anterior receiving region, and a dorsal hood. The dorsal hood (Fig. 6, DH) extends anteriorly over the posterior part of the stomach and ends blindly on the left dorsal side as a small coiled tube

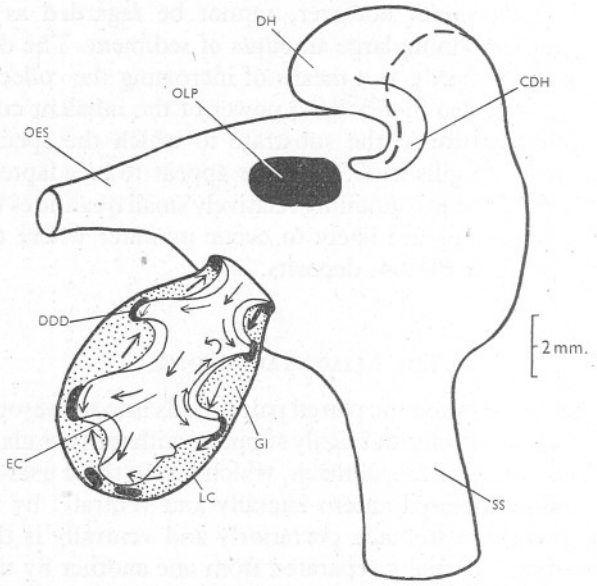


Fig. 6. *G. humanus*, diagram of the external appearance of the stomach. CDH, coiled region of dorsal hood; DDD, opening of duct from digestive diverticula; DH, dorsal hood; EC, extension of typhlosole within left caecum; GI, intestinal groove; LC, left caecum cut open; OES, oesophagus; OLP, opening of left pouch; SS, combined style sac and mid-gut. Arrows indicate ciliary currents within the left caecum.

(CDH). The greater part of the stomach consists of the posterior globular region (Fig. 7). Leaving this region ventrally is the combined style sac and mid-gut (SS). The minor typhlosole (T_2) ends at the junction of the style sac with the stomach while the major typhlosole (T_1) extends as a flap-like structure anteriorly across the floor of the stomach. As in the majority of Eulamellibranchia, it is continued into the right (ORC) and left (OLC) caeca (Graham, 1949) to form broad tongue-shaped structures (Fig. 6, EC). Continuous with the mid-gut and following the course of the major typhlosole is the intestinal groove (Fig. 7, GI). The posterior sorting area (SAP) is well developed, extending dorsally over the right wall of the stomach and on to the right wall of

the dorsal hood. The posterior margin of the sorting area is formed by a well-developed fold (PF) while the anterior margin is formed by the rejection groove (RG) which drains into the intestinal groove on the floor of the stomach. The anterior margin of the rejection groove is formed by a second fold bearing a number of small parallel ridges (AF).

The openings of the ducts to the digestive diverticula are to a considerable extent concentrated within the right and left caeca. The ducts of the right dorsal regions of the diverticula open independently on the right dorsal wall of the stomach (RD) while those of the left dorsal regions open into the left pouch. The pouch opens into the stomach by a single large aperture (OLP) near the gastric shield (GS). The gastric shield consists of a thickened convex portion between the apertures of the coiled region of the dorsal hood (OCH) and the left pouch (OLP) and a larger wing-like expansion extending over most of the left posterior wall of the stomach and the left ventral wall of the hood. A spur-like extension of the shield projects into each of the openings helping to maintain the shield firmly in position.

The anterior receiving region of the stomach is little more than an expansion of the oesophagus (OES). The entrance of the oesophagus into the stomach is marked by a circular groove (CG) which is continued posteriorly over the roof of the stomach (DG). This dorsal groove curves over the ventral lip of the hood, between the thickened portion of the gastric shield and the anterior fold, to terminate on the ventral floor of the hood (Fig. 8).

The posterior sorting area, together with the rejection groove and bounded by the anterior and posterior folds, extends on to the right wall of the dorsal hood (Fig. 8, SAP). The anterior fold curves round on to the antero-ventral face while the posterior fold forms a crest over the mid-dorsal line of the hood. Both anterior and posterior folds terminate within the coiled portion of the hood, the anterior in a curious circular protuberance. Extending over the roof of the hood to the left of the posterior fold is a powerfully ciliated acceptance tract which is continued ventrally over the posterior wall of the stomach (AT). Over the left wall of the hood and extending to the margin of the gastric shield is a series of poorly developed folds.

The Functioning of the Stomach

That in the Lamellibranchia both intracellular and extracellular digestion occur is generally agreed. The crystalline style is the source of certain extracellular enzymes which are liberated into the stomach by the dissolution of the style (Yonge, 1925). Intracellular digestion takes place within the cells of the digestive diverticula (Yonge, 1926*a*). With these facts it should be possible to estimate some of the more important mechanical requirements of the lamellibranch stomach. For the efficient utilization of the extracellular enzymes the ingested particles should be brought into intimate contact with the crystalline style. Secondly, for intracellular digestion, it is essential that

fine particles be presented to the digestive diverticula. This may be achieved either by an effective sorting mechanism capable of selecting suitable particles or by the ingested particles undergoing a process of trituration. Finally, the waste materials from the digestive diverticula, consisting of solid excretory products and rejected particles, should be prevented from returning to the gastric cavity.

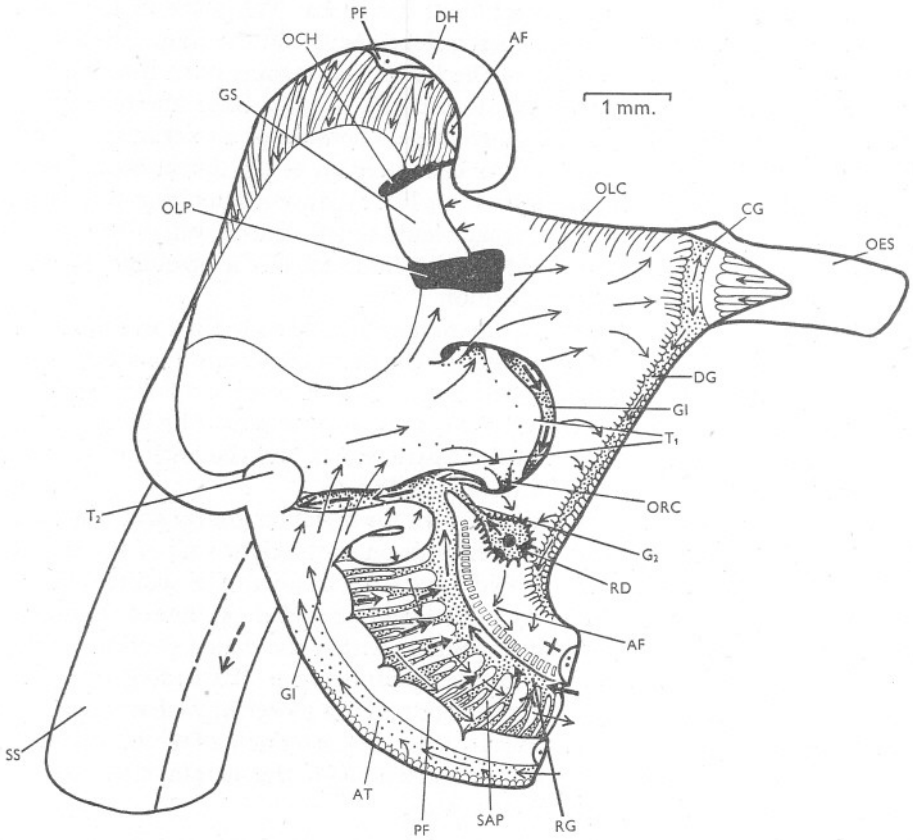


Fig. 7. *G. humanus*, stomach opened by a dorsal and posterior incision and the right wall deflected ventrally. AF, anterior fold; AT, acceptance tract; CG, circular groove; DG, dorsal groove; GS, gastric shield; G_2, rejection groove from isolated ducts of right side; OCH, opening of coiled region of dorsal hood; OLC, opening of left caecum; ORC, opening of right caecum; PF, posterior fold; RD, opening of isolated ducts of right side; RG, rejection groove; SAP, posterior sorting area; T_1, major typhlosole; T_2, minor typhlosole. Other lettering as before. The arrows indicate the direction of ciliary currents.

To understand the functioning of the stomach it is necessary to realize that the crystalline style rotates. Unfortunately the style has not been observed revolving in *G. humanus*, but investigation of the cilia of the style sac suggests that it revolves in a clockwise direction when viewed from above. This is

supported by the observations of workers who have viewed the rotation of the style in other species; Nelson (1918) in *Modiolus* and *Anodonta*; Yonge (1926*b*) in the spat of *Ostrea edulis*; Yonge (1949) in *Tellina tenuis*; Yonge (1951*a*) in *Sphenia binghami*; Yonge (1951*b*) in *Cryptomya californica*. The statement by Yonge (1951*b*) that the style in *Cryptomya* rotated in an anti-clockwise direction when viewed from the anterior end was printed in error (personal communication).

In *Glossus humanus* particles entering the stomach from the oesophagus are conveyed dorsally by the cilia of the circular groove and then directed posteriorly by the cilia of the dorsal groove (Fig. 7). At the ventral lip of the dorsal hood (the region marked x) the particles are directed against the ridges of the anterior fold (AF). Here, at least when the stomach has been opened for observation, the particles embedded in mucus collect and remain stationary. In the intact stomach the revolving crystalline style may reasonably be presumed to brush these particles on to the ridged surface of the posterior sorting area (SAP). The ciliary mechanisms of the grooves and ridges of this area exercise a quantitative selection. The heavier particles are carried by the cilia of the grooves to the rejection groove (RG) and so to the mid-gut by way of the intestinal groove (GI). The lighter particles are carried dorsally, being thrown from crest to crest across the ridges. Thus, only the finest particles are retained and reach the distal regions of the dorsal hood (Fig. 8). Within the dorsal hood they are directed to the edge of the gastric shield and included in the revolving mass at the head of the crystalline style. The style is relatively delicate and almost certainly incapable of causing any effective trituration of particles.

The acceptance tract (AT) conveys particles from the head of the crystalline style over the roof of the dorsal hood and ventrally over the posterior wall of the stomach (Fig. 7). Where this current has been described in previous accounts it has generally been considered as supplying particles to the mid-gut. In *G. humanus*, however, this current serves to carry particles on to the floor of the stomach. The entrance of particles into the mid-gut is prevented by the flap-like structure of the major typhlosole which extends over the intestinal groove. The currents on the floor of the stomach are directed towards the openings of the left pouch (OLP), the left (OLC) and right (ORC) caeca and the isolated ducts of the right side (RD). Within the right and left caeca the ciliary currents over the extensions of the major typhlosole direct particles towards the openings of the ducts into the digestive diverticula (Fig. 6, DDD).

The material carried by the acceptance tract should therefore consist of fine particles which have previously been in contact with the crystalline style. The pattern of ciliary activity within the dorsal hood is shown in Fig. 8. It was at first thought that the poorly developed folds over the left wall of the hood might constitute a sorting area. Detailed investigation of the ciliary currents did not reveal any selective action. The currents over the

ventral regions of the folds direct particles to the edge of the gastric shield while those of the dorsal region direct particles to the acceptance tract. An understanding of the function of these currents can obviously only be obtained through an appreciation of their dynamic relationships with the revolving crystalline style. Fig. 9 shows a simplified diagrammatic representation of the circulation of material within the stomach. The material

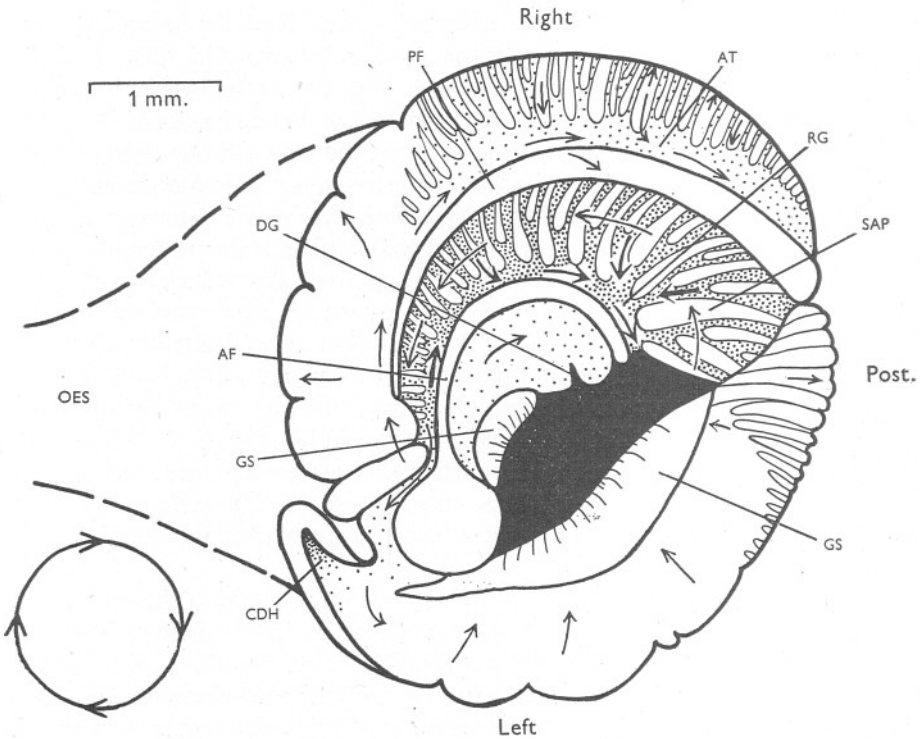


Fig. 8. *G. humanus*, dorsal hood of stomach opened along the broken line shown in Fig. 6 and viewed dorsally. Lettering as before. Arrows indicate the direction of ciliary currents; the circular arrow indicates the clockwise direction of rotation of the crystalline style when viewed dorsally.

entering from the oesophagus (OES) consists of a mixture of fine and coarse particles. These particles are caught up by the revolving crystalline style (CS) and brushed on to the posterior sorting area. The ciliary currents of the sorting area direct the coarse particles to the mid-gut (MG) by way of the rejection groove (RG) and the intestinal groove (GI). The fine particles are conveyed dorsally. Thus the material being revolved by the crystalline style consists of fine particles at the head of the style passing into a mixture of fine and coarse particles below. The ciliary currents over the dorsal regions of the folds within the dorsal hood therefore convey fine particles from the

head of the style to the acceptance tract (AT). The currents over the ventral regions of the folds act on a mixture of fine and coarse particles directing them to the edge of the gastric shield and so returning them to the mass around the crystalline style. Some coarse particles may be caught up in the ciliary currents of the acceptance tract as it passes ventrally over the posterior wall of the stomach, although the tract is 'protected' to some extent by the presence of the posterior fold along the right-hand margin. Particles too large to enter the openings of the left pouch, the right and left caeca, and the isolated ducts of the right side are carried anteriorly to join the incoming material and so circulate again. Within the right and left caeca waste products and rejected particles from the ducts of the diverticula are caught up by the ciliary currents in the intestinal groove (GI) and conveyed to the mid-gut. Rejected material from the isolated ducts of the right side is conveyed to the intestinal groove by way of the short groove G_2 (Fig. 7). The ducts of the diverticula which open within the left pouch return material to the general circulation within the stomach.

Most of the mechanical requirements mentioned earlier are fulfilled in the stomach of *G. humanus*. The ingested material is subjected to a rigorous sorting which appears to be entirely quantitative and the 'accepted' particles are brought into intimate contact with the style and so with the enzymes released from it. The flap-like major typhlosole isolates the currents in the intestinal groove. This agrees with the description by Graham (1949) of the intestinal groove as the 'private pathway of the digestive gland and sorting areas'. In the Eulamellibranchia there appears to be a progressive concentration of the ducts of the diverticula within the right and left caeca. This isolation of the ducts within the caeca may be associated with preventing material rejected by the diverticula from returning to the stomach. Even so, in the majority of the eulamellibranchs, the ducts opening into the left pouch return their waste products to the general circulation within the stomach. This retention of an isolated opening for the left pouch may well be correlated with the need for a firm fixation of the gastric shield. In most species the shield is anchored firmly by the spurs which project into the openings of the left pouch and the coiled region of the dorsal hood.

The stomach in *G. humanus* is well adapted for dealing rapidly with fine particles and is comparable with the stomachs of the suspension-feeders, *Paphia pullastra*, *Cardium edule* and *Venus fasciata*, as described by Graham (1949). Yonge (1949) has already discussed the reasons for not including *Tellina crassa* and *Solecurtus chamosolen* with the above. In both these species of the Tellinacea very coarse particles are taken into the stomach and the style acts as an efficient organ of trituration. Here the possession of an extensive sorting area would be a disadvantage. In fact, in *Tellina* and *Solecurtus*, the grooves and ridges of this region are very poorly developed or even absent.

The efficiency of the stomach mechanism may be judged by a consideration of the paths by which particles are able to enter the mid-gut. The material carried by the intestinal groove is derived from the rejection groove of the posterior sorting area and from the rejectory currents of the digestive diverticula. The rejection groove eliminates those particles rejected by the sorting area. All other particles remain within the gastric cavity until they have been

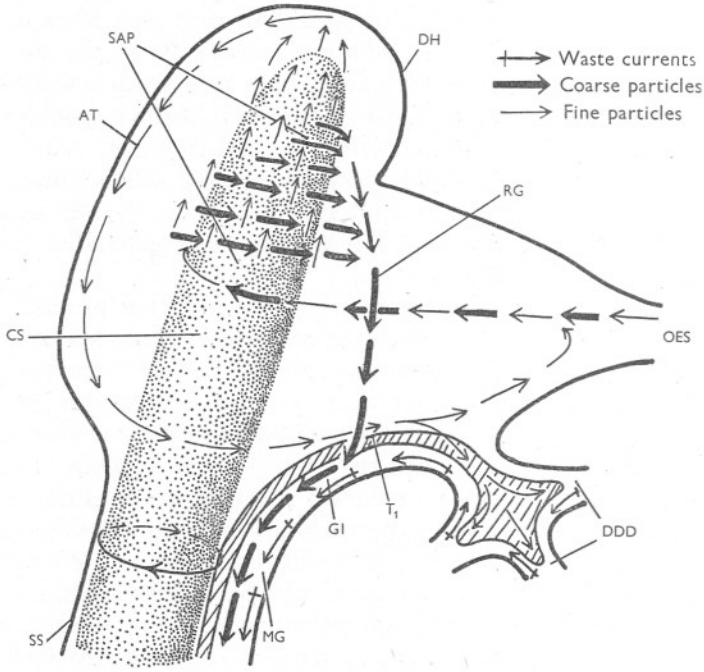


Fig. 9. *G. humanus*, diagrammatic representation of the circulation of particles within the stomach. CS, crystalline style; MG, mid-gut; SS, style sac. Other lettering as before. The heavy arrows indicate coarse particles, the light arrows fine particles, and the tailed arrows particles rejected by the ducts from the digestive diverticula.

'presented' either to the ducts of the digestive diverticula within the caeca or to the isolated ducts of the right side. This retention of particles within the stomach is possible because the currents in the intestinal groove are isolated by the flap-like major typhlosole.

Reproduction

Glossus humanus is unisexual and both the testes and ovaries are white when ripe. The gonads ripen towards the end of August and during September. Artificial fertilization was attempted on 3 September with some success, a small number of apparently normal trochophore larvae being obtained.

There was little doubt, however, that at this time the ovaries were not fully ripe. Thus, as in *Aloidis* and *Cuspidaria* (Yonge, 1946), spawning appears to occur at the end of September. Yonge has suggested that this autumnal spawning may indicate origin in deep water where the highest temperatures occur late in the season.

Discussion

Glossus humanus is specialized for life in a substratum of very soft mud. The poor elastic properties of the ligament, the inflated form of the shell, and the relatively weak musculature, all suggest a sedentary mode of life within a soft substratum. The siphons are extremely sensitive, and it is unlikely that large quantities of material are normally taken into the pallial cavity. The marginal grooves of the gills are very narrow and convey only fine particles to the labial palps and mouth. These adaptations indicate that *G. humanus* is normally an inhabitant of fairly deep water where there is little or no disturbance of the bottom deposits. Under such conditions the surrounding water will contain little suspended material. If the animal is to obtain sufficient food large volumes of water must be passed through the gills. Their well-developed plications must be considered primarily as an adaptation for dealing with large volumes of water containing little suspended material. This restriction of the species to deep water is further supported by the late autumnal spawning.

In *G. humanus* the mechanical functions of the stomach appear to be carried out by ciliary activity aided by the revolving crystalline style. In other Eulamellibranchia, however, the entry of particles into the ducts to the digestive diverticula appears to take place by some means other than cilia (Purchon, 1953). This may also occur to a lesser extent, in *G. humanus*. The part played by muscular activity in the functioning of the eulamellibranch stomach has, on the whole, been neglected. Patterson (1933) introduced a rubber balloon into the stomach of *Schizothoerus nuttallii* and so recorded pressure changes there. Slight stomach contractions occurred at the rate of one per minute regardless of the presence or absence of the crystalline style. While the experiment needs to be repeated, it is certainly true that slight changes in pressure within the stomach would aid the entry of particles into the ducts of the diverticula. The particles affected by such small changes in pressure would be those carried anteriorly over the floor of the stomach past the openings of the right and left caeca and the left pouch. These would be the fine particles from the head of the crystalline style which are carried ventrally over the posterior wall of the stomach by the acceptance tract.

Recently there has been much discussion regarding digestion in the Lamellibranchia. Mansour & Zaki (1947) claimed that lipolytic and proteolytic enzymes are secreted into the lumen of the stomach by the digestive diverticula. They suggested that the cells of the diverticula fragment and pass, together with their contained enzymes, into the stomach. George (1952),

investigating the digestion of fat in lamellibranchs, found that free droplets of neutral fat were hydrolysed in the stomach. Tests on the lipolytic activity of minced styles of several species all gave positive results, while only some species gave positive results in tests on minced digestive diverticula. Nevertheless, it is difficult to associate complete extracellular digestion with the organization of the stomach found in *Glossus humanus*. Graham (1949) discussed the functioning of the gastropod stomach and indicated the evolutionary trends associated with increased extracellular digestion and the secretory activity of the digestive gland in members of this class. In carnivorous species, such as members of the Stenoglossa, the stomach is little more than a bag where digestion takes place. There is no necessity for a separation of particles on a size basis and sorting areas are not present. The same conditions prevail in many of the pulmonates and opisthobranchs in which extracellular digestion predominates. This directly contrasts with conditions in the Eulamellibranchia, as exemplified by *G. humanus*, where there is an efficient sorting mechanism selecting particles on the basis of size.

The ciliary currents of the intestinal groove convey to the mid-gut particles discharged from the ducts of the diverticula within the right and left caeca. The flap-like major typhlosole prevents material carried by the intestinal groove from returning to the general circulation within the stomach. In contrast to Mansour & Zaki (1947), Coe (1947) suggested that the fragmented cells and minute brownish globules passed into the digestive canal from the diverticula were excretory. Morton (1951), investigating the gastropod family Struthiolariidae, came to the same conclusion. In members of this family fragmentation of the digestive cells results in tiny boluses of excretory matter containing nuclei, phagocytic cells and brown excretory spherules being passed into the gut. Morton emphasized that, although small traces of enzyme were doubtless liberated in this way into the stomach, the fragmented particles were essentially excreta.

Knowledge regarding digestion in the Lamellibranchia is confusing. There seems to be little doubt that extracellular lipases and proteases are present in the gastric cavity, although their source is uncertain. At the same time the complex ciliary mechanisms suggest a considerable degree of intracellular digestion as postulated by Yonge (1926*a*).

Summary

Glossus humanus (L.) (*Isocardia cor* Lam.), a large marine eulamellibranch, occurs in deep water in soft muddy substrates. Restriction of the species to a soft substrate is probably correlated with the inflated form of the shell, the weak musculature of the foot, and the poor elastic properties of the ligament.

Growth of the mantle/shell is the resultant of radial, transverse and tangential components, the normal axis being a turbinate spiral. The anterior

region of the ligament is progressively split during growth owing to the displacement posteriorly of the secreting surfaces. The relationships of the valves, ligament and mantle isthmus are discussed.

The deeply plicated ctenidia are specialized for dealing with large volumes of water containing little suspended material.

The anatomy and functioning of the stomach is described in detail. There is a complex ciliated mechanism selecting particles on the basis of size. Such a mechanism, it is suggested, must be associated with intracellular digestion. The flap-like major typhlosole prevents material carried by the intestinal groove from returning to the circulation within the stomach, while the retention of an isolated opening for the left pouch is probably correlated with the need for firm fixation of the gastric shield.

Spawning occurs at the end of September.

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THE GROWTH OF *BALANUS BALANOIDES* (L.) AND *B. CRENATUS* BRUG. UNDER VARYING CONDITIONS OF SUBMERSION

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(Text-figs. 1–7)

There is some information in the literature on the growth rate of *Balanus balanoides* (L.) under varying natural conditions, but very few comparable data on *B. crenatus* Brug. exist. Most of this work relates to natural populations where crowding and other factors make accurate measurement difficult. In general, samples of a population, sometimes of unknown or only estimated age-distribution, have been measured at intervals. In the work reported below, the growth of numerous individual barnacles of known age, exposed to both intertidal conditions and complete submersion, has been followed through several successive seasons at Millport. In addition, some observations are presented on the effect of tidal level on the intensity of settlement and subsequent mortality of *B. balanoides*.

The Experimental Methods

For the intertidal exposures (*B. balanoides* only), seven 15 × 10 in., rough, black, plastic panels were bolted on to a long metal frame which was lashed in a fixed vertical position to the outer face of a pier pile, the position of each panel in relation to Chart Datum being calculated from known Ordnance Datum. The level of the centre of each panel is shown in Table I.

Observations during periods of calm weather at the site have shown that, in general, there is a closer agreement between the heights actually reached and those predicted for Greenock from new data in the *Admiralty Tide Tables for 1952* (1951), than with the values obtained by applying the corrections given for Millport. Mean tidal levels at the site, relative to Chart Datum, are therefore similar to those for Greenock, which are as follows: M.H.W.S., +11·1 ft.; M.H.W.N., +9·3 ft.; M.T.L., +6·2 ft.; M.L.W.N., +3·2 ft.; M.L.W.S., +1·1 ft. The hours of exposure and submergence at the centre of each panel over the actual period of settlement (also shown in Table I), and over a typical full lunar period (data for Figure 4), were calculated from graphs of the tidal cycle drawn up from data for Greenock in the *Admiralty Tide Tables for 1951* (1950), with a correction based on the new mean levels given in the 1952 tables. Anemometer records near the site show that wind speeds during the

period of settlement were low and therefore the effect of wind on tidal levels has been neglected.

For the permanently submerged settlement (including both *B. balanoides* and *B. crenatus*), plain and ground glass slides of several sizes were exposed in a vertical position at a constant depth of 3 ft. from a nearby raft, a description of which has been given by Pyefinch (1948).

TABLE I. SETTLEMENT OF *BALANUS BALANOIDES* ON INTERTIDAL PANELS 6–26 APRIL 1951

The levels refer to the centres of panels and are given in feet relative to Chart Datum; the number of cyprids refer to the upper, middle and lower thirds of each panel, together with total numbers; the tides across are the number of times the water surface passed the centre of the panel each way during the settling period; exposure and submergence in hours and percentage of total (see text).

Panel No.	Level	Cyprids		Tides across	Exposure	Submergence
I	+10·8	0	0	34	460	30 (6%)
II	+8·9	50 154 159	363	76	368	122 (25%)
III	+7·2	1077 670 565	2312	80	283	207 (42%)
IV	+5·4	2509 1077 564	4150	80	212	278 (57%)
V	+3·6	425 254 38	717	74	132	358 (73%)
VI	+1·8	1837 555 148	2540	46	47	443 (90%)
VII	0·0	806 174 169	1149	0	0	490 (100%)

On all panels, after a quantity of spat had settled, the positions of a number (20–40 per set) of selected barnacles, uniformly distributed over the panels, were marked and these barnacles were thereafter measured at intervals. When in the laboratory for measurements, the panels and slides were kept in running sea water except while actually being measured and, with very few exceptions, were returned to the exposure sites within 24 hr. At each inspection the panels and slides, and with the older barnacles the surfaces of the barnacles themselves, were cleaned of debris, algae and settled animals.

Three dimensions were measured: the rostro-carinal axis (termed length), the greatest diameter at right angles to the length (termed breadth), and the perpendicular height of the tallest compartment (usually the carina) above the basis. The third dimension was measured on only a limited number of inspections, but the first two were measured throughout the observations. In the early stages of growth on both the large intertidal panels and the small submerged slides the lengths and breadths of the barnacles were measured

by means of a binocular microscope with low-power objective and an eye-piece micrometer. In the later stages on the submerged slides a vernier-measuring microscope was used. Heights of the submerged barnacles were measured throughout with a micrometer screw-gauge, allowance being made for the thickness of the slide. The use of small slides greatly facilitated accurate measurement of the submerged barnacles, but led in later stages of growth to the necessary sacrifice of some individuals which had to be removed to prevent overcrowding and consequent distortion of growth. With the intertidal barnacles, heights, and at the later inspections length and breadth also, had to be measured as accurately as possible with a pair of fine pointed dividers, because of the large size of the panels.

The observations on the submerged barnacles extended continuously from April 1950, and on the intertidal barnacles from April 1951, to October 1952, readings being taken monthly during periods of rapid growth.

In most studies of barnacles increase in length of the rostro-carinal axis has been used as a measure of growth (see p. 120). Moore (1934), however, measured length, breadth and height and computed the volume of an equivalent cone of basal diameter equal to the mean of the length and breadth, and with the measured height. As Moore points out, the increase in size of the whole animal is obviously better measured by volume changes, but since, as will be shown, the shape does not change greatly with age, for many purposes the length may be conveniently used. However, the fact that length has been used must be borne in mind; the volume will approximate to a function of the cube of the length. The advantages of using the length are that it does not involve any assumptions about length/volume relationship, and that the present results are more readily compared with those of other workers. Further, it is a convenient method for field work since the length can be measured quickly and with reasonable accuracy.

Settlement and Survival on the Intertidal Panels

In 1951 the first settlement of *B. balanoides* was observed on the pier piles and on the shore on 10 April, and settlement continued during the remainder of this month. The panels were exposed throughout this period, during which several inspections were made. At none of the inspections were any cyprids found on the topmost panel (Panel I, level + 10·8 ft.), although settlements took place on all of the remaining panels (II–VII). Before marking the selected barnacles on 27 April the distribution of *all* the cyprids and young barnacles on each panel was ascertained. In Table I (p. 108) the distributions (dividing the panels into upper, middle and lower thirds) are shown. With the exception of Panel V (level + 3·6 ft.), the total settlement is much less on Panel II than on the remainder; that on Panel V is apparently anomalous, and the only explanation that can be put forward is that it is

the result of different local conditions in the immediate vicinity of this panel brought about by a diagonal pier beam immediately behind the frame.

The numbers actually counted on any panel will represent the net result of cyprid settlement and mortality. It might be thought that cyprid settlement would increase with total submergence, and that the number of cyprids settling would be greater as the depth of panels increased. This is clearly at variance with the results (see Table I), and it seems difficult to find any neutralizing effect by which mortality could produce the observed net results in relation to panel depth. Exposure (to air), which might be expected to be the major factor responsible for cyprid mortality, since it is inversely related to submergence, clearly cannot produce the observed net result. The following theory is put forward to explain the observed distribution. Although it is known that *B. balanoides* will settle on a permanently submerged surface (indeed, quite good settlements are recorded here), under natural conditions it is usually an intertidal species and undoubtedly the greatest settlement takes place within the littoral zone. It is postulated that intertidal settlement takes place most readily from a thin layer of water, such as is normally obtained when water moves forwards and backwards over a solid surface. On vertically exposed panels this occurs while the tide is rising and falling past the panel. During this process the surface of the panel is continually being swept by films of water that drain away. The 'clinging reaction' of *B. balanoides* cyprids described by Pyefinch (1948) (in contrast to *B. crenatus*, which is mainly a sublittoral species) lends support to this hypothesis. On this basis, the important factor for settlement is the number of times the tide *crosses* the surface of the panel; this will be maximal in the centre of the tidal range, and less at the upper and lower levels (see Table I), and would lead to maximal settlement in the mid-tidal region. The major factor opposed to that producing settlement is considered to be mortality of cyprids caused by exposure to air, and the hours of exposure at the various levels during the period under consideration are shown in Table I. The low numbers on Panel II are therefore largely the result of high mortality consequent upon the very considerable exposure to air. The differences on Panels III and IV are due to the decreasing exposure factor, and the lower values on Panels VI and VII are due largely to the less frequent crossing of the panel by the tide, since the exposure factor and consequent mortality is here greatly reduced. The actual relation between exposure to air and mortality over the settling period is not quantitatively known, but mortality of cyprids and young barnacles is known to have been very high on Panel II. However, it is quite clear from the figures in Table I that the opposition of these two factors can produce a maximal net settlement about the level of mid-tide, in accordance with the observed results. A similar distribution of the settlements of *B. balanoides* over the littoral zone has been observed by other workers (see, for example, Hatton & Fischer-Piette, 1932; Moore, 1935*b*; Hatton, 1938). An extension

of this hypothesis would also seem capable of explaining in part the well-known fact that *B. balanoides* tends to settle in greater numbers in situations with considerable exposure to wave action. In such places, increased surge and breaking waves will repeatedly spread a thin layer of water over a larger area than in sheltered situations.

The complete lack of survival on Panel I must be due to the extremely high exposure factor; but at this level on the adjacent pier pile, which is just above the upper limit of *B. balanoides* in quantity (10·2 ft.), a very slight permanent new settlement was observed, and this is ascribed to the rougher surface texture of the wooden piles. Such a surface affords a better key for settlement (see Barnes, Crisp & Powell, 1951), and also is less easily desiccated (see Pomerat & Reiner, 1942; Pomerat & Weiss, 1946; Barnes & Crisp, 1953).

A further noticeable feature of the settlement, with the exception of the high-level Panel II, is the distinctly greater settlement on the upper third of each panel (see Table I). This can readily be explained in terms of a light response, since it has been shown (Barnes *et al.* 1951) that the crawling cyprids react positively to light, and this accumulation could therefore be the result of the general movement of the cyprids up the vertical panels. Further, the accumulation is most marked on the lower panels, and perhaps may be related to the total submersion time, longer periods of submersion allowing more time for migration.

Mortality of Marked Barnacles

Of the forty-seven marked barnacles uniformly distributed on Panel II (situated at about M.H.W.N. level) all except ten were dead after 1 month's exposure. Only four survived 2 months' exposure and these continued to grow slowly (see below) until late summer. They were all absent by October and there was no evidence of any accident or predation; so that, although this is below the level at which barnacles occur in quantity on the adjacent pier piles, conditions on the smooth panel during the higher summer air temperatures were apparently too severe for survival.

On the remaining panels all the marked barnacles, except those deliberately removed to prevent overcrowding, survived for at least 1 year. It should be remembered that all panels were kept free of such predators as *Nucella lapillus* (L.) and *Asterias rubens* L. However, in July of the second season many were dead, probably as a result of infection by the parasitic isopod *Hemioniscus balani* (Spence Bate). The resultant mortality was 56% on the uppermost panel at this time (Panel III) and 20% on the lower panels (IV–VII), and these figures indicate that this parasitism may here not only cause considerable mortality of adult barnacles, but also exert a differential effect with respect to tidal level. Prenant (1923) records that approximately 20% (which he regards as a minimum) of *Balanus balanoides* growing on rock masses near Roscoff were parasitized by *Hemioniscus balani*.

Seasonal Growth and Specific Growth Rates

Balanus crenatus *on Submerged Panels*

Pyefinch (1948) has pointed out that, although the major liberation of nauplii and subsequent settlement of cyprids takes place in the spring in this area, ripe larvae are present in a proportion of the adults (although in diminishing numbers) throughout the summer; periodic outbursts of cyprid larvae are found in the plankton throughout the summer and early autumn. In 1950, when the present observations were begun, quite considerable settlements took place during the late summer and autumn—as late as October.

In all, the growth of seven distinct settlements was followed throughout three successive seasons. For comparison of growth each settlement (Series I–VII) has been considered separately, and the mean length and standard error calculated for each Series at each date of observation; the mean lengths are plotted against age in Fig. 1.

It is clear from the figure that rapid growth occurs after metamorphosis, and increase in length continues during the spring and summer months. In the animals settling from mid-April to mid-June (Series I–IV, termed Group I) growth continued until mid-September, by which time the mean length of the earliest settlers (Series I) had reached 21·7 mm. and this was only a few millimetres less than the maximum mean length attained over three seasons; the later Series IV of this Group had reached a mean length of 17·7 mm. by mid-September of the first season. In none of this Group was any significant growth recorded after mid-September, that is during the first autumn and winter. The second season's growth did not begin until the latter half of May 1951, a month later than the time at which rapid growth began in the previous season. In the second season the same general growth pattern was repeated in Group I but with greatly reduced intensity, growth taking place during the summer and ceasing in the autumn. The mean increase in length, however, was very small during this second season, not exceeding 4–5 mm.: clearly Group I individuals had almost reached their maximum size in the first season's growth.

Series V–VII (Group II) settled during the summer and autumn of 1950. Growth followed settlement as with the spring spat but, in marked contrast, increase in length continued much longer, some being recorded in late autumn and winter (October to December). Indeed Group I had virtually ceased to grow before the settlements of Series VI and VII occurred. Further, in sharp contrast to Group I, the latest settled individuals (Series VII) were showing some growth between January and February of the following season, more growth from February to March and rapid growth, comparable with the initial growth of the earliest settlements of the previous year, from March to April. Growth continued until early autumn of the second year, when the barnacles appeared to have reached their maximum size, which was com-

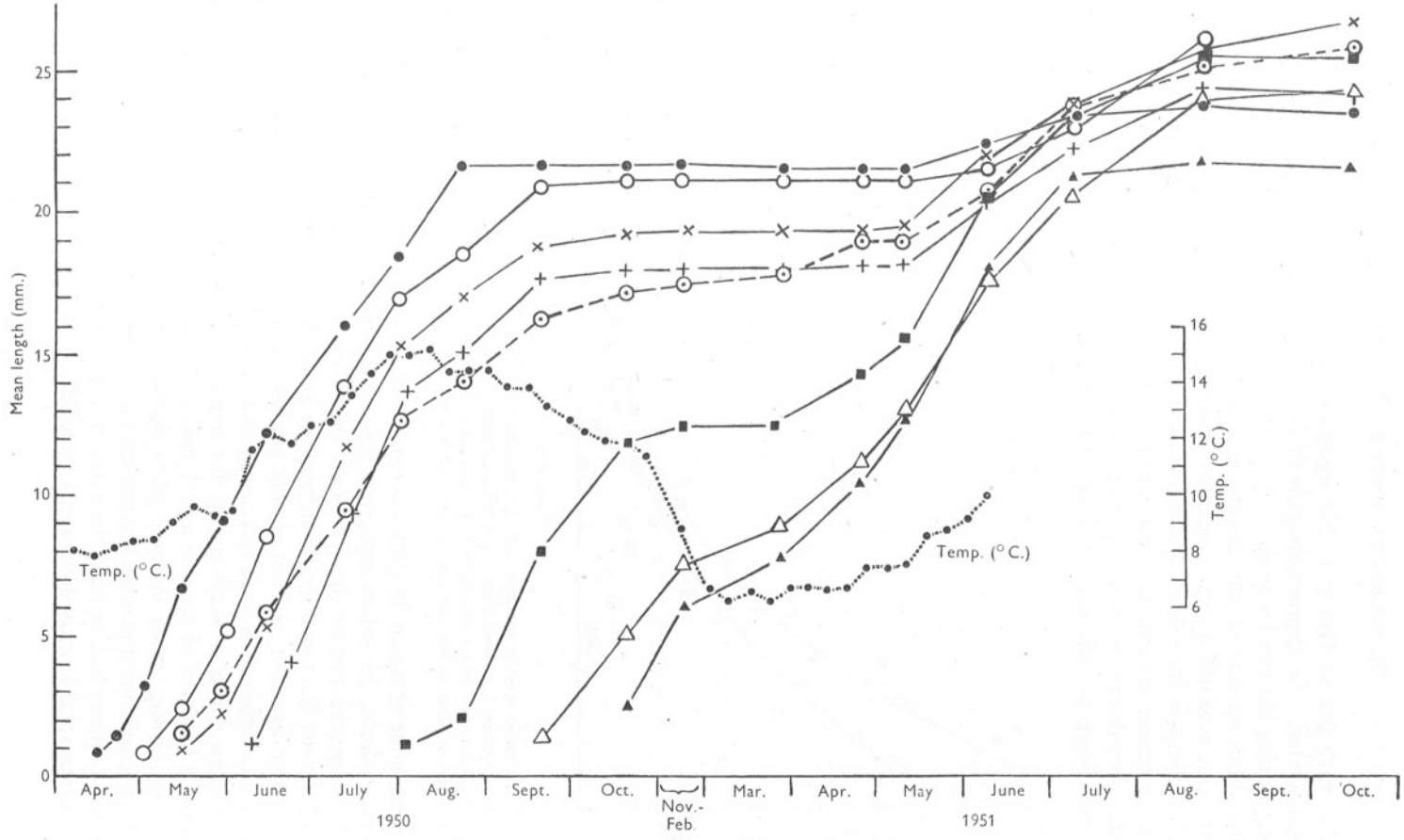

Fig. 1. Growth of *Balanus crenatus* and of *B. balanoides* over several seasons under conditions of total submersion. The mean lengths of each settlement (Series I–VII) at each date of inspection are shown: the almost negligible growth in the third season is omitted. The weekly mean sea-water temperatures (° C.) are also shown. *B. crenatus*: Series I, ●; II, ○; III, ×; IV, +; V, ■; VI, △; VII, ▲; *B. balanoides*, ⊙.

parable with that of Group I. No significant growth took place during the second winter. No appreciable growth (< 1·0–2·0 mm.) occurred in either Group during the third season.

The above account of the seasonal growth has been based on increases in length, and a similar picture emerges if the breadth is used. However, in order to compare the relative growth at different times, the size of the animal at the particular time under consideration must be taken into account. The specific growth-rate is used for such comparisons. The increase in length per unit length per day has therefore been calculated for each individual and

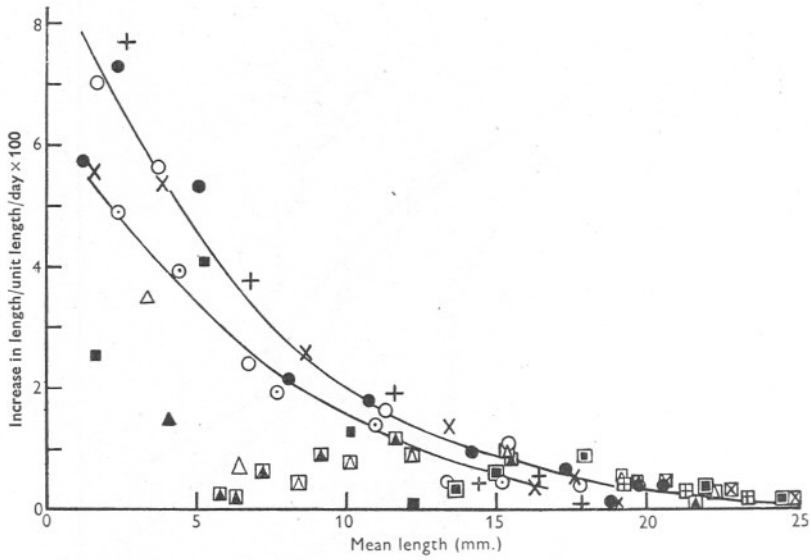

Fig. 2. The mean specific growth-rate (i.e. increase in length per unit length per day) × 100, plotted against the mean length, for all settlements of *B. crenatus* and a single settlement of *B. balanoides* under conditions of constant submersion. Symbols as in Fig. 1; those enclosed in squares are records for second season.

the mean value obtained for each interval for all Series. (If multiplied by 100, for convenience, the values represent percentage increase in length per day.) All the values, except for those periods when there was no increase in length, plotted against the mean length during the interval concerned, are shown in Fig. 2. The intervals are not always exactly the same. The mean specific growth-rate diminished with increasing size. The points for Group I all fall on a smooth curve, indicating that the mean specific growth-rate over this period is a function of the size and is independent of the time of settlement within the group, since in these plots the ages are not considered. Food supply, temperature and other environmental factors which varied throughout the season appear not to have been overriding factors. However, the values of the mean specific growth-rate of Group II late in the season are less than

the corresponding values for Group I at the same length in the spring and early summer, suggesting that environmental factors were effective in limiting growth late in the season (see p. 124). Further, in the second season the mean specific growth-rates of Group II in the very early spring are less than those of Group I at the same size, growing rather later in the previous season. Indeed, during the latter part of the spring of the second season's growth, the mean specific growth-rate of Group II actually rises with an increase in mean length.

Balanus balanoides *on Intertidal and Submerged Panels*

In contrast to *B. crenatus*, there is only one liberation of nauplii during the year, and the settlement of cyprids usually takes place over the latter part of March and throughout April. There is therefore nothing comparable with the seven Series of *B. crenatus*, but only observations on the growth of a single

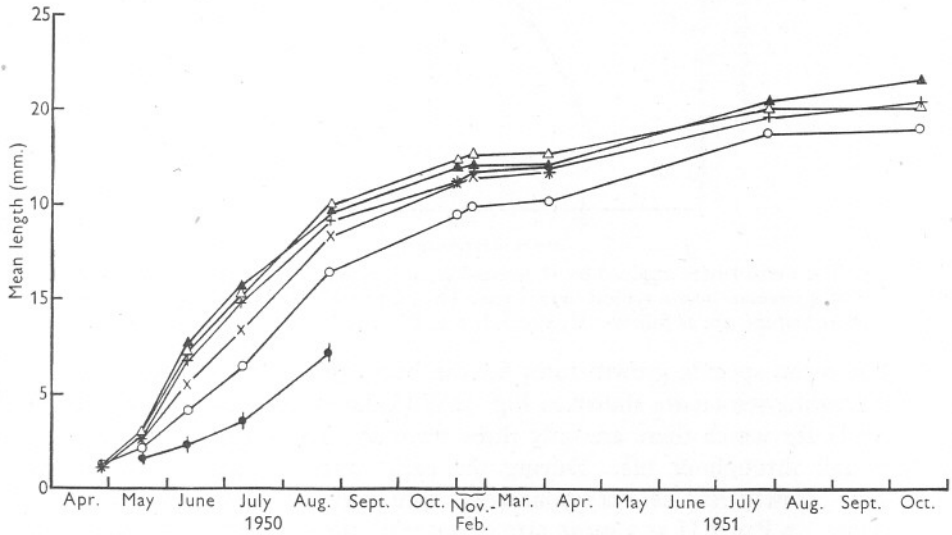

Fig. 3. Growth of *B. balanoides* over two seasons on intertidal panels II–VII (see Table I). The mean lengths on each panel at each date of inspection are shown. Panel II, ♦; III, ○; IV, ×; V, +; VI, △; VII, ▲.

settlement for each panel at different tidal levels. The mean lengths plotted against age are shown in Figs. 1 and 3. It should be remembered that growth of the intertidal barnacles was measured in the season following the observations on the submerged barnacles. The growth pattern is similar to that just discussed for *B. crenatus*. Increase in length takes place throughout the spring and summer months, but in contrast, some growth, although very small, appears to continue throughout the first winter on the intertidal panels, this continued growth being more marked on the upper panels where the

barnacles were smaller. In the second season growth continued on all panels. By October of the first season the mean length attained by *B. balanoides* on the submerged exposures was 17·2 mm., and on the intertidal panels the mean length varied from 17·3 mm. on the lower Panel VII to 14·4 mm. on the upper Panel III (no barnacles remaining on Panel II at this date). Complete submersion has thus given optimum conditions for growth, and the growth on the lower intertidal panels closely approaches that for complete submersion. This is clearly brought out in Fig. 4 in which the mean length attained by 24 August 1951 (i.e. after approximately 4 months' growth) is plotted against the hours of submersion during one typical full lunar period.

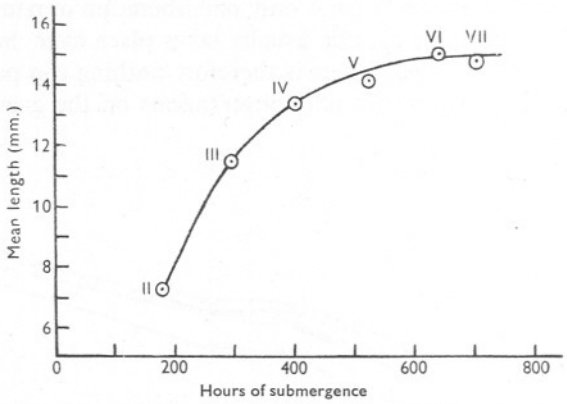

Fig. 4. The mean length attained by *B. balanoides* on intertidal panels, plotted against hours of submergence over a typical lunar cycle. The heights of the Panels, in feet (relative to Chart Datum) are as follows: II, 8·9; III, 7·2; IV, 5·4; V, 3·6; VI, 1·8; VII, 0·0.

The mean specific growth-rates for the barnacles on each panel over the first growing season are shown in Fig. 5. With the exception of the high level Panel II for which there are only three readings, the mean specific growth-rates fall throughout life. During the early stages of growth the mean specific growth-rate is very markedly influenced by the exposure. For example, on Panel II at a mean size of 2·0 mm. the specific growth-rate was 0·0141, while on Panel VII at 2·17 mm. it was 0·0387, and on the totally submerged panels at a mean length of 2·37 mm. it was 0·0489. The other intertidal panels give values intermediate between these extremes. At the levels of Panels V–VII there is little evident distinction between the growth-rates, although the points lie below those for complete submersion (raft) derived during the previous year. The mean specific growth-rates, however, fall more steeply on those panels with greater submersion, so that by the time a size of about 9 mm. is reached the mean specific growth-rate and the change of mean specific growth-rate with increasing length have become virtually identical, and at a slightly increased length are coincident with the values for complete submersion.

Apparently *B. balanoides*, unlike *B. crenatus*, does not reach its virtual maximum size in a single season, and therefore it is not surprising to find that the length began to increase significantly quite early in the second season and that relatively more growth took place during the second season, even though this was small in comparison with the first season's growth. Thus on the submerged panels there was an increase in the mean length from 17·2 to 25·8 mm. from October 1950 to October 1951, that is during the second

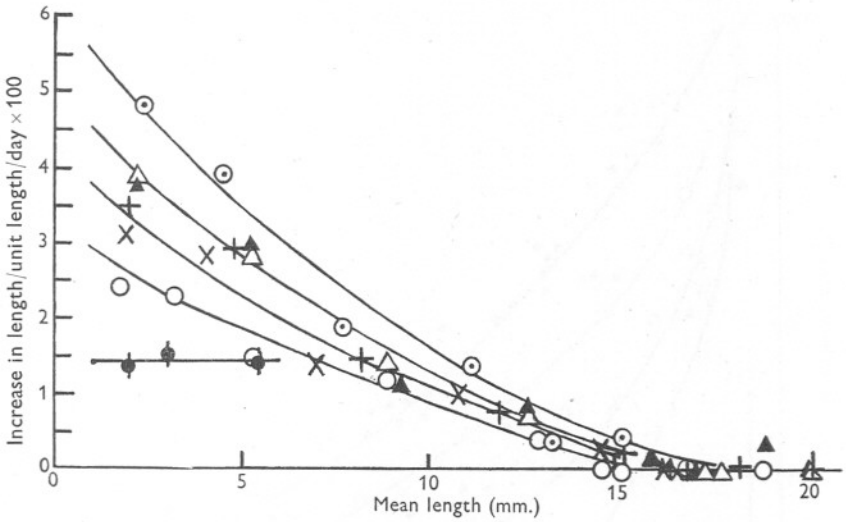

Fig. 5. The mean specific growth-rate (i.e. increase in length per unit length per day) × 100 plotted against mean length, for *B. balanoides* on intertidal Panels II–VII, together with that for the completely submerged specimens for the previous year. Symbols as in Fig. 3; submerged *B. balanoides* shown as dot-and-circle.

season, and even a slight increase to 26·3 mm. during the third season, whereas in the intertidal exposures the second season's increase in length was of the order of 4 mm. (the maximum mean length reached at the end of the second season was 21·7 mm. on Panel VII and 19·1 mm. on Panel III), the relative increase being greatest on the higher panels where there had been only slow growth in the first season.

The Length, Breadth and Height Relations during Growth

Runnström (1925) has stated that *B. balanoides* at Herdla were relatively higher in the second than in the first year, but he gives no measurements. Moore (1934) observed no change in the apical angle of individuals on the shore, but recorded a change in others growing under estuarine conditions.

The periodic measurement of length, breadth and height of individuals in the present work allows the possibility of such changes in shape to be investigated.

For all raft and intertidal exposures the length/breadth and length/height ratios were calculated for each individual on each separate date of inspection during the growing period; the mean values of the ratios and their standard errors were then found for each set on all the inspections. In Figs. 6 and 7 these mean ratios are plotted against the appropriate mean length: the pooled standard error of the means is also shown. The curves were drawn by inspection.

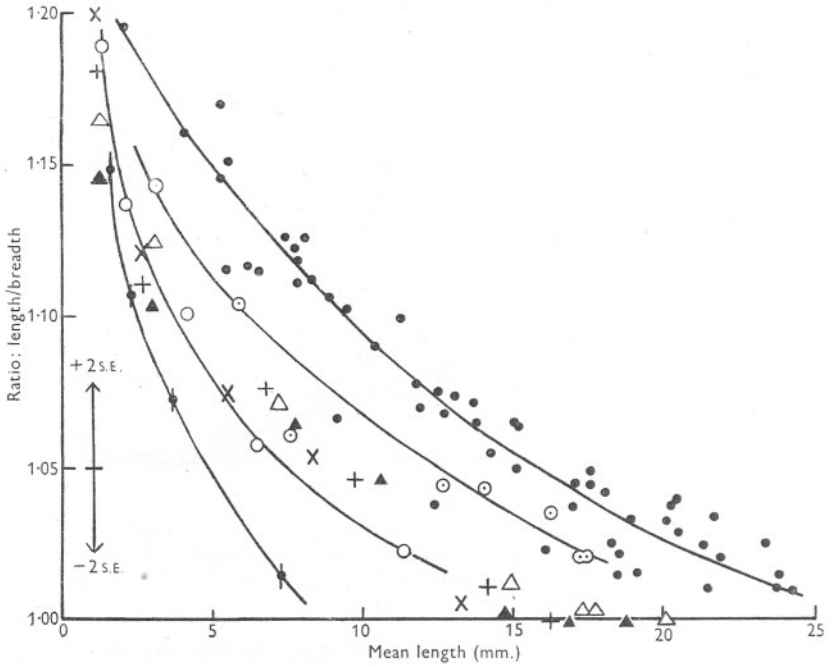

Fig. 6. The mean ratio of length to breadth plotted against mean length for *B. crenatus* totally submerged and *B. balanoides* on intertidal and totally submerged panels. Limits of 2 × standard error shown by arrows. *B. crenatus*, all series shown as black circles, since there was no significant difference in ratio (see text); *B. balanoides*, as in Fig. 5.

No account is taken of age in these Figures. With *B. crenatus*, with its several Series, the barnacles represented for a given mean length are often of quite different total age, as a result of taking into account all the settlements which were spread over several months.

B. crenatus will be considered first. Fig. 6 indicates that the length/breadth ratios decrease with increasing size and, within the limits of random sampling errors, can be considered to lie on a single curve, i.e. there is no indication that the ratio changes from Series to Series: in other words, the ratio is not a function of the time since settlement but only of the size attained by the animal. The rate of change of the ratio with length is greater at the lower

mean lengths, i.e. during the period of most vigorous growth (see p. 112). The total change in the mean ratio is not great, from 1·200 at a mean length of 2 mm. to 1·010 at a mean length of 24 mm. In terms of change of shape, this decrease in the ratio with increasing size means that throughout growth the outline of the base changes, most rapidly at first, from more or less elliptical to almost circular. (If the actual cyprid itself were considered then the initial change would be greater.)

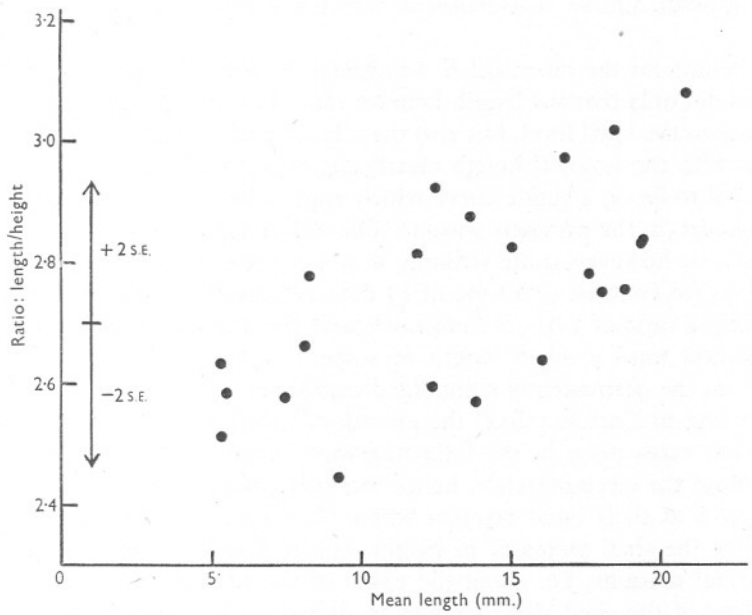

Fig. 7. The mean ratio of length to height plotted against mean length for totally submerged *B. crenatus*. Limits of 2 × standard error shown by arrows.

The method of measuring the height is far less accurate and satisfactory than that of the length and breadth, for the upper part of the compartments, particularly of the carina which was usually measured, is liable to damage. These inaccuracies are reflected in the large standard errors, the pooled value of which was 0·119, compared with a value of 0·014 for the length/breadth ratio. However, quite clearly the ratio increases with increasing mean length, the barnacles becoming relatively squatter as they become older. As far as can be ascertained, the relation is linear throughout the growth of the barnacle.

The form of the curve for length/breadth ratio of submerged *B. balanoides* plotted against mean length is similar to that for *B. crenatus*, that is, there is a decrease in the ratio as the animal increases in size, and the rate of change of the ratio with length decreases with increasing size of the barnacle. Again, therefore, there is a change from an elliptical to a circular outline of the base. The values of the ratio at a given length all lie below those for *B. crenatus* at

the same length and a separate curve has therefore been drawn for *B. balanoides*. The position of this curve, below that for *B. crenatus*, implies that at a given length *B. balanoides* is more circular than *B. crenatus*.

The length/height ratios for the submerged *B. balanoides* were too few to graph: the results again showed a wide spread. However, the value of the ratio at a length of 3·1 mm. was 2·42, at 6·0 mm. 3·27, and thereafter there was no significant change. The major change therefore took place very early during growth, unlike *B. crenatus* in which the ratio showed a progressive change.

The results for the intertidal *B. balanoides* are also shown in Fig. 6, which indicates not only that the length/breadth ratios at a given length tend to vary according to the tidal level, but also that the rate of change of the ratios also changes with the level, although clearly the values on Panels V–VII can be considered to lie on a single curve which approaches that for the submerged *B. balanoides* (in the previous season). The difference in Panel II, the highest of the set, is, however, quite striking: at a mean length of 7·25 mm. reached in 98 days (in contrast to a time of 45 days required to reach this length on Panel VII) a ratio of 1·015 is found, whereas this ratio is not reached on the lower panels until a mean length of some 14 mm. has been reached and 19 mm. on the permanently submerged exposures.

According to Darwin (1854) the growth of the shell of a barnacle such as *B. crenatus* takes place in the following way. Accretions to the basis occur only around the circumference, hence increasing the diameter; the compartments grow at their basal margins where they are in contact with the base and hence the shell increases in height; the compartments also grow along their lateral margins, i.e. along the edges of the radii and alae, so that the upper part of the shell also increases in diameter. The foregoing results on the changes in shape imply that there are differential changes in the rate at which calcareous material is laid down during the life of the barnacle. For the length/breadth ratio to decrease during growth there must be a relative increase in growth-rate at the basal margins of the lateral compartments, as well as at the lateral parts of the calcareous basis in *B. crenatus* in order to maintain the continuity of the shell. The relative changes in height, which it should be remembered refer to the carinal compartment, also indicate changes in relative growth-rates in this part of the shell with respect to the overall length.

Summary of Literature on Growth-rate of *Balanus balanoides* and *B. crenatus*

Runnström (1925), working at Herdla, has given data for the rostro-carinal diameter of intertidal *B. balanoides* over a period of 2 years, making no distinction with regard to tidal levels: the mean spring tidal range, however,

is only 4 ft. The settlement took place in late April (29th) and growth followed during the first spring and early summer, but had almost ceased by July, by which time the mean length was only 3·0–4·0 mm. He states that growth began again in October of the first year and by the following January the mean length was 7·0 mm. Further growth apparently took place in the following spring and decreased very rapidly in the summer, at the end of which a mean size of 10·4 mm. had been attained. He states that the first year is a vegetative year and that maturation during the second season is followed by fertilization in October, and the liberation of nauplii in the third spring. Further, he considered that *B. balanoides* lives for two years, occasionally three.

It is clear from Runnström's figures that individual barnacles were not followed, since the numbers measured at each inspection vary irregularly, e.g. on 15 February 1925 twenty-six were measured, while on 7 April 1925 102 were measured; and in view of this fact and the lack of data between July and October in the first season, it is doubtful if the information is sufficient to permit a critical assessment of the growth. Any marked growth during the period October to December of the first season would also seem to be somewhat doubtful. The spat was not followed into the second year but the growth-rate over 2 years was deduced by the measurement of older individuals inferred to be of the second year-class from the different appearance of the shells due to algal infection (cf. Parke & Moore, 1935). Indeed his graph, when the absence of data between 24 June and 15 October is taken into account, can be interpreted to follow quite closely the form of Figs. 2 and 4, with a gradual slowing of the growth in the late summer, although his actual growth-rates at any one time are very much smaller. The only real discrepancy is the period of rapid growth which he suggests takes place in the first mid-winter and which, in view of the great similarity of environmental conditions (see below) and of the method of deriving these figures, i.e. from two entirely different sets of barnacles, may be questioned.

The *B. balanoides* measured by Moore (1934) were natural populations growing at the top, centre and near the bottom of their intertidal range. The growth was not measured from settlement and the actual ages of the barnacles were deduced. Indeed his growth curves extending over 3 years are hypothetical curves based on measurements over 1 year only. However, for the first year he indicates that rostro-carinal lengths of 5·2, 5·3 and 4·7 mm. were reached on his upper, middle and lower patches, respectively. In addition, samples of his O and Q groups (of known age but from a level not specified) were measured and the growth curve for the O group is similar to those of the present investigation. Thus he found rapid growth from April to July, but virtually none during the late summer and winter, and that growth was renewed by May of the second season. There are no results from the early part of the second spring, so it is not known when the second season's growth began. Moore's other group (Q) showed very little growth in the first season,

and he suggests that they settled so late that their growth was retarded during the hot summer period.

Hatton & Fischer-Piette (1932) have also studied the growth of intertidal *B. balanoides*, on blocks placed at a number of tidal levels and subject to varying conditions of wave-action, in the St Malo region. The rostro-carinal lengths were measured. Settlement in this region takes place from March to May and the graphs of two individual barnacles measured during two successive seasons (neglecting certain points which show a decrease in size and which are explained as due to erosion) show growth curves similar to those in Figs. 2 and 4. Rapid increase in length took place during the spring and slowed down between July and October. The pattern was repeated in the second season, but growth began between February and March. A length of about 3·0 mm. was reached during the first season and 5·5 mm. by the end of the second season. Further, Hatton & Fischer-Piette point out that the greatest growth is at the lower levels where the animals are immersed for longer periods. Hatton (1938) confirms and extends these observations.

Corlett (1948) reporting on work in the Mersey estuary found that *B. balanoides* settling in April attained a mean rostro-carinal length of 5·9 mm. by December of the first season, and 6·55 mm. by the following April and states that growth was most rapid between May and September. He also records the occurrence of extremely large individuals (20·35 mm. mean length) of unknown age on concrete piles.

Fuller (1946) reports a mean length of 4·0, 6·0 and 8·0 mm. in 4, 8 and 12 weeks respectively, for *B. balanoides* settling between May and July on panels exposed to continuous submersion at Frenchman's Bay, Maine; but he also records that the growth of barnacles settling a year later was much less, approximately 1·0, 1·5 and 2·0 mm. in the same periods. He suggests that the smaller growth on the later occasion was due to the barnacles being situated on the lower surface of deep panels.

There are fewer data available for *B. crenatus*. Pyefinch (1948), working at Millport, has measured samples growing on panels submerged from a raft, and his figures expressing the modal lengths show rapid increase in length during the spring, with a falling off during the late summer and, by interpolation, a rapid growth in autumn of a late settled spat. He finds that the maximum size can be attained in the first season's growth as has been confirmed in the present observations. Topsent (1911) states that at Luc-sur-Mer, apparently in an intertidal, but low zone, *B. crenatus* can attain a size of 3·0–3·5 mm. in 50–60 days, and 5·0–6·0 mm. after 105 days.

Corlett (1948) found that 100 specimens of *B. crenatus*, settled some time between late April and early July 1946, had a mean length of 5·6 mm. (maximum 11·5 mm.) by October 1946; another group had a mean length of 6·6 mm. (maximum 11·0 mm.) in April 1947.

Barnes & Bagenal (1951), studying *B. crenatus* settled on *Nephrops nor-*

vegicus (L.), have deduced that spring-settling *Balanus crenatus* grow to a mean length of 4·4 mm. by October and 8·5 mm. by the following October, while those settling in the autumn showed some growth during the winter and reached a mean length of 1·8 mm. by January and 4·9 mm. by the following July.

Discussion

When due account is taken of the methods of size estimation used by many workers on populations whose age was not accurately known, the results here presented seem to give a picture of the seasonal growth which is valid for all the regions from which results are available, from Herdla to St Malo and Luc-sur-Mer, and in the Gulf of Maine. This result is not surprising since the relative seasonal changes of water temperature and other environmental factors (with the possible exception of summer air temperatures) are not widely different in any of these regions.

Settlement in the spring is followed in both species by a rapid increase in length during late spring and early summer, after which the rate of increase in length falls off in the late summer and autumn and begins again during the following spring. In *B. crenatus*, under conditions of permanent submersion, the early spat appears to be able to reach a virtual maximum size in the first season, that is by October of the first year, and the small growth taking place in the second season begins somewhat later than in the first. In *B. balanoides*, however, on submerged panels, the maximum length is not reached in the first season, and since the specific growth-rate is higher in the smaller animals growth continues further into the winter, and starts earlier and is more vigorous than in *B. crenatus* during the second season.

There is no doubt from a comparison of the present results with those summarized above that, when growing free from competitive and other restricting environmental factors, *B. balanoides* grows more rapidly under conditions of complete immersion, at any rate when the constant depth is no greater than 3 ft. The evidence from the intertidal exposures suggests that at any level the maximum size reached is approximately the same, since by the end of the second season there is little difference at the five levels. This was a conclusion reached by Hatton & Fischer-Piette (1932) in studying *B. balanoides* settled on blocks exposed intertidally. It seems, therefore, that the observation of Moore (1935*b*) and of Hatton (1938) that the optimum level for growth is at low water for newly settled barnacles, but moves progressively up to high water as they grow older, is due in part to the specific growth-rate varying with size. The older barnacles at the top of their distribution will be growing more in their second season simply because they have grown slowly during the first, although other environmental factors, present on the shore and absent on intertidal panels kept relatively free from other growth, may play a part.

Quite evidently it is not the unsuitability of complete submersion for growth and development that usually limits *B. balanoides* to the intertidal zone.

At all levels on the intertidal exposures, observations on barnacles other than those measured showed that maturation followed by fertilization and development of nauplii had taken place during the first season. Fertilization of *B. balanoides* in this area takes place in the autumn when the lowest mean size attained (by those on Panel III) was between 11·0 and 14·0 mm. Other work has shown that, even though growth is much less, on the adjacent shore barnacles produce nauplii during their first year at all levels. It seems that the vegetative year observed by Runnström (1925) is due to the very low growth-rate, indicated by the small size reached at the end of the first summer's growth (some 3 mm.). The observations of Moore (1935*a*) that the barnacles at the lower tidal levels matured in their first year, but at higher levels not until a year later, could possibly also be explained in this way. Again, the *B. balanoides* measured by Corlett (1948) had only reached a mean length of 5·9 mm. in December, and he records that rather less than 10% contained developing nauplii. Neither Moore nor Corlett, however, indicate whether the sterile barnacles in question were in fact sufficiently close together to permit cross-fertilization. In our observations the high-level *B. balanoides* on both the pier panels and on the shore were close enough to have been fertilized by adjacent barnacles, but frequently high-level barnacles are so scattered as to be incapable of cross-fertilization.

Further, under the conditions of these experiments, far greater sizes were attained than are usually recorded under what may be termed more natural conditions. Occasionally isolated and presumably very old individuals are found at the upper levels on the adjacent pier piles (see also Corlett), the size of which approaches those recorded here. Presumably, therefore, under natural competitive conditions death frequently intervenes before the possible maximum size is reached. It should, of course, be emphasized that, from the point of view of continued survival of the barnacle population as a whole, the isolated individuals here considered are atypical since cross-fertilization would never be achieved.

The exceptional growth of *B. crenatus* on submerged panels might be expected since it is normally a sublittoral but not a deep-water species. The sizes recorded by Barnes & Bagenal (1951), and indeed general experience of *B. crenatus* in the field, indicate that these maximum possible sizes are not normally reached. The environmental factors, perhaps in particular food supply, seem to be more favourable under raft conditions.

The fact that the specific growth-rates for all the Group I *B. crenatus* lie on a single curve, although there were 2 months between the times of settlement of Series I and IV, together with the lack of marked discontinuities in the curve, suggests that environmental conditions which varied throughout

this period were not limiting the growth-rate. The possibility, however, cannot be ruled out that there were two different environmental factors acting in opposition. Thus, the sea temperature when Series I had reached a mean length of 12·0 mm. was 11·0° C., but was 15° C. when Series IV were growing at this mean length (see Fig. 1), and, although the specific growth-rates were identical, possibly an increased food supply at the lower temperature in June was equivalent to a reduced food supply for Series IV at the end of July with its higher temperature. There is some evidence that food supply becomes a limiting factor later in the season, since Series V in August, with the temperatures still comparatively high, had lower specific growth-rates than expected (from Group I data) at their mean length. Indeed the lower specific growth-rates for Group II as a whole, between August and October, even though the temperatures during this period were always higher than those in the spring, suggest that food supply may then have been a controlling factor. Lack of food also seems to be a major factor affecting growth during the winter period since the mean temperatures during January are almost as high as those during the spring, an opinion further substantiated by the increase in the specific growth-rate during the following March, when food becomes available with the advent of the spring diatom increase.

Summary

The length, breadth, and in part the height, of individual *Balanus balanoides* and *B. crenatus* have been measured over several seasons, and the mean specific growth-rates calculated. The *B. balanoides* had settled at various known intertidal levels, and both species under conditions of permanent submersion.

Under all conditions, except on the highest intertidal panels, length increases rapidly subsequent to settlement and during the early summer. *B. crenatus* can reach its virtual maximum size (20–25 mm. rostro-carinal length) in a single growing season, under the conditions studied. Late-settled *B. crenatus* continue to grow further into the autumn, and begin to grow again early in the spring of the second season at the end of which the virtual maximum size is reached. The seasonal growth of both intertidal and submerged *B. balanoides* is similar, but the growth-rate is a function of submersion, which is, of course, dependent upon the tidal level. However, maximum growth is not reached in the first season and there is consequently relatively more growth in the second season than in *B. crenatus*.

The mean specific growth-rates in both species decrease with increasing size and, with permanently submerged *B. crenatus*, there is no suggestion that during the first spring and early summer any change in the environmental factors was effective. On the intertidal panels, the mean specific growth-rates at a given size vary with the tidal level, and this effect is most marked in the smaller barnacles.

The ratio of length to breadth decreases, at first rapidly, then more slowly, with increase in length in both species, so that the outline of the base approaches that of a circle. The relation varies according to the tidal level. The height measurements were less satisfactory, but it is evident that the ratio increases with increasing size, the barnacles becoming relatively squatter.

The net settlement of *B. balanoides* on the intertidal panels was maximal at about mid-tide level, and it is suggested that this is the result of two opposing factors, namely, gain due to settlement when the tide *crosses* the panel and loss due to desiccation during exposure to air. The hypothesis is based upon the suggestion that the cyprids of an intertidal species such as *B. balanoides* settle most readily from relatively thin layers of water draining from solid surfaces.

A high incidence of infection by *Hemioniscus balani* was noted in the intertidal *B. balanoides* in the second season.

A comparison with the results of other workers shows that the growth-rates recorded and sizes reached on these experimental exposures are far greater than have hitherto been observed under natural conditions, although the seasonal growth cycle is similar over a wide area. In *B. balanoides* it is clearly not the unsuitability of complete submersion for growth and development that usually limits this species to the intertidal zone.

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COMPARISON OF THE DIFFERENT METHODS OF ESTIMATING NANOPLANKTON

By Dorothy Ballantine

From the Plymouth Laboratory

An assessment of the abundance of nanoplankton in the sea cannot be made until a reliable method for counting has been found. The object of the present investigation was to compare the efficiency of a number of methods. The organisms concerned range in size from less than 1 to about 10 μ, most being less than 5 μ, and belong to the classes Chrysophyceae, Cryptophyceae, Dinophyceae and Chlorophyceae.

The larger planktonic diatoms and armoured dinoflagellates, which occur in the plankton in numbers insufficient to give accurate results with the methods under comparison, have been omitted. Utermöhl's (1931) sedimentation method gives the best results for these larger forms (Nielsen, 1933); but it is not suitable for naked nanoplankton, since no adequate fixative has been discovered which will leave these organisms in a state recognizable for identification. A second method, suggested by Goldberg, Baker & Fox (1952), of filtering samples through a molecular filter, is convenient for treating all size-ranges of organisms at sea. Counts may then be made in the laboratory without any prior treatment of the sample, but the majority of the naked forms up to 5 μ are unidentifiable.

Some means of concentrating the sample is usually necessary in most counting methods since the depth of the water film which would be required to give any figure at all from a raw sample is too thick to allow an accurate count. This statement does not apply to Dr R. H. Millar's technique of counting under dark-ground illumination.

The methods compared here are the use of either a centrifuge or filters for concentrating the sample, followed by counting; a counting method using dark-ground illumination without previous concentration of the sample; and the utilization of dilution techniques.

My most sincere thanks are due to Dr M. Parke, of Plymouth, for her many helpful suggestions during the course of this investigation; to Dr R. H. Millar, of Millport, to whom I am indebted for an account of his method for counting flagellates under dark-ground illumination; and to Dr E. D. Goldberg, of the Scripps Institution of Oceanography, California, for a description of the use of the molecular filter, and for a supply of these filter membranes.

My thanks are also due to the Director, Mr F. S. Russell, and staff of the Laboratory of the Marine Biological Association at Plymouth, where this work was carried out when the author was in receipt of a Development Commission Fisheries Training Grant.

Methods

Much has been said in the past about the use of a centrifuge for concentrating nanoplankton samples, and many different methods have been used. Gran (1929) states that 'test experiments with the centrifuge method show that the source of error involved by vortex formation in the tubes, as mentioned by Wulff (1926) and Utermöhl (1927), can be avoided altogether if care is taken first to ensure that the motion is not too suddenly checked, and tubes with a narrow bottom part used, and the clear liquid drawn up cautiously with a pipette'. The buoyancy of certain species (Kofoid, 1897; Gross & Zeuthen, 1948), due to a specific gravity less than that of water, causes selective action with the centrifuge, but the majority of such species are diatoms or water-bloom-forming organisms such as blue-green algae, which can be estimated with the sedimentation method after fixation.

The method of centrifugation adopted here is based upon that of Bruce, Knight & Parke (1940). 10 ml. samples are centrifuged for 15–30 min. at 1500 r.p.m., a relative centrifugal force of 358 × gravity. The centrifuge is then stopped very slowly, to avoid currents developing in the tubes, and the top 9 ml. are withdrawn by means of an upturned pipette attached to a pump. The use of the pump and a pipette with an upturned end enables the water to be drawn off evenly, and any suction currents which may develop will be only at the surface of the water. The remaining 1 ml. of water is then thoroughly mixed, and counts made in a haemacytometer, objectives up to $\frac{1}{6}$ in. being used if necessary. Average values, based on counts from four tubes, were recorded for each sample.

Several experiments have been carried out to determine the speed and duration of centrifugation which gives the maximum results (Table I). In these experiments no attempt was made to count all the organisms in the concentrate, as Lohmann (1908) did, but an average figure was obtained for each sample.

Nielsen & v. Brand (1933–34) describe the use of a precipitate to aid in the sedimentation of preserved cells while centrifugation is in progress, and it was thought that this principle might be applicable to the centrifugation of living cells. Accordingly, before centrifugation, 0·05 ml. of a 1% solution of potassium aluminium sulphate crystals was added to the 10 ml. sample of sea water (Atkins & Parke, 1951). The flocculent precipitate thus formed does not kill the organisms, nor does it hinder subsequent counting. Further treatment of the precipitate is therefore unnecessary. The results from this

method using the floc, compared with results from untreated samples (labelled 'normal') are found in Table I, Sample 1, p. 134, and Table IV, samples 2 and 3, p. 137.

As a check on the sedimentation of living organisms during centrifugation 10 ml. samples were centrifuged and split up immediately into three sections (i.e. the top 5 ml., middle 4 ml. and bottom 1 ml., the latter being the section usually used for estimating the numbers). These separate sections were then placed in dilute Erdschreiber[1] culture solution and left to grow. Never did more than two different organisms appear in the flask containing the top 5 ml. of the centrifuged sample, or more than three in the flask containing the middle 4 ml., and the time taken for these cultures to develop was comparable with that for cultures started from a single-cell inoculum at the same period of the year. The flask containing the bottom 1 ml. of the sample, however, produced a thick mixed culture in a short time. This evidence, together with that obtained from centrifuging cultures (Table II), proves that the bulk of living nanoplankton organisms will settle during centrifugation.

The second method of concentrating sea-water samples was by means of the 'Stefi' filter, using a collodion membrane having an average pore diameter of 0·5 μ (Cole & Knight-Jones, 1949). With this apparatus samples were concentrated to one-fiftieth of their original volume, and samples of the concentrate were counted in a haemacytometer.

To facilitate the counting of a large number of motile organisms under direct illumination, fixation with osmic vapour is necessary. This treatment leaves the organisms in a recognizable state, but cannot be used for large volumes of water. The percentage of motile and non-motile forms in each size-group can be determined later if required.

The third method tested for concentrating the nanoplankton was by means of the molecular filter suggested by Goldberg *et al.* (1952). This filter-membrane is composed of incompletely cross-linked high polymer molecules of partially substituted cellulose acetate and cellulose nitrate. The filters used in this work had an average pore-diameter of 0·5 μ. Following the method of Goldberg *et al.* all solutions used in the preparation of the sample were molecular-filtered before use to remove any particles. Organisms in the sample were then fixed by means of a saturated solution of iodine in potassium iodide, at a concentration of 40 ml. of fixative to a litre of sea water. Throughout, samples of 50 ml. were used. After fixation the sample was filtered, and the sea water was gradually replaced by distilled water by washing with a series of sea water/distilled water mixtures. 20 ml. of Fast Green were added to the filter, which was then disconnected from the pump, and left for 30 min. The Fast Green was then drawn through, and the membrane was dehydrated with ethyl alcohol and cleared with cedar oil.

[1] Dilute Erdschreiber culture solution (Plymouth formula): sea water 1 l., $NaNO_3$ 0·15 g., $Na_2HPO_4 . 12H_2O$ 0·015 g., soil extract 25 ml.

The membrane was then removed from the filter, cut in half, and each half mounted in balsam on a microscope slide. If the number of diatoms and larger dinoflagellates in the sample is required, the entire area of the filter is used. These organisms can usually be easily identified on the membrane. To estimate the numbers of nanoplankton four fields only were counted, each being taken at random from the filtering area of the membrane. The mean of these counts was multiplied by $83 \times 95/50{,}000$ to bring the figure to numbers per cubic millimetre of the original sample. This correction factor must be calculated for each filtering apparatus and microscope used when counting.

The next method (unpublished), designed and used by Dr R. H. Millar of Millport, for the counting of flagellates, makes use of the highly refractive nature of the nanoplankton organisms. The details of this method have been communicated to me by Dr Millar, for use in these comparative studies. In his method a drop of water is placed in the cell of a counting slide (Thoma haemacytometer), and, with a phase-contrast microscope adjusted to give dark-ground illumination with a low-power objective, all the moving flagellates are rapidly counted in the whole area of the cell, which is scanned by means of a mechanical stage. Any microscope fitted with dark-ground equipment can be used for this method, but the advantage of a phase-contrast microscope is the ease with which it can be altered to give either direct illumination, or actual phase contrast, so that particular organisms can be observed more clearly. The count must be made as soon as possible after the drop of water is placed on the slide, as many organisms settle very rapidly.

The next two methods tested for estimating nanoplankton involve the use of cultures. The first was proposed by Allen (1919) and the second by Knight-Jones (1951). Allen's method consists simply of adding 0·5 ml. of the sea-water sample to 1·5 l. culture solution, shaking thoroughly, and distributing this into a large number of flasks, which are then left in a north light for the organisms to grow. It is then assumed that every species which grows in each flask was started from a single cell, thus giving a minimal count. Knight Jones's method utilizes the serial dilution technique used by bacteriologists, with Erdschreiber culture solution as the nutrient medium. Three series of five tubes are used in each estimation, each series containing one-tenth of the volume of the sample that is present in the previous series. The tubes are placed in a north light and left to grow for 1–2 months in the summer, and longer in the winter. On the basis of the number of tubes in each series which show growth the final results are worked out from Swaroop's (1938) tables of the most probable numbers in the original sample. This serial dilution method has been used with sea-water samples, where the numbers obtained have been compared with counts after concentrating with the membrane filter and centrifuge, and also with actively growing cultures, where the number of cells was counted prior to dilution.

Results

Centrifugation

(Tables I, II, IV sample 2, VIII, and X sample 2)

The experiments to test the efficiency of centrifugation at different speeds and for different periods of time were carried out using both samples treated with potassium alum and untreated ('normal') samples. The results of these experiments are shown in Table I and the figures from which they were obtained are in Table VIII (p. 143).

Sample 1 shows that there is no significant difference between untreated samples and those which have been treated with potassium alum. This conclusion is confirmed by results shown in Table IV (p. 137), and from other results not given here, including some from experiments using cultures.

Results of centrifugation for different periods at 1500 r.p.m. (Sample 1) show that a 10 min. period does not give a maximum count, but from 15 min. onwards there is no significant increase. Increasing the centrifuge speed to 2000 r.p.m. does not improve the efficiency of sedimentation (Samples 2 and 3).

After the relative centrifugal force and time above which no further sedimentation occurs had been established, the actual efficiency of centrifugation was tested by the use of cultures. Cultures of a density that permitted an accurate count in a haemacytometer were centrifuged for 20–30 min., and counted. The results from these experiments are shown in Table II. The culture had usually to be diluted to one-tenth of its original strength prior to centrifugation, as the excessive number of organisms after concentration would otherwise have precluded an accurate count.

Throughout Table II the expected standard deviation (σ), based on the Poisson distribution, has been given, the actual standard deviation being unknown. Where thick cultures were used in this series of experiments a lower count than that obtained from the original sample resulted after centrifugation. This was particularly true with flagellate no. 3 (Plymouth collection) and *Hemiselmis rufescens*. In *Hemiselmis* the count from the centrifuged diluted culture is considerably larger than that from the centrifuged undiluted culture, and it seems possible that the density of the culture limits the efficiency of sedimentation during centrifugation.

These results strongly suggest that the centrifuge sediments the great majority of the nanoplankton, particularly in samples in which the organisms are not very numerous, as in raw sea water itself.

Centrifugation and membrane filter

(Tables III and IX)

Table III shows the comparison of results obtained by counting after samples had been concentrated with the membrane filter and the centrifuge.

TABLE I. COMPARISON OF SPEEDS AND TIMES OF CENTRIFUGATION

(Figures throughout refer to numbers per cubic millimetre, and results marked * are significantly different.)

Sample 1: 1500 r.p.m. (relative centrifugal force = 358)

Duration (min.)	'Normal' or 'Floc'	Mean	Standard deviation (s)	Difference between means	Standard error of difference
10	N	7·25	1·83		
	F	7·62	2·98	0·37	±1·24
15	N	10·25	3·18		
	F	9·9	3·04	0·35	±1·55
30	N	11·25	2·71		
	F	10·0	2·4	1·25	±1·28
60	N	10·0	2·83		

Treatments compared	'Normal' or 'Floc'	Difference between means	Standard error of difference
10 and 15 min.	N	3·0	±1·34*
	F	2·28	±1·51
15 and 30 min.	N	1·0	±1·475
	F	0·1	±1·42
30 and 60 min.	N	1·25	±1·38
10 and 30 min.	N	4·0	±1·15*
15 and 60 min.	N	0·25	±1·5

As there is no significant difference between 'normal' and 'floc' in any series, these two sets of figures have been treated together for each length of time, and the following results are obtained.

Duration (min.)	Mean	Standard deviation
10	7·44	2·36
15	10·1	3·0
30	10·62	2·56
60	10·0	2·83

Treatments compared	Difference between means	Standard error of difference
10 and 15 min.	2·66	±0·96*
15 and 30 min.	0·52	±0·99
30 and 60 min.	0·62	±1·19
10 and 30 min.	3·18	±0·87*
15 and 60 min.	0·1	±1·25

Sample 2: collected from Knap Buoy, 3 June 1952

Treatment	Mean	Standard deviation (s)
1500 r.p.m. for 15 min.	25·75	2·49
2000 r.p.m. for 15 min.	25·22	3·42
2000 r.p.m. for 30 min.	25·4	4·11

Sample 3: 15 min. at 1500 and at 2000 r.p.m. compared

Speed in r.p.m.	Mean	Standard deviation	Difference between means	Standard error of difference
1500	10·4	2·45		
2000	9·0	2·07	1·4	±1·12

N.B. At 2000 r.p.m. the relative centrifugal force is equal to 636 × gravity.

It will be seen that the theoretical Poisson standard deviation, the root of the mean, is frequently much less than the observed standard deviation for membrane-filtered samples, a fact which suggests that the organisms tend to aggregate when subjected to this treatment. χ^2 has then been calculated, and the probability found from Tables. This figure is often very low. This evidence

TABLE II. RESULTS FROM THE CENTRIFUGATION OF CULTURES

(Centrifugation at 1500 r.p.m. for 20–30 min. The results are expressed per cubic milli metre of the original culture.)

	Original count		Culture centrifuged		Culture diluted and centrifuged	
Organism	Mean	Theoretical standard error (σ)	Mean	Theoretical standard error (σ)	Mean	Theoretical standard error (σ)
Chlorophyceae						
Chlamydomonas sp. 1	4440	221	..	..	4300	208
Pyramimonas grossii Parke	220	47	..	..	200	44·7
Chrysophyceae						
Flagellate no. 3	3230	180	..	..	2760	167
Chromulina pleiades Parke	7770	279	..	..	7310	270
Cryptophyceae						
Flagellate no. 6	5300	230	..	..	4900	222
Hemiselmis rufescens Parke	3420	186	2800	53	3210	179
Dinophyceae						
Peridinium trochoideum (Stein) Lemm.	143	37·8	151	12·6	146	38·2
Gymnodinium sp. 2	180	42·5	..	..	190	43·2

of aggregation is rarely found in centrifuged samples. In all the results from centrifuged samples there is only one (Table III, sample 4) in which the probability is less than 0·05, a result well within chance expectations. The counts from which these figures are derived will be found in Table IX (p. 144).

The figures in Table III show little, if any, difference between the actual results from the two methods of concentration, except for the sample taken on 3 June 1952, which was full of detritus. The time involved in using the two methods is, however, very different. The centrifuge is simple to operate, and the counts can be made fairly rapidly, as there are only twenty or thirty organisms per field, whereas with the membrane filter great care must be taken to ensure that the membrane does not dry out completely, for in so doing a number of the naked flagellates would be destroyed. The number of organisms per field after this latter treatment is also large, and the time taken to count them is therefore much greater. The additional time spent on the method appears not to be justified by any greater, or more reliable, estimate of the number of organisms.

TABLE III. COMPARISON OF THE RESULTS OBTAINED BY COUNTING AFTER CONCENTRATING WITH THE MEMBRANE FILTER AND THE CENTRIFUGE

(All samples are from Knap Buoy.)

	Counts per haemacytometer field					Numbers per cubic millimetre of the original sample	
	Mean	Standard deviation (s)	Deviation (σ)	χ^2	Probability	Mean	s
Sample 1, 27. v. 52							
Membrane filter							
Total	101·7	20·95	10·1	21·5	< 0·001	20·3	4·19
Under 2 μ	64·6	15·95	8·04	19·6	0·01–0·001	12·9	3·19
2–under 5 μ	28·7	6·15	5·35	..	..	5·7	1·23
Over 5 μ	8·3	2·34	2·88	..	..	1·7	0·47
Centrifuge							
Total	20·13	4·375	4·58	..	..	20·13	4·375
Under 2 μ	9·19	2·4	3·03	..	..	9·19	2·4
2–under 5 μ	7·75	2·98	2·78	..	..	7·75	2·98
Over 5 μ	3·19	1·56	1·79	..	..	3·19	1·56
Serial dilution	..	..	..	..	..	3·9	2·1
Sample 2, 29. v. 52							
Membrane filter							
Total	89·7	16·8	9·7	15·7	0·01–0·001	17·94	3·36
Under 2 μ	53·9	9·7	7·35	..	..	10·78	1·94
2–under 5 μ	25·0	4·52	5·0	..	..	5·0	0·9
Over 5 μ	10·8	5·17	3·29	12·3	0·05–0·02	2·16	1·03
Centrifuge							
Total	22·0	4·76	4·68	..	..	22·0	4·76
Under 2 μ	11·12	2·25	3·34	..	..	11·12	2·25
2–under 5 μ	6·63	2·78	2·58	..	..	6·63	2·78
Over 5 μ	4·25	1·895	2·06	..	..	4·25	1·895
Serial dilution	..	..	..	..	..	2·0	1·0
Sample 3, 3. vi. 52							
Membrane filter							
Total	202	16·9	14·2	..	..	40·4	3·4
Under 2 μ	131	17·4	11·45	11·5	0·05–0·02	26·2	3·5
2–under 5 μ	44·3	18·5	6·66	38·6	< 0·001	8·9	3·7
Over 5 μ	26·7	5·46	5·17	..	..	5·3	1·09
Centrifuge							
Total	33·1	6·77	5·75	..	..	33·1	6·77
Under 2 μ	21·0	4·62	4·58	..	..	21·0	4·62
2–under 5 μ	6·7	2·33	2·59	..	..	6·7	2·33
Over 5 μ	5·4	1·96	2·32	..	..	5·4	1·96
Sample 4, 6. vi. 52							
Membrane filter							
Total	173·0	24·75	13·2	17·7	0·01–0·001	34·6	4·95
Under 2 μ	124·0	20·6	11·3	17·01	0·01–0·001	24·8	4·12
2–under 5 μ	26·0	6·32	5·1	..	..	5·2	1·26
Over 5 μ	23·0	5·43	4·8	..	..	4·6	1·1
Centrifuge							
Total	36·0	8·57	6·0	14·22	0·05–0·02	36·0	8·57
Under 2 μ	25·9	6·86	5·1	..	..	25·9	6·86
2–under 5 μ	4·5	2·0	2·12	..	..	4·5	2·0
Over 5 μ	5·6	2·51	2·37	..	..	5·6	2·51
Sample 5, 9. vi. 52							
Membrane filter							
Total	135·9	38·7	11·7	55·0	< 0·001	27·18	7·7
Under 2 μ	100·5	32·7	10·03	53·0	< 0·001	20·1	6·5
2–under 5 μ	23·7	5·27	4·87	..	..	4·74	1·05
Over 5 μ	11·7	3·88	3·42	..	..	2·34	0·78
Centrifuge							
Total	27·1	5·35	5·21	..	..	27·1	5·35
Under 2 μ	19·0	3·02	4·36	..	..	19·0	3·02
2–under 5 μ	4·7	2·45	2·17	..	..	4·7	2·45
Over 5 μ	3·4	1·65	1·84	..	..	3·4	1·65

N.B. This sample was full of detritus, and very difficult to count, and this fact may account for the divergent results.

Centrifugation, membrane filter, dilution techniques

(Tables III samples 1 and 2, IV, V, IX samples 1 and 2, X, and XI)

In Table III, samples 1 and 2 show a third estimate of numbers, obtained by the serial dilution method. Further figures obtained from sea-water samples by this method are shown in Table IV, in which the figures are derived from counts shown in Table X (p. 145).

TABLE IV. TABLE OF RESULTS FROM SAMPLES ESTIMATED BY (*a*) CENTRIFUGATION, FOLLOWED BY COUNTING IN A HAEMACYTOMETER, AND (*b*) BY DILUTION

(Numbers are given per cubic millimetre of the original sample.)

Method	Mean	Standard deviation	Difference between means	Standard error of difference
	Sample 1, 9. iv. 51			
Centrifuge	32·0	8·26	..	..
Serial dilution	0·79	0·45	..	..
Allen's dilution	0·204	..	..	..
	Sample 2, 2. iv. 52			
Centrifuge 'Normal'	8·12	1·46	0·88	±0·66
Centrifuge 'Floc'	9·0	0·815		
Serial dilution	2·1	1·0	..	..
	Sample 3, 9. iv. 52			
Centrifuge 'Normal'	9·5	2·93	2·0	±1·48
Centrifuge 'Floc'	11·5	2·83		
Serial dilution	1·3	0·7	..	..

Tables III and IV show that the estimates from the dilution methods are extremely low. It appears that this method of estimation cannot be generally applied until a more suitable culture solution and optimum cultural conditions for all the organisms are available—that is, if it is desired to estimate the total number of organisms in the phytoplankton. Dr H. A. Cole, of Conway (private communication), states that 'We have found that figures for the density of flagellates in our tanks, obtained by direct counts of concentrated samples using the membrane filter technique, are almost invariably between about four and ten times those obtained by serial dilutions.'

Further corroboration of this fact is found in Table V, which shows the results of serial dilutions set up using cultures. The daily counts from these cultures over the period when the dilutions were set up show them all to be in a state of active growth (see Table XI, p. 145).

In this series of experiments, as in those using sea-water samples, a suitable preliminary dilution was made before the serial dilutions were set up. This preliminary dilution varied, 1 ml. of the sample to 100 ml. culture solution for a sea-water sample, and 1 : 10,000 for cultures, except *Pyramimonas*, where the preliminary dilution was 1 : 1000.

With the exception of flagellate K, a chrysomonad with three flagella, the numbers obtained by the dilution method were very low throughout these experiments.

TABLE V. THE RESULTS OF THE SERIAL DILUTION TECHNIQUE USED ON CULTURES OF FLAGELLATES, COMPARED WITH THE KNOWN COUNTS FROM THE CULTURES PRIOR TO DILUTION

(Results are expressed per cubic millimetre of the original culture.)

	Count from culture		Dilution method	
Organism	Mean	Expected σ	Most probable no.	σ
Pyramimonas sp.	90	30	39	21
Gymnodinium sp. 2	200	44·8	63	37
Pseudopedinella sp. 1	180	42·5	18	9
Flagellate K	340	58·4	240	121
Flagellate no. 16	255	50·5	33	17

TABLE VI. COMPARISON OF THE RESULTS OBTAINED BY CONCENTRATING SAMPLES BY CENTRIFUGATION AND THE USE OF THE MOLECULAR FILTER

(All samples are from Knap Buoy, and counts are expressed per cubic millimetre of the original sample.)

	Centrifuge		Molecular filter	
Sample	Mean	s	Mean	s
13. xi. 52. Total	20·0	3·8	18·06	3·97
Under 2 μ	12·5	2·64	14·85	3·0
2–under 5 μ	4·3	1·38	2·09	0·32
Over 5 μ	3·2	1·74	1·12	0·223
19. xi. 52. Total	19·8	4·76	16·8	2·1
Under 2 μ	14·3	3·4	14·2	1·91
2–under 5 μ	3·5	1·35	1·85	0·395
Over 5 μ	2·0	0·95	0·75	0·199
21. xi. 52. Total	21·8	4·06	21·6	1·35
Under 2 μ	14·4	3·44	18·1	0·9
2–under 5 μ	4·9	1·6	2·6	0·58
Over 5 μ	2·5	0·975	0·9	0·25
28. xi. 52. Total	24·3	4·94	22·8	0·94
Under 2 μ	16·3	3·44	16·9	0·92
2–under 5 μ	5·8	1·83	4·55	0·47
Over 5 μ	2·2	1·475	1·35	0·42
4. xii. 52. Total	23·3	3·06	24·6	1·65
Under 2 μ	15·4	3·47	19·98	1·79
2–under 5 μ	4·8	1·34	3·0	0·56
Over 5 μ	3·1	1·2	1·62	0·152

Centrifugation, molecular filter

(Tables VI and XII)

Results from the use of the molecular filter are very similar to those obtained after centrifugation. The figures in Table VI showing these results are derived from counts given in Table XII (p. 146).

This method has certain advantages in that diatoms may be estimated in

the same sample as nanoplankton, and that the sample may be made into a permanent preparation which can be counted at a later date. Its disadvantages are two, namely that for routine sampling the time involved in the preparation of a sample for counting is about 3 hr., and the actual counting is rather difficult, as the naked forms tend to shrink after killing, and so make identification difficult, if not impossible.

Centrifugation, Dr Millar's method

(Tables VII and XIII)

The last method used in this comparative study is the use of dark-ground illumination for counting an unconcentrated sample. The Thoma cell used has a total volume of 8·66 cubic millimetres, and this is the volume of water in which organisms were counted. The figures for these counts, together with those from samples after centrifuging, will be found in Table XIII (p. 147).

The results from the counts of cultures, given in the first part of Table VII, show that when only motile cells were counted under dark-ground illumination a large number of the individuals, at least of the two species used, were missed, because they had become non-motile. In other samples when both motile and non-motile cells were counted, a figure was obtained which was very near to the count from the culture prior to dilution. The counting of non-motile cells does, however, need considerable care, because a speck of detritus the same size as an organism will produce an image very similar to that of a living cell under dark-ground illumination.

Throughout the estimations of sea-water samples by this method, the results of which are given in the second part of Table VII, both motile and non-motile forms have been counted, and it can be seen that the agreement between the results obtained by the two methods is very close.

Discussion

In considering the relative merits of the above six methods for estimating nanoplankton the ultimate aim of the investigation must be clearly defined. There is very little difference between the efficiencies of the four counting methods with regard to the final estimate of numbers given. If the purpose is to determine only the total number of organisms in a sample of sea water, then counting under dark-ground illumination is the most rapid method. Counts after concentrating with the centrifuge or filters will also give this figure. If, however, the aim is to count the organisms, place them in size groups, and assign them as far as possible to their systematic position, the centrifuge or membrane filter for concentrating living samples should be used. Centrifugation is better because of its speed, and the uniformity of the subsequent counts, as there is no evidence of aggregation of the organisms after this treatment, as there was with the membrane filter.

TABLE VII. COMPARISON OF COUNTS USING DARK-GROUND ILLUMINATION WITH COUNTS FROM CULTURES AND AFTER CONCENTRATION BY CENTRIFUGATION

(a) Results obtained from cultures

(Numbers are per 0·1 mm.³.)

Organism	Straight count in haemacytometer	Dark-ground counts: Motile only	Dark-ground counts: Motile and non-motile
Hemiselmis rufescens Parke	189	138	179
Chromulina pusilla Butcher	426	230	450

In both cases the cultures were diluted prior to the dark-ground count: the dilution was 1:50 in the case of *Hemiselmis*, and 1:100 in the case of *Chromulina*.

(b) Results obtained from sea-water samples

(Numbers per cubic millimetre of the original sample.)

Sample	Size-group	Centrifuge and count	Dark-ground count
10. xii. 52. West end of breakwater	Total	17·6	16·3
	Under 2 μ	9·9	10·3
	2–under 5 μ	4·5	3·7
	5–under 10 μ	2·3	1·5
	Over 10 μ	0·9	0·8
11. xii. 52.	Total	19·3	19·2
	Under 2 μ	10·1	11·7
	2–under 5 μ	6·1	4·5
	5–under 10 μ	2·2	2·08
	Over 10 μ	0·9	0·92
12. xii. 52. Tank water from the Aquarium	Total	6·4	7·75
	Under 2 μ	4·3	5·15
	2–under 5 μ	1·2	1·62
	5–under 10 μ	0·5	0·64
	Over 10 μ	0·4	0·34
15. xii. 52. Knap Buoy	Total	11·4	10·2
	Under 2 μ	7·4	6·2
	2–under 5 μ	2·5	2·2
	5–under 10 μ	1·1	1·16
	Over 10 μ	0·4	0·64

Sample		Straight count	Diluted to 1/10 prior to dark-ground count
Mixed culture of diatoms and flagellates	Total	11·6	13·4
	Under 5 μ	7·9	8·9
	5–under 10 μ	2·6	3·1
	Over 10 μ	1·1	1·4

In this last sample the numbers are given per 0·1 mm.³.

The dilution methods give very low estimates of the numbers of nanoplankton in sea water, and the time elapsing between setting up the dilutions and the end of the period required for the organisms to give a reasonably thick culture may be several months. This makes the method cumbersome if a large number of samples are to be estimated.

The importance of cultures of nanoplankton should not, however, be underestimated, in view of the numbers of hitherto undescribed species which occur in sea water. It is very necessary to pick out organisms and grow them in species-pure culture in order to study them more closely. The two dilution methods can also be of great use in isolating certain organisms, as they save the tedious labour involved in isolating individual organisms by means of a micropipette.

Summary

Six methods for estimating the numbers of nanoplankton organisms in sea water have been compared. Of these, centrifugation of a living sample, followed by counts of the numbers of organisms in the concentrate, appears to be the most satisfactory method from all points of view. The method is rapid, simple to operate, and gives a result which is strictly comparable with results from counts made under dark-ground illumination of unconcentrated samples, and from counts after concentrating with filters. By this method the organisms counted can usually be assigned to the class and order to which they belong, even if their systematic position cannot be determined more definitely.

The use of species-pure cultures is a necessary adjunct to the direct examination of sea-water samples when studying the distribution, abundance, and annual fluctuations of nanoplankton, as so little is known of the systematics of these organisms.

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TABLE VIII. THE COMPARISON OF DIFFERENT SPEEDS AND TIMES OF CENTRIFUGATION USING BOTH 'NORMAL' SAMPLES AND THOSE TREATED WITH POTASSIUM ALUM ('FLOC')*

Sample 1

	'Floc'								'Normal'							
1500 r.p.m. for 10 min. (R.C.F. = 358)																
Total	5	10	12	7	5	6	5	11	5	10	8	8	9	7	5	6
Under 2 μ	..	4	3	3	3	3	3	5	2	5	3	4	4	5	3	4
2–under 5 μ	5	4	3	2	1	2	2	4	1	3	2	3	4	2	2	1
Over 5 μ	..	2	6	2	1	1	..	2	2	2	3	1	1	..	..	1
1500 r.p.m. for 15 min. (R.C.F. = 358)																
Total	7	15	7	11	10	11	12	6	15	11	9	12	8	13	9	5
Under 2 μ	4	8	3	5	4	5	3	3	7	4	2	6	5	5	3	..
2–under 5 μ	2	4	2	3	1	3	5	3	5	4	4	4	2	6	4	4
Over 5 μ	1	3	2	3	5	3	4	..	3	3	3	2	1	2	2	1
1500 r.p.m. for 30 min. (R.C.F. = 358)																
Total	8	11	9	6	11	14	11	10	11	12	14	11	10	6	11	15
Under 2 μ	3	5	4	4	6	4	4	7	6	2	4	3	4	4	3	5
2–under 5 μ	4	5	3	2	4	6	5	3	3	4	5	4	4	2	5	7
Over 5 μ	1	1	2	..	1	4	2	..	2	6	5	4	2	..	3	3
1500 r.p.m. for 1 hr. (R.C.F. = 358)																
Total									12	9	13	12	8	7	6	13
Under 2 μ									5	4	6	6	4	5	2	6
2–under 5 μ									6	4	5	5	3	2	4	5
Over 5 μ									1	1	2	1	1	..	..	2

Sample 2, Knap Buoy, 3. vi. 52

(All counts made with untreated samples)

1500 r.p.m. for 15 min. (R.C.F. = 358)								
Total	24	22	25	29	29	24	26	27
Under 2 μ	13	4	15	18	18	18	17	17
2–under 5 μ	6	8	4	4	9	4	5	5
Over 5 μ	5	10	6	7	2	2	4	5
2000 r.p.m. for 15 min. (R.C.F. = 636)								
Total	25	24	19	30	24	28	24	28
Under 2 μ	16	13	14	19	16	18	13	24
2–under 5 μ	3	3	3	6	4	2	3	2
Over 5 μ	6	8	2	5	4	8	8	2
2000 r.p.m. for 30 min. (R.C.F. = 636)								
Total	24	21	26	23	23	23	33	30
Under 2 μ	12	15	15	17	16	14	22	19
2–under 5 μ	4	4	5	2	4	3	5	7
Over 5 μ	8	2	6	4	3	6	6	4

Sample 3

(Both untreated samples, i.e. 'Normal', and only the totals are given)

1500 r.p.m. for 15 min.	12	14	10	10	12	7	11	7
2000 r.p.m. for 15 min.	11	5	7	10	11	9	9	10

* Throughout these tables (VIII–XIII), unless otherwise stated, the figures given refer to one field of the Thoma haemacytometer, i.e. they are equivalent to 1 mm.^{3} of the original sample in the case of a centrifuged sample and 5 mm.^{3} of a filtered sample.

TABLE IX. COUNTS FROM THE SAME SAMPLES AFTER CONCENTRATING TO 1/10 WITH THE CENTRIFUGE, AND TO 1/50 WITH THE MEMBRANE FILTER

(All samples are from Knap Buoy)

Sample 1, 27. v. 52

Centrifuge																
Total	18	16	17	32	27	19	22	24	17	18	16	22	19	17	19	19
Under 2 μ	8	7	9	14	13	9	11	11	6	7	6	10	7	11	10	8
2–under 5 μ	6	7	6	16	12	8	8	10	5	7	7	8	9	5	6	4
Over 5 μ	4	2	2	2	2	2	3	3	6	4	3	4	3	1	3	7
Membrane filter																
Total	130	122	106	88	82	82										
Under 2 μ	78	86	72	54	48	50										
2–under 5 μ	40	30	28	26	26	22										
Over 5 μ	12	6	6	8	8	10										

Sample 2, 29. v. 52

Centrifuge																
Total	19	15	30	24	24	25	25	19	26	24	22	29	15	22	17	16
Under 2 μ	10	9	14	12	10	13	13	10	13	12	13	14	9	11	8	7
2–under 5 μ	4	3	13	7	10	8	8	6	8	8	8	6	4	7	3	3
Over 5 μ	5	3	3	5	4	4	4	3	5	4	1	9	2	4	6	6
Membrane filter																
Total	63	95	104	90	78	108										
Under 2 μ	40	55	64	60	44	60										
2–under 5 μ	18	30	28	22	24	28										
Over 5 μ	5	10	12	8	10	20										

Sample 3, 3. vi. 52

Centrifuge																
Total	21	45	39	29	34	37	35	29	41	35	40	31	34	22	25	33
Under 2 μ	11	31	20	18	22	26	23	21	23	23	25	19	21	14	19	21
2–under 5 μ	7	6	12	8	6	7	6	4	10	7	6	6	9	5	2	6
Over 5 μ	3	8	7	3	6	4	6	4	8	5	9	6	4	3	4	6
Membrane filter																
Total	222	186	213	205	180	205										
Under 2 μ	124	110	119	145	130	157										
2–under 5 μ	70	50	60	30	32	24										
Over 5 μ	28	26	34	30	18	24										

Sample 4, 6. vi. 52

Centrifuge								
Total	31	48	49	32	37	36	25	30
Under 2 μ	22	36	35	23	25	29	19	18
2–under 5 μ	4	4	7	2	8	4	4	3
Over 5 μ	5	8	7	7	4	3	2	9
Membrane filter								
Total	126	181	191	186	165	188		
Under 2 μ	86	138	124	138	118	140		
2–under 5 μ	26	22	38	26	24	20		
Over 5 μ	14	21	29	22	23	28		

Sample 5, 9. vi. 52

Centrifuge										
Total	26	39	24	23	24	27	25	33	21	29
Under 2 μ	16	24	17	16	20	20	16	21	17	23
2–under 5 μ	7	10	4	4	2	4	6	5	3	2
Over 5 μ	3	5	3	3	2	3	3	7	1	4
Membrane filter										
Total	80	169	100	172	161	133				
Under 2 μ	51	130	74	128	124	96				
2–under 5 μ	20	20	18	32	26	26				
Over 5 μ	9	19	8	12	11	11				

TABLE X. TABLE OF COUNTS FROM CENTRIFUGED SAMPLES FROM WHICH SERIAL DILUTIONS WERE ALSO SET UP

Sample 1, 3 miles south of Looe Island, 9. iv. 51

Total	30	39	43	22	39	24	27
Under 2 μ	16	9	8	8	17	12	13
2–under 5 μ	9	11	24	12	11	9	11
Over 5 μ	5	19	11	2	11	3	3

Sample 2, 2. iv. 52

	'Floc'				'Normal'							
Total	9	9	10	8	7	10	9	8	7	10	8	6
Under 2 μ	7	5	7	5	5	5	5	3	5	4	4	3
2–under 5 μ	2	4	3	3	2	4	4	4	2	3	3	2
Over 5 μ	..	..	..	..	..	1	..	1	..	3	1	1

Sample 3, 2 miles south of Rame, 9. iv. 52

	'Floc'								'Normal'							
Total	15	12	10	9	16	8	12	10	9	13	15	9	7	8	7	8
Under 2 μ	7	3	5	6	10	4	7	4	4	6	6	4	2	4	3	3
2–under 5 μ	6	4	3	3	5	4	4	5	2	4	5	4	2	3	3	3
Over 5 μ	2	5	2	..	1	..	1	1	3	3	4	1	3	1	1	2

TABLE XI. TABLE OF COUNTS TO DETERMINE GROWTH-RATES OF CULTURES USED TO SET UP SERIAL DILUTIONS

Days after inoculation	*Pyramimonas* sp.		*Gymnodinium* sp. 2		*Pseudopedinella* sp. 1	
	Mean	σ (theor.)	Mean	σ (theor.)	Mean	σ (theor.)
0	..	..	59	24·3	75	27·4
1	..	..	74	27·2	82	28·6
2	20	14·3	87	29·5	118	34·4
3	36	19·0	110	33·2	131	36·2
4	61	20·47	152	39·0	162	40·3
5	90*	30·0	200*	44·8	180*	42·5
6	..	..	292	54·0	260	51·0
7	400	63·3	..	..	..	..
8	710	84·4	..	..	..	..

	Flagellate K		Flagellate 16	
	Mean	σ (theor.)	Mean	σ (theor.)
0	200	44·7	200	44·7
1	213	46·2	220	47·0
2	..	..	255*	50·5
3	250	50·0	..	..
4	340*	58·4	290	54·0
5	520	72·0	310	55·7

The standard deviation given here is theoretical, based on the Poisson Distribution, and equal to $\sqrt{(\text{Mean of counts})}$.

* Indicates the density of the culture at which the serial dilution was set up. Numbers are given per cubic millimetre of the culture.

TABLE XII. COUNTS OBTAINED FROM THE SAME SAMPLE AFTER CONCENTRATION TO 1/10 WITH THE CENTRIFUGE AND TO 1/63 WITH THE MOLECULAR FILTER

(The figures for the counts on the molecular filter refer to one field of the microscope. All samples are from Knap Buoy.)

	Centrifuge										Molecular filter			
Sample 1, 13. xi. 52														
Total	20	17	25	27	19	16	21	16	22	17	132	140	89	97
Under 2 μ	11	10	18	15	12	12	14	9	13	11	112	117	70	78
2–under 5 μ	3	4	5	7	6	3	4	3	4	4	15	15	11	12
Over 5 μ	6	3	2	5	1	1	3	4	5	2	5	8	8	7
Sample 2, 19. xi. 52														
Total	21	19	26	17	13	12	19	23	25	23	117	119	94	96
Under 2 μ	14	13	19	13	9	10	13	16	18	18	100	101	79	80
2–under 5 μ	6	3	5	3	3	1	3	4	4	3	12	15	9	11
Over 5 μ	1	3	2	1	1	1	3	3	3	2	5	3	6	5
Sample 3, 21. xi. 52														
Total	25	24	24	18	25	18	25	15	26	18	149	134	129	135
Under 2 μ	15	12	18	11	19	12	19	10	16	12	121	115	107	115
2–under 5 μ	7	8	5	4	4	4	4	3	6	4	21	13	18	14
Over 5 μ	3	4	1	3	2	2	2	2	4	2	7	6	4	6
Sample 4, 28. xi. 52														
Total	30	33	20	19	29	26	23	20	20	23	149	137	150	144
Under 2 μ	20	17	15	12	22	20	15	14	12	16	111	101	114	105
2–under 5 μ	7	10	3	5	5	5	6	5	6	6	33	26	28	28
Over 5 μ	3	6	2	2	2	1	2	1	2	1	5	10	8	11
Sample 5, 4. xii. 52														
Total	27	25	22	23	24	18	20	26	27	21	163	152	165	143
Under 2 μ	17	18	15	16	16	11	10	18	21	12	138	118	135	115
2–under 5 μ	5	5	4	4	3	5	6	6	4	6	16	24	19	17
Over 5 μ	5	2	3	3	5	2	4	2	2	3	9	10	11	11

TABLE XIII. COUNTS OBTAINED FROM THE SAME SAMPLE BY (*a*) CONCENTRATING TO 1/10 WITH THE CENTRIFUGE AND (*b*) COUNTING UNDER DARK-GROUND ILLUMINATION

(Numbers here are equivalent to 0·1 mm.³ of the original sample for a straight count, 1 mm.³ for a sample concentrated by centrifuging and 8·66 mm.³ in the dark-ground count.)

(*a*) *Counts of cultures*

Organisms	Straight count				Culture diluted: dark-ground counts: Motile cells		Motile and non-motile cells		
Chromulina pusilla Butcher	386	480	412		190	206	419	359	
Hemiselmis rufescens Parke	184	170	212	190	243	234	327	268	332

(*b*) *Counts of samples*

	Centrifuge and count										Dark-ground count		
Sample 1, west end of the Breakwater, 10. xii. 52													
Total	16	17	19	19	19	13	20	15	18	20	107	190	124
Under 2 μ	8	9	10	11	12	8	11	10	9	11	65	138	64
2–under 5 μ	5	4	6	3	3	4	6	3	6	5	21	36	39
5–under 10 μ	3	2	3	2	3	0	3	1	3	3	14	11	14
Over 10 μ	0	2	0	3	1	1	0	1	0	1	7	5	7
Sample 2, 11. xii. 52													
Total	20	22	16	17	21	20	17	19	22	19	182	150	
Under 2 μ	13	11	10	8	12	11	8	9	10	9	113	89	
2–under 5 μ	5	6	4	5	6	8	5	7	8	7	40	38	
5–under 10 μ	1	3	2	3	2	0	3	2	3	3	19	17	
Over 10 μ	1	2	0	1	1	1	1	1	1	0	10	6	
Sample 3, aquarium tank water, 12. xii. 52													
Total	8	6	5	7	6	4	8	7	6	7	62	72	
Under 2 μ	5	4	4	5	4	4	4	4	5	4	41	48	
2–under 5 μ	1	1	1	1	2	0	2	2	1	1	12	16	
5–under 10 μ	1	0	0	1	0	0	1	1	0	1	5	6	
Over 10 μ	1	1	0	0	0	0	1	0	0	1	4	2	
Sample 4, Knap Buoy, 15. xii. 52													
Total	12	10	14	9	12	14	10	8	13	12	96	80	
Under 2 μ	10	7	10	6	7	7	6	6	7	8	56	51	
2–under 5 μ	1	2	3	2	2	3	3	2	4	3	21	17	
5–under 10 μ	1	1	0	1	2	3	0	0	2	1	13	7	
Over 10 μ	0	0	1	0	1	1	1	0	0	0	6	5	

	Straight count										Dark-ground count after dilution to 1/10	
Sample 5, mixed culture of diatoms and flagellates												
Total	11	13	14	13	9	10	13	11	12	10	108	124
Under 5 μ	8	10	9	9	6	8	9	6	7	7	70	82
5–under 10 μ	2	3	3	4	2	1	2	3	4	2	29	27
Over 10 μ	1	0	2	0	1	1	2	2	1	1	9	15

THE FEEDING HABITS OF PLAICE POST-LARVAE IN THE SOUTHERN BIGHT

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

Introduction

The overfishing of valuable plaice grounds in the southern North Sea, has diverted attention from the natural history of the species (*Pleuronectes platessa*) towards a better understanding of its population dynamics. The early statistical studies of Buchanan-Wollaston (1914–26) have been followed by the recent work of my colleague Mr A. C. Simpson, at the Fisheries Laboratory, Lowestoft, on egg production and survival of larvae over the plaice spawning ground in the Southern Bight.

Observations of a purely biological nature are often needed to clarify the pattern of population fluctuations derived from statistical data. For instance, efforts to assess the mortality of plaice in the Southern Bight had been somewhat impeded by gaps in our knowledge of the food and feeding behaviour of the post-larval phase. In 1950 I was given the opportunity to fill in these gaps.

A survey of literature on this subject revealed a variation in food preferences of post-larvae from different coastal areas of Great Britain. Kyle (1898), in his investigation of the Scottish plaice fishery, discovered young annelid larvae, crustacean ova, *Evadne nordmanni*, and larval molluscs in the post-larval stomachs. Lebour (1919), working at Plymouth, concluded that certain copepods and other Entomostraca formed the main food of nearly all young flatfish caught, though the plaice was poorly represented in this examination. Scott (1922), in the Irish Sea area, found harpacticoids, small copepods and algal spores to be the chief food constituents of the plaice stomachs.

As a result of the dissection of some hundreds of specimens taken in four different years the diet of plaice post-larvae from the Southern Bight is now known, in normal seasons, to be markedly different from that in the areas examined by earlier workers (Table I).

Collection and Dissection Methods

In the early months of 1946 and 1947, the Hensen net was used to catch planktonic plaice post-larvae during egg-sampling cruises. The Heligoland larva net and the Agassiz trawl were introduced in 1950 and 1951, as work

progressed on the post-hatching stages. Larvae and post-larvae were preserved in 2% formalin, to be later measured and classified into developmental stages 1–5. Stage 1 is the larva in which the yolk-sac is not yet resorbed. Post-larval stages 2 and 3 are planktonic and still symmetrical, whereas stage 4 covers the movement of the left eye on to the edge of the head. Stage 5, with both eyes on the right side, is thought to be the first wholely bottom stage, approaching completion of metamorphosis.

TABLE I. SUMMARY OF PLAICE POST-LARVAL FEEDING

Date of cruise	No. of post-larvae examined	Empty stomachs	Post-larvae feeding on *Oikopleura*	% feeding post-larvae containing *Oikopleura*	Other food
1946					
20–22 Jan.	158	80	65	82·5	Few copepod nauplii, significant numbers *Coscinodisus* spp., green food remains
1947					
22–28 Jan.	1	—	1	—	Nil
13–18 Feb.	35	11	—	—	Copepod nauplii, invertebrate eggs, bivalve veliger, *Coscinodiscus* frustules, green food remains
11–14 Mar.	19	7	—	—	Copepod nauplii, copepodites, small copepods, diatom debris
22–28 Mar.	6	1	—	—	*Temora longicornis* nauplii and polychaete bristles
1950					
9–12 Mar.	83	23	47	78·3	2 *Temora longicornis*, 3 copepod fragments, 1 polychaete larva, occasional diatom frustule
1951					
10–20 Feb.	32	17	12	80·0	Only few diatom frustules
27 Feb.–7 Mar.	34	14	19	95·0	1 gastropod veliger, few diatom frustules
20–31 Mar.	129	65	51	79·7	2 *Temora longicornis*, 3 fragmentary copepods, 5 with palps of *Magelona papillicornis*, occasional copepod eggs, single gastropod veliger

In 1950 and subsequent years, the gut contents were examined as follows. Specimens were placed on a black background, in order to emphasize the opaque outlines of the coiled alimentary canal. The body wall was dissected away on one side with needles; the gut severed in the anterior oesophageal and posterior anal regions, then removed into water in a watch-glass. No optical aids were needed. After disengaging liver and muscular tissue under a dissecting microscope, the gut was transferred to a drop of water on a slide and divided into three sections, by transverse cuts at the anterior and posterior stomach sphincters. These sections were laid open with needles, the lumen contents separated from gut tissue by gentle teasing, and the former examined under high power.

The Diet in Recent Years

In February–March 1950, eighty-three undamaged post-larvae, between 5·5 and 15·5 mm. in length, were taken in the Agassiz trawl off the Dutch coast. Subsequent examination of the gut contents showed that forty-seven specimens contained black cigar-shaped pellets, always associated with transparent, indigestible matrix vesicles, arranged along the length of the gut like peas in a pod (Plate I, fig. 1). Only five specimens held the chitinous remains of copepods; in two these remains were identified as *Temora longicornis*, in the other three they were too fragmentary for identification. Twenty-three stomachs were empty. Diatoms were usually found associated with animal food remains, the principal genera being *Coscinodiscus*, *Biddulphia*, *Navicula* and *Nitzschia*. The larger thin-walled diatoms are readily taken by the yolk-sac stage larvae and the early stage-2 post-larvae. They probably represent the 'green food remains' observed in the past by a number of workers.

The black pellets and surrounding matrix vesicles were studied more closely (Pl. I, fig. 2). Pellets varied greatly in length (0·12–0·60 mm.), but their shape was more consistent. Their number varied from specimen to specimen, and their distribution within the gut was not constant. As many as sixty-two pellets were found in one gut alone, forty-two in the stomach and twenty in the intestine. As fish post-larvae are not known to produce compact faeces, these pellets were judged to be formed by a certain type of animal prey. The prey would have been soft, easily and almost completely digestible, leaving characteristically shaped pellets and a thin matrix vesicle as the only evidence of its nature. Zooplankton samples from the spawning area were examined for such an animal. *Oikopleura dioica* (Pl. I, fig. 4) fitted the description and was present at all stations where post-larvae occurred. The shape variation of pellets from the gut of *Oikopleura* and from the gut of post-larvae taken in the same locality are compared in Text-fig. 1. There is a distinct relationship. The indigestible matrix vesicles were, without doubt, the developing 'houses' of *Oikopleura* eaten by the young plaice. Lohmann (1903), in his work on the Appendicularia, noted the resistant nature of these structures. The cuticular secretions of the tunicates are now known to have a chemical composition closely allied to cellulose. The absence of cellulase from the enzymic system of the plaice post-larva would explain the indigestibility of the 'house'.

During the spring of 1951, a further series of Agassiz hauls was made over the plaice spawning grounds. A total of 195 post-larvae were caught, eleven of which bore undigested *Oikopleura* in the oesophagus (Pl. I, fig. 3), whilst a further seventy-one contained typical pellets and vesicles. Seventeen specimens held other animal food, principally *Temora longicornis*, copepod eggs and molluscan veligers. The palps of *Magelona papillicornis*, a sedentary polychaete, were found in the stomachs of a few late stage-5 post-larvae.

TABLE II. ABSTRACT OF PLANKTON ANALYSIS FOR THE SOUTHERN BIGHT, 1947

Main potential food organisms of plaice post-larvae per cubic metre. Vertical Hensen net hauls (60 meshes/1 in.).

Species	Station no. 1	2	3	4	5	6	7	8
Month of cruise: January								
Small copepods	N.D.	87	205	234	214	445	868	N.D.
Polychaete metatrochophores	N.D.	—	—	—	—	—	—	N.D.
Polychaete post-larvae	N.D.	—	—	—	—	—	20	N.D.
Lamellibranch post-larvae	N.D.	4	—	21	164	70	39	N.D.
Oikopleura spp.	N.D.	4	—	9	7	3	20	N.D.
Coscinodiscus concinnus	N.D.	—	—	—	33	127	296	N.D.
Biddulphia regia	N.D.	—	—	47	296	19,826	26,250	N.D.
Month of cruise: March								
Small copepods	103	56	77	77	3,796	345	329	129
Polychaete metatrochophores	4	9	—	—	56	—	16	4
Polychaete post-larvae	—	—	—	—	—	—	—	—
Lamellibranch post-larvae	—	—	22	—	422	—	41	—
Oikopleura spp.	—	—	11	—	—	—	—	—
Coscinodiscus concinnus	123	59	33	131	12,656	181	1,376	16,286
Biddulphia regia	—	2	153	1,796	1,026,563	3,055	7,904	129
Month of cruise: April								
Small copepods	1	131	112	136	129	173	94	92
Polychaete metatrochophores	—	6	13	—	—	12	23	—
Polychaete post-larvae	—	—	—	14	—	—	—	26
Lamellibranch post-larvae	—	—	—	—	—	—	—	—
Oikopleura spp.	—	—	—	—	—	—	—	—
Coscinodiscus concinnus	59	938	108	32	246	190	598	99
Biddulphia regia	—	47	15,361	289	43	138	105	59
Month of cruise: May								
Small copepods	2,918	9	11	82	195	664	1,059	N.D.
Polychaete metatrochophores	14	—	—	—	—	—	4	N.D.
Polychaete post-larvae	—	—	—	3	—	—	20	N.D.
Lamellibranch post-larvae	—	—	—	—	—	—	16	N.D.
Oikopleura spp.	—	—	—	—	—	—	—	N.D.
Coscinodiscus concinnus	879	104	203	—	3	—	4	N.D.
Biddulphia regia	8	66	53	—	25	—	—	N.D.

N.D. = no data.

Station nos. 1–8 represent a line of stations 12 miles apart stretching across the Southern Bight, from Smith's Knoll to the Hook of Holland.

TABLE III. ABSTRACT OF PLANKTON ANALYSIS FOR SOUTHERN BIGHT, EARLY MARCH 1950

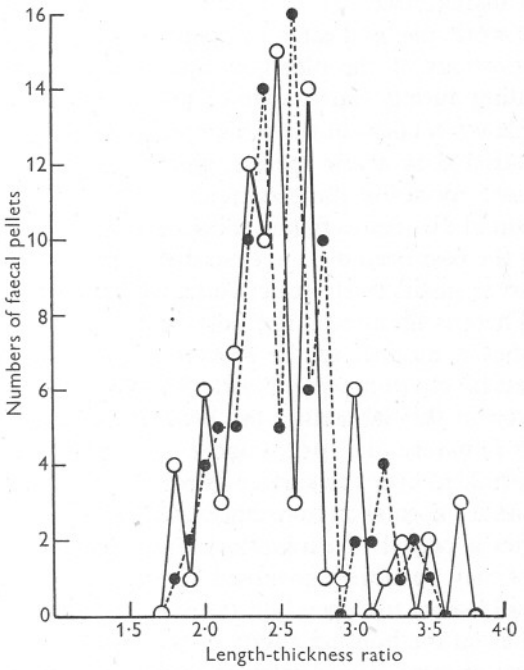
Main potential food organisms of plaice post-larvae per m.^{3}. Vertical Hensen net hauls (60 meshes/1 in.).

Species	Station no. 11	12	23	24	25	34	35	38	39	48
Small copepods	951	1,987	191	613	239	234	244	285	204	40
Lamellibranch post-larvae	—	—	6	5	7	—	3	—	—	2
Oikopleura spp.	82	52	181	—	83	6	54	7	41	13
Coscinodiscus concinnus	—	—	—	—	—	—	—	—	—	10
Biddulphia regia	74,320	142,629	2,694	2,885	128,892	89	7,398	152	38	31

Plankton organisms less than 0·35 mm. in length are not filtered quantitatively by the Hensen net. However, size measurements of the faecal pellets taken from plaice stomachs suggest that smaller *Oikopleura* were either relatively scarce or were not used as food by plaice post-larvae.

Ninety-six stomachs were empty. Towards the end of March 1951, fifty completely metamorphosed young plaice (15–17 mm. long), collected at stations about 25 miles west of Ijmuiden, were found to be feeding mainly on the palps of *M. papillicornis*.

Records for January 1946 show that a high proportion of feeding post-larvae of an early stage, had 'detrital pellets' in the alimentary canal. On the other hand, post-larvae caught with a Hensen net during 1947 appear to have fed on a mixed diet of large diatoms and small copepods, with an occasional molluscan veliger and polychaete larva.



Text-fig. 1. Variation in length/thickness ratio of equal numbers of pellets, from the gut of plaice post-larvae (black circles—continuous line) and of *Oikopleura dioica* (open circles—broken line).

Thus in 1946, 1950 and 1951, plaice post-larvae from the Southern Bight seem to have utilized the small population of *Oikopleura* present as a principal source of animal food during their planktonic life (Table I). Preliminary observations for 1952 give a similar picture.

The anomalous situation in early 1947, when other species assumed principal importance as food, has yet to be explained. Probably the prolonged easterly gales and icy conditions in the plaice spawning area delayed the annual increase of *Oikopleura* stocks. The organisms comprising this alternative diet are usually more abundant than *Oikopleura* in the plankton during the post-larval development period of the plaice (Tables II and III).

The Phenomenon of Food Selection

There is much evidence to support the view that flat-fish post-larvae are discriminate feeders. Dannevig (1897) was of the opinion that only one food species was eaten for a time by baby plaice, though different individuals may eat different species. Petersen (1894) observed that dabs, plaice and flounders living under the same food conditions, had distinctly different food preferences. Lebour (1919) concluded that most young fish select their food from what is commonest in the plankton at the time, indiscriminate feeding seldom or never taking place.

In the present work, the food eaten by plaice post-larvae bore little relationship to the proportions of the plankton constituents. As an example, the summary of feeding during March 1950 (Table I), should be compared with the analysis of plankton taken in the same area at the same time (Table III). Feeding was restricted to a few species, and was not in proportion to the abundance of those species in the plankton.

The mechanism of diet restriction is still in doubt. The popular term 'food selection' has in the past been used to describe an effect, with the implication that fish post-larvae instinctively select certain food species when presented with a choice. There is no question of post-larvae being inherently fitted to utilize only a limited number of the species of prey normally available to them in their natural environment. North Sea plaice post-larvae have been successfully reared in this laboratory on *Artemia salina* nauplii, which they do not encounter in nature. In 1951, stage-2 post-larvae fed eagerly on dried egg fragments sprinkled over the surface of sea water in tanks.

Conceivably, under normal circumstances, young fish are able to recognize suitable food after a period of exploratory hunting activity, and the extent of this suitability may be largely governed by the vulnerability of the prey. Vulnerability would seem to depend on the size, structure and behaviour of the prey, as well as on the hunting ability of the predator.

The importance of prey size in relation to the mouth and gullet proportions of a post-larva was discussed by Lebour (1919), and elaborated recently by Wiborg (1948) for cod larvae. Observations made at sea in March 1952 revealed that stage-3 plaice post-larvae were feeding on *Oikopleura*, *Temora* nauplii, metanauplii and copepodites, which were particularly abundant, and on the large diatoms *Coscinodiscus concinnus* and *Biddulphia regia*. These organisms were occasionally found together in the same stomach. Post-larvae in earlier stages of development were eating smaller *Oikopleura*, nauplii and the usual diatoms. Advanced larval and early post-larval forms fed on diatoms only, the smallest of these food types.

Structure and behaviour play an equally important part in determining the vulnerability of prey. Lebour (1918) noticed that decapod larvae were rarely eaten by young fish, and attributed this to the spinous nature of the prey.

The number and efficiency of sense organs, conspicuousness, special escape reactions, secretion of repellents, and the swarming habit, are but a few of the factors which might well decide how far a particular prey is open to attack by a particular predator.

With the concept of vulnerability in mind, it is easy to see why the soft-bodied, slow-moving, but conspicuously vibratile *Oikopleura* should fall a ready victim to a visual feeder, as the plaice post-larva appears to be (see below).

Diurnal Feeding Activity

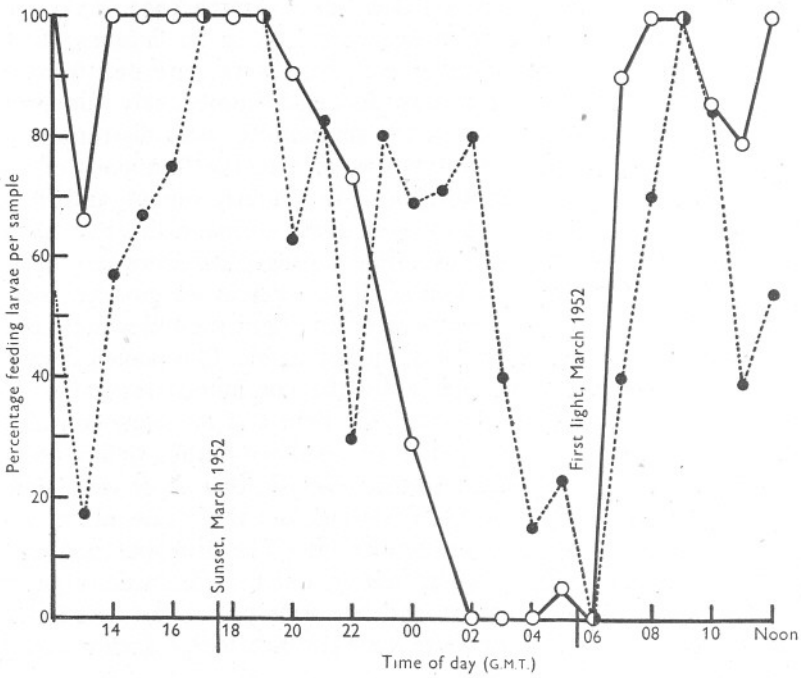
Forty-four per cent of all post-larval stomachs were empty. Other workers have referred to the lack of food traces in a high proportion of the total stomachs they investigated. Scott (1922) thought that the easy rupture of the stomachs might account for emptiness in the early planktonic forms, and that regurgitation in the net, or on addition of preservative, took place with metamorphosing and later stages. Lebour (1921) commented on the high numbers of empty stomachs encountered during work at Plymouth, and considered that infrequent feeding coupled with rapid digestion, might serve as a protective measure in young clupeoids. In these circumstances, feeding fish would only occasionally be caught.

The regurgitation theory was tested at sea in March 1952. Two vertical Heligoland larva-net hauls were made every 2 hr. in 20 fathoms of water. The plaice larvae and post-larvae of each haul were separated from other planktonic material. One catch was dissected immediately for stomach contents, and the other preserved in 2% formalin for later dissection. After 24 hr. work, no significant difference was seen in the frequency of empty stomachs between the two samples from any 2-hourly station, and the conclusion was reached that the addition of dilute formalin did not stimulate antiperistalsis. However, as the experiment progressed from day to night, a distinct falling off in the proportion of filled stomachs was noticed, followed by a sudden burst of feeding activity after first light the following morning. The experiment was continued for a further 24 hr. The pooled results for both days are represented in Fig. 6 by the continuous line. Post-larval feeding was most intense shortly after first light and for some considerable time before darkness fell, with a period of desultory feeding before and after noon. Data for the 4 years under consideration in this paper were analysed in a similar way to those of the 48 hr. station, and the results are presented in Table IV and Text-fig. 2 by the broken line. The principal gradients are not as sharply defined as for the 48 hr. station, due to differences in the extent of daylight hours between January and late March, but the first light and afternoon peaks of feeding activity are quite distinct, with a decline occurring in the early hours of the morning.

Light conditions seem to play an important part in determining the

TABLE IV. INCIDENCE OF PLAICE POST-LARVAE WITH FOOD IN THE STOMACH

	48 hr. station		Other stations	
Hour	Total larvae	No. feeding	Total larvae	No. feeding
13.00	6	4	12	2
14.00	11	11	7	4
15.00	4	4	9	6
16.00	16	16	28	21
17.00	1	1	8	8
18.00	10	10	—	—
19.00	4	4	9	9
20.00	37	34	40	25
21.00	—	—	41	34
22.00	46	35	70	21
23.00	—	—	15	12
Midnight	14	4	16	11
01.00	—	—	35	25
02.00	3	0	20	16
03.00	7	0	20	8
04.00	19	0	13	2
05.00	19	1	17	4
06.00	24	0	2	0
07.00	51	46	15	6
08.00	9	9	13	9
09.00	19	18	2	2
10.00	7	6	13	11
11.00	8	7	26	10
Noon	12	12	67	36



Text-fig. 2. Feeding periodicity of plaice post-larvae. Percentage of larvae in each sample with food in the gut, from the 48 hr. station, March 1952 (open circles—continuous line), and from other stations of recent years (black circles—dotted line).

periodicity of feeding activity in plaice post-larvae. This conclusion is reinforced by laboratory work, in which the post-larvae of plaice and plaice-flounder hybrids were subjected to changes in light conditions, other environmental factors being equal. The experiment was carried out at 9° C. using *Artemia salina* nauplii as food. On the first day of the experiment, five beakers containing batches of post-larvae were standing in tank *A*, surrounded by circulating water at 9° C., open to artificial and diffuse daylight. That same night, three of these beakers (7, 12 and 15*a*) were transferred to tank *B*, in which conditions were the same as in tank *A*, except for the complete exclusion of light. These three beakers were returned to tank *A* the following

TABLE V. THE FEEDING ACTIVITY OF POST-LARVAE IN RELATION TO LIGHT

1952	Time	Plaice 7	Plaice 11	Hybrid 12	Hybrid 15*a*	Hybrid 16*b*
29 Jan.	11.00	Light 1/7	Light 1/6	Light 9/9	Light 4/10	Light 1/10
	15.20	Light —	Light —	Light —	Light —	Light 7/10
	17.30	Light 2/7	Light 3/6	Light 8/9	Light 7/10	Light 7/10
30 Jan.	11.00	Dark 0/7	Light 2/6	Dark 0/9	Dark 0/10	Light —
	13.00	Dark 0/7	Light —	Dark 0/9	Dark 0/10	Light 9/10
	15.15	Dark 0/7	Light 3/6	Dark 0/9	Dark 0/10	Light 8/10
	18.00	Dark 0/7	Light 4/6	Dark 0/9	Dark 0/10	Light 8/10
31 Jan.	10.30	Light 1/7	Dark 0/6	Light 5/9	Light 6/10	Dark 0/10
	14.30	Light 5/7	Dark 0/6	Light 7/9	Light 8/10	Dark 0/10
	18.00	Light 7/7	Dark 0/6	Light 7/9	Light 8/10	Dark 0/10
1 Feb.	10.40	Light 2/7	Light 0/6	Light 4/9	Light 6/10	Light 8/10
	18.00	Light 4/7	Light 2/6	Light 7/9	Light 4/10	Light 9/10

The fractions give the number of post-larvae with food in their stomachs (numerator) and the total number of post-larvae in the beaker (denominator).

evening, whilst the remaining two beakers (11 and 15*b*) took their place in darkened tank *B*. On the evening of the third day, beakers 11 and 15*b* were transferred back to tank *A*. Observations on post-larval feeding activity were made each day at approximately 10.30, 15.00 and 18.00 hr., and the results are recorded in Table V. On no occasion were post-larvae found feeding in darkness; in lighted conditions plaice-flounder hybrids were more vigorous feeders than plaice.

Thus there are good grounds for concluding that plaice post-larvae are visual feeders, although the possibility of an inherent daily feeding rhythm cannot be entirely overlooked. For present purposes, it is sufficient to say that a large proportion of the empty stomachs encountered during the investigation of their diet in the Southern Bight, can be accounted for by big diurnal fluctuations in feeding activity influenced by light conditions.

I am indebted to members of the staff of the Fisheries Laboratory at Lowestoft, in particular to Mr A. C. Simpson, for the experimental data on the feeding periodicity of post-larvae in tanks and for access to material from his plaice and larva surveys of 1946–52. I would also like to thank Mr J. C. Cattley for the plankton analyses and Dr H. A. Cole of the Shellfish Experimental Station, Conway, for his criticism of the manuscript.

Summary

An examination was made of the stomach contents of plaice post-larvae taken from the Southern Bight spawning ground in the spring of 1950. They were found to be feeding mainly on a soft-bodied prey, which left characteristically shaped faecal pellets and an undigested matrix vesicle as the only evidence of its nature. Pellets from the post-larvae compared closely in shape with those of *Oikopleura dioica*, found in the plankton of the spawning area during the period of post-larval development.

In 1951, undigested *Oikopleura* were seen in the oesophageal regions of a few dissected specimens, in addition to typical indigestible remains in a large proportion of the total feeding post-larvae examined.

Records for 1946 suggest a similar exploitation of the *Oikopleura* population by plaice post-larvae, although the phenomenon was not repeated in 1947, when inclement weather may have interfered with the annual outburst of this prey.

The phenomenon of food selection by plaice post-larvae is discussed, with special reference to the part that vulnerability may play in limiting the number of species of prey available as food.

A feature of this and other investigations on post-larval feeding, was the occurrence of a high proportion of empty stomachs. An experiment designed to test a regurgitation hypothesis, revealed the existence of a marked diurnal pattern of feeding activity, apparently influenced by light conditions. The importance of light in feeding behaviour was confirmed by tank experiments. In this investigation a large proportion of the empty stomachs encountered can be explained by reference to diurnal fluctuations in feeding activity, supporting the view that plaice post-larvae are visual feeders.

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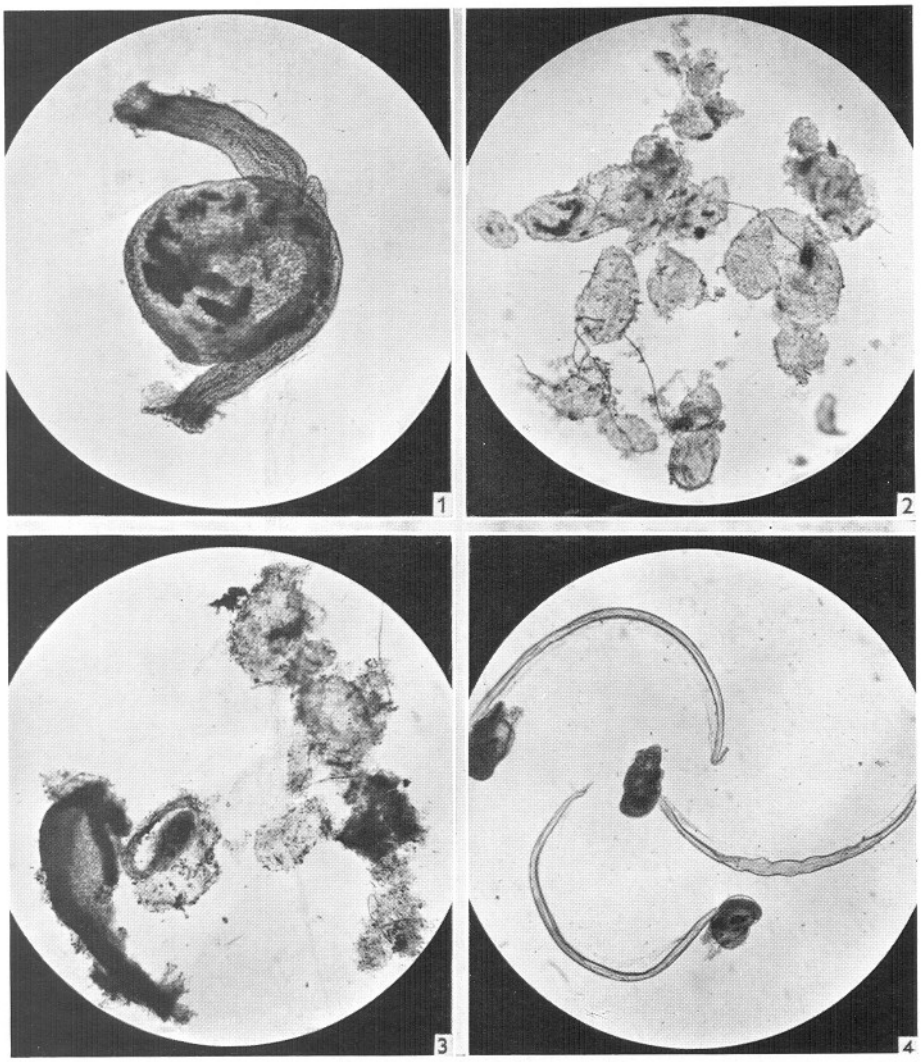
EXPLANATION OF PLATE I

Fig. 1. Complete gut of plaice post-larva, showing large pellets and matrix vesicles *in situ*; × 40.

Fig. 2. Small pellets and vesicles after removal of the gut; × 50.

Fig. 3. Undigested *Oikopleura dioica* (left centre) removed from the oesophagus (left), and matrix vesicles (right) from the stomach; × 50.

Fig. 4. Selected *Oikopleura dioica* from plankton sample. Southern Bight, February 1951; × 50.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STUDIES IN THE PHYSIOLOGY OF COMMENSALISM. III. THE POLYNOID GENERA *ACHOLOË*, *GATTYANA* AND *LEPIDASTHENIA*

By Demorest Davenport

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(Text-fig. 1)

The following investigations were conducted at the Plymouth Laboratory under a grant from the John Simon Guggenheim Memorial Foundation. The author wishes to express his appreciation to the Foundation for its support; to the Director and members of the staff of the Plymouth Laboratory for their friendly co-operation and assistance; and in particular to Dr D. P. Wilson for his continued interest and aid.

Introduction

Previous experiments (Davenport, 1950; Davenport & Hickok, 1951) gave evidence that in certain echinoderm-polynoid partnerships the specificity in part depended upon a positive response of the commensals to a substance or substances released by their hosts. In the partnership between the starfish *Evasterias troschelii* Stimpson and the polynoid *Arctonoë fragilis* (Baird) the attractant was found to be non-dialysable and relatively unstable; but, as demonstrated by the Y-tube choice-apparatus employed, enough attractant was continuously released by the host to affect the activity of the commensal at a considerable distance.

Experiments with the scale-worm *Halosydna brevisetosa* Kinberg, commensal with the terebellid *Amphitrite robusta* (Johnson), gave no evidence that a chemical attraction plays an important part in governing this association, at least when the partners are adult. However, a single unpublished experiment conducted during the summer of 1952 at the Kerckhoff Marine Laboratory in California showed that the commensal *Hesperonoë adventor*, which lives in the tube of the echiuroid *Urechis caupo*, is effectively 'tied' to its host with a powerful bond. An *Hesperonoë* can unerringly distinguish water in which its host is housed from plain sea water, and will make an active search for its host when separated from it. In this strict association the polynoid is perhaps conditioned to whatever substance it is that gives *Urechis* its familiar, powerful, aromatic smell.

The present work constitutes an effort to elucidate the mechanism of control of additional commensal partnerships.

Acholoë astericola (Delle Chiaje)

Material and Methods

At Plymouth the partnership between the starfish *Astropecten irregularis* (Pennant) and the polynoid *Acholoë astericola* (Delle Chiaje) is well known (Fig. 1), and the animals are readily available for investigation. In collections regularly brought into the Laboratory from the Eddystone Grounds, at times

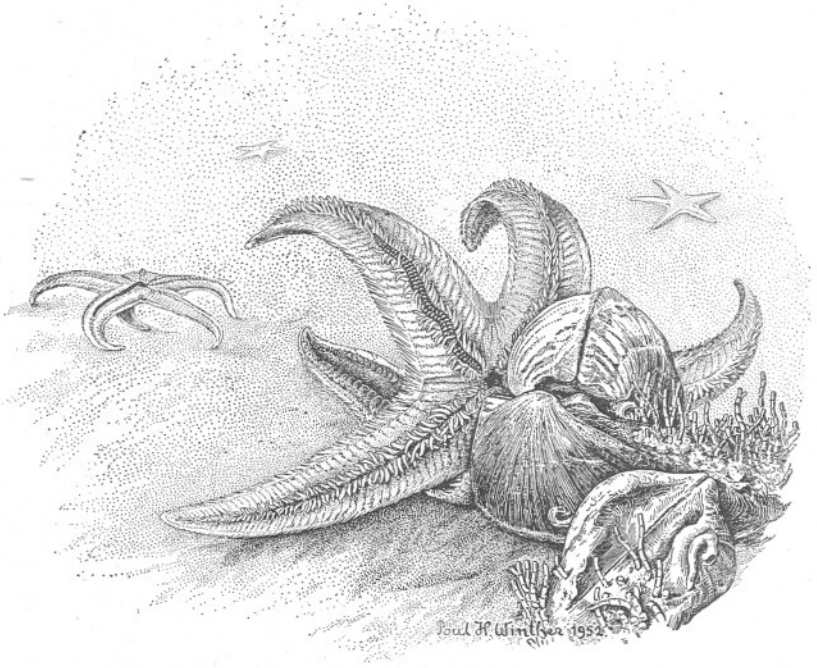

Fig. 1. The host starfish *Astropecten irregularis* showing the commensal *Acholoë astericola* in the ambulacral groove with head directed towards the stomach of the host, into which it may extend when feeding.
(By Courtesy of Gunnar Thorson, from studies made in Dakar, Senegal, April 1952. Drawn by Poul H. Winther.)

as many as 75% of the starfish have the small orange polychaete in the ambulacral groove. Ordinarily only one worm per starfish is found, but very occasionally two are present, which may be markedly different in size and age.

Astropecten is the only known host of *Acholoë* at Plymouth (Marine Biological Association, 1931). In the Mediterranean the polynoid occurs on *Astropecten aurantiacus* and *A. pentacanthus* (McIntosh, 1900). Panceri (1874) records the occurrence of *Acholoë* on the starfish *Luidia ciliaris*

(Philippi) (=*L. fragilissima* Forbes) in the Mediterranean, but no such association is known in Plymouth waters, in spite of the fact that at times *Luidia* is relatively common in the same environment as commensal-carrying *Astropecten*.

At the outset it was obviously important to determine whether the commensal made any constant response to the host. As observed by many workers, if the *Acholoë* were removed from the host and the tip of the arm of the host placed in close proximity to the head, the worms almost immediately became activated and fastened themselves to the host, either climbing quickly on to the aboral surface, or into the ambulacral groove, or wrapping themselves entirely around the tip of the host's arm. Brief experiments indicated that single tube-feet removed from the host would elicit a typical response. If a tube-foot was presented to its head, the *Acholoë* became active and with a twisting motion quickly wrapped itself around the tube-foot and the tip of the holding forceps. This response could only be elicited by contact. In many experiments tube-feet were held within a millimetre or less of the head of the commensal without eliciting any reaction. In addition, several experiments demonstrated that a small quantity of non-circulating sea water which had surrounded two host starfishes in a 200-ml. beaker for 2 hr. had no effect whatever on the behaviour of the commensals when brought near the head by a fine pipette. However, under normal conditions contact of host tissue with the head of the commensal 'triggered' an immediate and typical reaction.

The facility with which this typical and constant response can be evoked from a random sample of worms, and easily manipulatable materials can be presented while the commensals are under observation, has made the partnership particularly productive for investigating the problem of specificity and the nature, source and effect of the attractant.

In the following experiments standard procedure was to isolate six *Acholoë*, selected at random, in shallow dishes of fresh 'outside' sea water. Responses were elicited by presenting materials held in the tip of fine-pointed forceps. An effort was made to keep all materials in one series of tests of approximately the same size. In each experiment materials were presented in rapid order to the six worms and the responses recorded. Between tests of each material the commensals were washed in clean 'outside' water and instruments in alcohol-acetone.

Responses were recorded as follows. If on presentation of the test material the commensal was immediately activated and responded by quickly moving on to or wrapping itself around the material, the response was recorded as +. If a response appeared only after the material had been in contact with the head for several seconds or if the activity produced was sluggish, this was recorded as (+). No response whatever, after insistent presentation, was recorded as 0.

Observations

Experiment No. 1. Is the response specific? Single tube-feet from members of the genera of starfish represented in Plymouth waters were presented; and reactions recorded in Table I.

As can be seen, the response is only relatively specific. The majority of *Acholoë* behave consistently in the presence of each starfish presented, but an occasional individual (e.g. A, 3) seems unable to 'tell the difference', a response being elicited by the tube-foot of any starfish.

TABLE IA

Worm	*Astropecten irregularis* (host)	*Asterias rubens*	*Marthasterias glacialis*	*Luidia ciliaris*	*Porania pulvillus*
1	+	o	o	+	(+)
2	+	o	o	(+)	(+)
3	+	+	(+)	+	(+)
4	+	o	o	+	(+)
5	+	o	o	+	o
6	+	o	o	(+)	o

TABLE IB

Worm	Host control	*Palmipes membranaceus*	*Solaster papposus*	*Henricia sanguinolenta**	*Stichastrella rosea**	*Asterina gibbosa**
1	+	(+)	o	(+)	o	(+)
2	+	(+)	o	(+)	o	(+)
3	+	(+)	(+)	(+)	o	(+)
4	+	(+)	o	(+)	o	(+)
5	+	(+)	o	(+)	o	(+)
6	+	(+)	o	(+)	o	(+)

* Small size of tube-feet necessitated presentation of tip of arm of starfish.

Unfortunately, because of the limited availability of experimental animals, experiments could not be conducted with a large enough sample of commensals to give a statistically significant quantitative evaluation of the difference of intensity of response to each form. However, from the above data certain conclusions can be drawn.

It can be seen that *Acholoë* respond immediately and with highest intensity to host tube-foot, and with about equal intensity to the tube-feet of *Luidia*. This is of particular interest in view of the absence of *Acholoë* on the *Luidia* of Plymouth waters, where *Luidia* may be taken in the same dredge-haul with *Astropecten*. Curiously, the above-cited reference to the occurrence of *Acholoë* on *Luidia* in the Mediterranean was not found in the literature until after the above experiments had been completed. At the time of writing, *Acholoë* placed on a large *Luidia ciliaris* have lived 5 months in the 'Drake's Island' Tank at Plymouth. The worms do not appear to take up position in the ambulacral grooves, but cling to the sides and aboral surface of the unaccustomed host.

The above data also indicate that the *Acholoë* respond to some extent to all members of the asteroid order Phanerozonia available at Plymouth (*Astro-*

pecten, *Luidia*, *Porania*), to certain Spinulosa (*Asterina*, *Palmipes*, *Henricia*), but not ordinarily to Forcipulata (*Asterias*, *Marthasterias*, *Stichastrella*). This perhaps indicates some biochemical similarity among these members of the Phanerozonia and Spinulosa. Evidently the starfish *Porania*, *Palmipes*, *Asterina* and *Henricia*, which elicited a delayed or weak response, either produce a smaller amount of attractant or one slightly different from that of the host or *Luidia*. At any rate their attraction is enough to cause the commensals to become attached to them when contact occurs. The answer to the strict specificity of *Acholoë* and *Astropecten* in Plymouth waters, in view of the attraction which a number of starfish show for this polynoid, can only be sought in further laboratory and field observations.

Experiment No. 2. What can be determined concerning the source of the attractant in the host? Small pieces of host tissue which had been thoroughly washed in clean sea water were presented, with the following results:

Worm	Spine	Aboral integument	Stomach	Gastric caecum	Tube-foot control
1	+	+	+	o	+
2	+	+	+	+	+
3	+	+	+	+	+
4	(+)	+	+	(+)	+
5	+	+	+	+	+
6	+	(+)	+	(+)	+

These clearly show that all parts tested had some attraction for commensals. Again, the sample of worms available for testing was not large enough for a quantitative evaluation of the difference in intensity of attraction of each tissue. It appeared that stomach triggered off the most active and enthusiastic response, but this may have been the result of using slightly larger masses of stomach. However, because of the ready response elicited, a number of subsequent experiments have been conducted with stomach as the source of attractant.

These data also offer rather persuasive evidence that texture or consistency of surface, in which the tissues differed widely, have little to do with the response.

Experiment No. 3. The question was now asked whether the attractant produced by the stomach is released into the stomach cavity, and whether a response can be elicited by the presentation of material that has been soaked in stomach contents.

Small pieces of cotton-wool were introduced into the stomachs of six *Astropecten* and left for 1 hr. They were then presented to six *Acholoë*, whose reactions were:

Worm	Test cotton-wool from stomach	Control cotton-wool	Control stomach
1	(+)	o	+
2	o	o	o
3	+	o	+
4	+	o	+
5	+	o	+
6	(+)	o	(+)

Thus apparently attractant is present in stomach contents. That attractant absorbed on cotton-wool will elicit a response presents additional evidence that surface texture plays little part in the response and provides a tool with which it is possible to investigate the presence of attractant in extracts, etc.

The above results would scarcely have been obtained had there not been a quantity of attractant in the stomach contents. That not enough attractant diffuses from the general surface of the host to affect the behaviour of the commensals at a distance has already been noted. Attractant may collect in the stomach to give a higher concentration than occurs in the water immediately surrounding the host, or in the stomach the attractant may be protected (from oxidation?).

No indication has been given, however, that attractant diffuses from isolated stomach more readily than from other tissues. Although, as noted above, stomach elicits a strong response from commensals, contact still appears to be necessary. This was indicated in the following experiment.

Experiment No. 4. A small bag of 100-mesh bolting-silk was firmly filled with isolated stomach. The bag was presented.

Worm	Bag of stomach	Control stomach	Control bag
1	(+)	+	o
2	o	+	o
3	(+)	+	o
4	(+)	+	o
5	(+)	+	o
6	o	+	o

The results clearly show that when bolting-silk separates the head of the worm from stomach tissue the response is markedly weakened. The weakened responses elicited from four of the worms may have been the result of contact with small bits of stomach protruding through the holes in the silk. One set of experiments has already shown the unimportance of surface texture, and here the failure of the normal response appears to be due to the separation of the head by the bolting silk from the surface of the material to which attractant is closely bound.

These experiments indicate that for normal responses to be elicited contact with a surface to which the attractant appears to be closely bound is necessary.

The effects were then tested of certain physical factors (time duration, temperature change) on the attracting ability of whole isolated host tissue.

Experiment No. 5. Does the passage of some hours decrease the ability of isolated tissue to attract, provided it stays fresh? Fresh stomach pieces elicited a positive response from six commensals. After the passage of 18 hr. in clean sea water at 15° C. the pieces were as effective as ever.

Experiment No. 6. Can a response be elicited to refrigerated whole host tissue? Pieces of stomach were placed in 300 ml. of clean sea water, refrigerated overnight at 1° C. and tested at 2° and 8° C. Worms were tested as usual in shallow dishes of sea water at 15·5° C. The pieces of stomach were

removed from the refrigerated sea water and rapidly presented to the heads of the commensals. Before each worm was solicited, the piece of stomach was briefly put back into the refrigerated sea water to chill its surface again.

Worm	2° C. stomach	8° C. stomach	15·5° C. stomach (control)
1	o	+	+
2	o	+	+
3	o	(+)	+
4	(+)	+	+
5	(+)	+	+
6	(+)	+	+

As can be seen, a weak response was elicited from half the worms at close to freezing temperature, while stomach at 8° C. seemed as effective as controls.

Experiment No. 7. How do high temperatures affect the ability of whole stomach to elicit a response? Pieces of stomach were placed in sea water at 44° and 64° C. for 10 min., then placed in sea water at the temperature of control stomach (15·5° C.) to cool them off, and presented, with results as follows:

Worm	15·5° C. stomach	44° C. stomach	64° C. stomach
1	+	+	o
2	+	o	o
3	+	(+)	o
4	+	+	o
5	+	+	o
6	+	o	o

The data indicate that raising the temperature to 44° C. either slightly alters the attractant or allows just about the quantity to be released which is at the threshold sensitivity of the worms. Temperatures higher than 64° C. either completely destroy or alter the attractant, eliminating all response.

It is apparent, then, from the above experiments that passage of time or reduction of temperature do not markedly alter the attraction of whole isolated host tissue, but that temperatures much higher than 45° C. cannot be used in efforts to extract the attractant factor.

Experiment No. 8. What effect does grinding up the tissue have? About twenty *Astropecten* arms were ground up in a Waring Blendor in 150 ml. of 'outside' sea water. This material was then centrifuged at 1500 r.p.m. for 15 min. Cotton-wool was soaked in the supernatant and presented. No response whatever occurred, while control whole arms elicited typical responses from all six commensals.

Experiment No. 9. Five *Astropecten* stomachs were ground up in 5 ml. of clean sea water and sand and passed through filter-paper. Presentation of cotton-wool soaked in this filtrate resulted in no response whatever. Control pieces of whole stomach elicited typical responses from all commensals.

Experiment No. 10. A large number of tube-feet were rapidly ground up in 5 ml. of clean sea water and sand. This material was centrifuged at 1500 r.p.m. for 15 min. and the supernatant presented soaked in cotton-wool. Again, there were no responses whatever.

Experiment No. 11. What if the ground material is not filtered? Five *Astropecten* stomachs were ground up in 8 ml. of clean sea water in sand and the preparation allowed to stand for a few minutes to settle out, before presentation:

Worm	Control stomach	Control cotton-wool	Cotton-wool soaked in preparation
1	+	o	(+)
2	+	o	o
3	+	o	(+)
4	+	o	o
5	+	o	(+)
6	+	o	o

Here a weak response is occasionally elicited, which may result from contact with minute bits of tissue remaining in the supernatant. Clearly, no very effective amount of attractant remains in the preparation.

The above experiments indicate that grinding obliterates the attraction of host tissue and that the attractant present is rapidly altered or destroyed. Efforts to protect the attractant by introducing a crystal of ascorbic acid in the preparation or by grinding, centrifuging and testing at low temperatures (1–9° C.) resulted in failure.

However, two experiments gave evidence that the attractant is rapidly oxidized.

Experiment No. 12. Five *Astropecten* stomachs were isolated and placed in a test-tube with 8 ml. of sea water and a small quantity of washed sand. Through this preparation nitrogen was bubbled for 3 hr. At the end of this time the stomachs were quickly ground up in the nitrogen atmosphere, the supernatant poured off and tested:

Worm	Control stomach	Control cotton-wool	Cotton-wool soaked in preparation
1	+	o	(+)
2	+	o	(+)
3	+	o	(+)
4	+	o	+
5	+	o	o
6	+	o	+

Here again, as a result of the small sample tested and the appearance of a number of delayed or weak responses, one cannot make a definite statement on the relative effectiveness of the material which had been prepared in nitrogen. Yet the five responses, two of which were typical, appear to indicate that cotton-wool soaked in material prepared in nitrogen was slightly more effective than cotton-wool soaked in material prepared in air (Exp. No. 11).

Experiment No. 13. Finally, cotton-wool soaked in stomach contents and then exposed to air in a moist chamber was found very quickly to lose its attraction. Bits of cotton-wool were placed in the stomachs of hosts for

90 min., quickly removed, placed in a moist chamber in air, and tested. After the passage of 5 min. in air bits elicited normal responses, but others which were tested after they had been in air for 15 min. had lost all their attraction.

GATTYANA CIRROSA (PALLAS) AND *LEPIDASTHENIA ARGUS* HODGSON

The association of these two handsome polynoids respectively with the terebellids *Amphitrite johnstoni* Malmgren and *A. edwardsi* Quatrefages is well known.

In a series of experiments, with material collected at the estuary of the Yealm and at Salcombe, the ability of the commensals to discern the presence of their host terebellids at a distance was tested with the Y-tube choice-apparatus used in the author's 1950 and 1951 experiments. In no tests was it possible to demonstrate that the commensals chose the host-water arm a statistically significant number of times, even when as many as seven or eight host terebellids were placed in the test aquarium, when 'outside' sea water was used and when the commensals were partially protected against the adverse effect of light stimuli by wrapping the Y-tube in red cellophane.

This result strictly accords with that obtained (Davenport & Hickok, 1951) with another terebellid-polynoid association, that of *Amphitrite* (*Neoamphitrite*) *robusta* (Johnson) and *Halosydna brevisetosa* Kinberg of Puget Sound.

Orton and Smith (1935), using *Gattyana* and *Lepidasthenia*, conducted brief experiments which they believed 'appear to indicate...that there is a tropic response on the part of the polynoid, causing it to enter an *Amphitrite* burrow whenever possible.' No data is presented, however, to support this hypothesis. In a personal communication on unpublished work R. Phillips Dales states that he has been able to demonstrate a positive response by *Gattyana* to water from the host, when using only the freshest material and taking the greatest precautions to prevent the disturbing effects of other stimuli (light, contact, etc.). It is clear, however, that while in these terebellid-polynoid associations the commensal's behaviour may be affected by a chemotaxis to the host, certainly not nearly as strong a response can be demonstrated as in the echinoderm-polynoid partnerships. The author has been unable to duplicate, when using *Amphitrite johnstoni* and *Gattyana*, his experiments demonstrating the response to host body wall or tentacle exhibited by *Halosydna brevisetosa*.

Observations made in the field and on material in glass U-tubes indicate that the activity of commensals is perhaps limited more by the powerful thigmotaxis they demonstrate to the wall of the tube and the body-wall of the host than by responses to attractants secreted by the host.

That a chemotaxis may be involved in enabling the young commensals to find their hosts cannot be discounted.

Discussion

In the above experiments the importance of an unstable attractant in regulating the behaviour of the commensal partner in an association has become apparent for a second time. A marked difference exists, however, between the control of the *Evasterias-Arctonoë* partnership of Puget Sound and that of the *Astropecten-Acholoë* partnership of European waters. In both of them the commensal annelid is bound to the host asteroid by a powerful chemotactic response to an unstable substance or substances; but, whereas in the former the attractant will take effect at some distance from the host, in the latter a response can be elicited only by contact with the host. That the response of *Acholoë* may be purely a chemotaxis, in spite of contact being necessary, has been strongly suggested by the tests in which it was elicited by stomach juices soaked on cotton-wool.

Unfortunately in the 1950 Puget Sound experiments no tests were made similar to those in which *Acholoë* was found to respond to several starfish that appear never to be its natural host; however, in the Y-tube choice-apparatus used, *Arctonoë* was found not to respond to water from the star *Pisaster*, closely related to the host *Evasterias* and living in the same ecological niche. The lack of specificity of response in *Acholoë* is difficult to explain in view of the fact that it has been found only on *Astropecten* and *Luidia* in European waters.

It must be kept in mind, however, that under ordinary conditions during adulthood of the commensal, the chemotaxis serves merely to keep the commensal on the surface of the host. In a personal communication, G. Thorson, who has recently made some interesting observations on this partnership and to whom I am indebted for the excellent figure of the animals, says that when *Astropecten* are dying in an aquarium their *Acholoë* will leave them and may visit *Asterina*. This is quite in accord with our observations of the sensitivity of *Acholoë* to *Asterina*, on which the polynoid never occurs in nature. Under natural conditions an annelid during its adult lifetime would seldom be forced to seek a new host when its own is damaged or dying. Such an event is probably too rare to be considered a factor in the evolution of the powerful chemotactic response; but its occasional occurrence could conceivably initiate a new host habit, when the searching commensal finds itself able to 'colonize' a new host species.

Probably no answer will be found to the problems of the observable differences in the distances at which particular commensals respond, of the specificity of their responses, and of their specificity of habit, until the detailed ecology of the developmental stages of the respective commensals is known, including the manner in which the host is sought out and found.

During study of the effect of the host on the early stages of the commensal, Thorpe's (1939) olfactory conditioning factor must be kept in mind as a possible important element in the control of marine associations. In this

work Thorpe demonstrated that in certain insects host selection in part depends upon the conditioning of the parasite's early stages to host substance. For such conditioning to be effective the early stages must obviously spend some time in intimate contact with host tissue. Work at present in progress indicates that the prototrochophore and trochophore stages of *Acholoë* are in the plankton for not less than 10 days. It is a little difficult to see how any conditioning of the early stages can occur in forms with as long a planktonic stage as *Acholoë*.

In considering the evolution of a specific commensal habit it is admittedly dangerous to generalize from an evaluation of the economics of the association based largely on guess-work. It should be possible to determine precisely, in a number of partnerships, just how the commensal is attracted and bound to the host and how the host is forced to tolerate it. Turning to the *consequences* of such an association, the remarks of Maurice Caullery (1952), referring to lichens, are quite as applicable here. He says that we will 'find that the question is really one of analyzing, by precise experiments, the relations' (of the partners) 'and of careful comparison of their behaviour in an isolated state and in association.' Such a careful comparison of the relations and requirements of the partners of specific associations of the type investigated by the author has to his knowledge never been made.

A possible course of evolution of intimate animal associations in general may be suggested. When one animal becomes associated with another to the advantage of the former, then the latter (the host) may have a number of possible fates. It may be able to evolve defences against the partner about as fast as the partner is able to evolve modifications to live at its expense, in which case the association may be considered one of commensalism or balanced parasitism. Or it may not be able, and the association may then be thought of as one of extreme parasitism, internal predatism or disease. Or, again, if *after the association has been unilaterally initiated*, some benefit is thereby given to the host, the host individuals which are best able to take advantage of the situation will perhaps be selected and the association will then tend to become truly mutualistic or symbiotic. However, it is difficult to take any other view than that at the initiation of the association only one of the two associated species is benefited.

With the polynoid-echinoderm partnerships investigated by the author, it appears most unlikely, since such a high percentage of host individuals live quite successfully without commensals, that some selective advantage has accrued to species which developed a substance whereby commensals were attracted. It is most difficult to imagine any way in which the echinoderm benefits from this association and therefore to label the partnership mutualistic. Should not then—the question is often put—these echinoderm-annelid associations be considered parasitic? If commensalism is defined classically as simply 'feeding at the same table', and if it can be demonstrated that no damage is done to the starfish by a commensal that occasionally removes

food from its stomach, the answer is 'no'. Obviously, however, these associations are very close to parasitism and probably are excellent examples of the manner in which truly parasitic associations develop.

In the author's opinion, from the point of view of the evolution of the association, the echinoderm has been passive. It has, in the course of the annelid's adaptation to commensal life, been unable to eliminate the commensal.

On the other hand, an organism that has become physiologically adapted both to finding, by evolving a positive response to a particular chemical secreted by a host, and to successfully 'colonizing' this host, has doubtless been given an advantage of selective importance. The finding itself and the successful 'colonization' must be investigated without undue regard to the economics of the association and with careful consideration of the manner of evolution of sensitivities to specific substances and, undoubtedly, of certain immunities. The author has observed *Acholoë* fully two-thirds within the stomach of the host, and Thorson states that they will keep this position for 10 min. or more. The annelids must be quite immune to host digestive enzymes. It may be that immunological studies will cast light on the apparent anomaly of a commensal that responds positively to a number of starfish (perhaps biochemically related) but is found in nature on only one or two of these.

That various intimate associations among terrestrial organisms (parasitic protozoa, trematodes, insects, etc.) are controlled by chemical sensitivities and immune reactions is well known; so far little attention has been paid to the role of these phenomena in marine associations.

Summary

The commensal polynoid *Acholoë astericola* (Delle Chiaje) exhibits a powerful positive response on contact with its host, *Astropecten irregularis* (Pennant).

This response is only relatively specific, *Acholoë* demonstrating it to a number of starfish that do not serve as host in Plymouth waters.

All living host tissue has some attraction for the commensal; and the attractant is apparently released in quantity in the stomach of the host.

Passage of time (apparently as long as tissue remains alive) and chilling do not markedly alter the attractiveness of isolated host tissue. Temperatures above 45° C. alter or abolish its attraction.

The extreme instability of the attractant has so far made it impossible to demonstrate its presence in extracts.

Evidence is presented that the attractant is rapidly oxidized.

Experiments with the scale-worms *Gattyana cirrosa* (Pallas) and *Lepidasthenia argus* Hodgson, commensal respectively with *Amphitrite johnstoni* Malmgren and *A. edwardsi* Quatrefages, indicate that a chemotaxis to the host may be of relatively minor importance in governing the behaviour of these commensals.

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SOME EXPERIMENTS ON THE FUNCTION OF THE PERICARDIAL ORGANS IN CRUSTACEA

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(Text-figs. 1–11)

The experiments reported here were designed to test the assumption that the nervous organs found in the pericardial cavity of the Crustacea, and termed 'pericardial organs'[1] (Alexandrowicz, 1953*a*, *b*), have a secretory function. Their situation suggested that the substances liberated by them into the blood of the pericardial cavity probably act on the heart. Accordingly, the aim of this series of experiments was to determine what might be the effect of these postulated hormones on the crustacean heart.

Method

The method employed consisted in preparing an extract of the pericardial organs and testing its action by adding it to the fluid perfusing the isolated heart.

The observations were chiefly made with the two species of large crabs easily available at Plymouth, *Cancer pagurus* and *Maia squinado*. The lobster (*Homarus vulgaris*) proved to be less suitable experimentally. Some experiments were performed at Naples[2] with a stomatopod, *Squilla mantis*. There were perfused in all 15 hearts of *Cancer*, 26 of *Maia*, 4 of *Homarus* and 10 of *Squilla*.

As pointed out in the paper quoted above, the pericardial organs can be found most easily in the pericardial cavity of crabs, where they lie at the openings of the branchio-cardiac veins. With *Maia* there is a chance of removing all the elements of the organs of one side hanging together, i.e. the

[1] R. I. Smith in a figure illustrating the course of the cardiac nerves in *Cancer irroratus* (*Biol. Bull. Woods Hole*, 1947, Vol. 93, p. 77) has shown nerves designated as 'lateral pericardial plexus', which are certainly the elements of the pericardial organs. This 'lateral pericardial plexus' was also observed in *Pugettia producta* by J. P. Heath (*Journ. Morph.*, 1941, Vol. 69, p. 481). The term 'pericardial organs', proposed by one of the present writers (J. S. A.) in his previous publication, and adopted without knowledge of the designation previously given, seems more appropriate in view of the unusual structure and function of these elements: this term will also embrace the analogous elements in stomatopods first described as parts of the 'system of dorsal trunks'.

[2] We wish to express our gratitude to Prof. R. Dohrn for the facilities offered to us. One of us (D. B. C.) is indebted to the Browne Fund of the Royal Society for financial assistance, which made his visit to Naples possible.

anterior and posterior bars with their prolongations and the longitudinal trunks. In *Cancer* the organs must be taken out in two portions separately, i.e. the anterior and posterior bars each with the adjoining parts. This procedure is also often more convenient in *Maia* since it is easier, and since in the preparation of extracts the organ need not be in one piece.

The isolated organs were first rinsed in the saline used for perfusion and then triturated with sand in a little of the same solution. The suspension was filtered and the residue washed with several more portions of fluid totalling 10 ml. The filtrate was used, in most of the experiments, as the stock solution from which certain amounts were taken and added to the perfusion fluid. The stock solution was prepared fresh before each series of tests.

In *Homarus* only a part of the pericardial organs is arranged in discrete trunks: the other part is spread as plexuses over the wall of the pericardium. Moreover, even the trunks themselves, which are much shorter than in crabs, cannot be easily distinguished from other tissues. As the dissection is difficult and its result uncertain only a few trials were made.

In *Squilla mantis* the pericardial organs are present in the 1st–5th abdominal segments and are of two kinds. Those of one kind, short trunks flattened to lamellae spanning the cleft between the dorsal muscles, can be easily found. To expose them, parts of the terga have to be removed, care being taken not to tear away the tissues in the mid-line. The lamellae are recognizable by their shape and position, sometimes also by their slightly greyish colour differing from the yellowish hue of the muscles. The elements of second kind, spreading over the pericardial wall, are in practice scarcely distinguishable in unstained preparations.

The saline used for perfusion in all four species was prepared according to the formula of Welsh (1939*a*) (modified Pantin's solution). The perfusion of the heart of the decapod crustaceans was performed according to the method used by Welsh (1939*a*) with some minor modifications of the apparatus and procedure.

The routine was as follows.

(i) The legs were cut off and the dorsal part of the carapace and of the pericardium removed.

(ii) The pericardial organs were exposed by cutting the lateral heart ligaments. The organs were excised and the extracts prepared.

(iii) The heart, with parts of the arteries, was cut out and put into a flat dish with perfusion fluid to rinse. Sea water may also be used for rinsing the heart, as it seems not to affect its later behaviour.

(iv) A cannula already fixed into the rubber stopper was introduced into the heart through the sternal artery. A cannula with a spherical enlargement of the tip may meet resistance at the arterial valve, and only when it can be pushed freely farther into the heart cavity is there a certainty that the valve has been passed.

(v) A ligature was tied round the sternal artery to fix the cannula in place, and a second one round the anterior aorta.

(vi) The heart was put into a short glass tube with a capacity of 80 ml., the stopper fixed in place, the tube filled with perfusion fluid, and the thread of the anterior ligature attached to the writing lever.

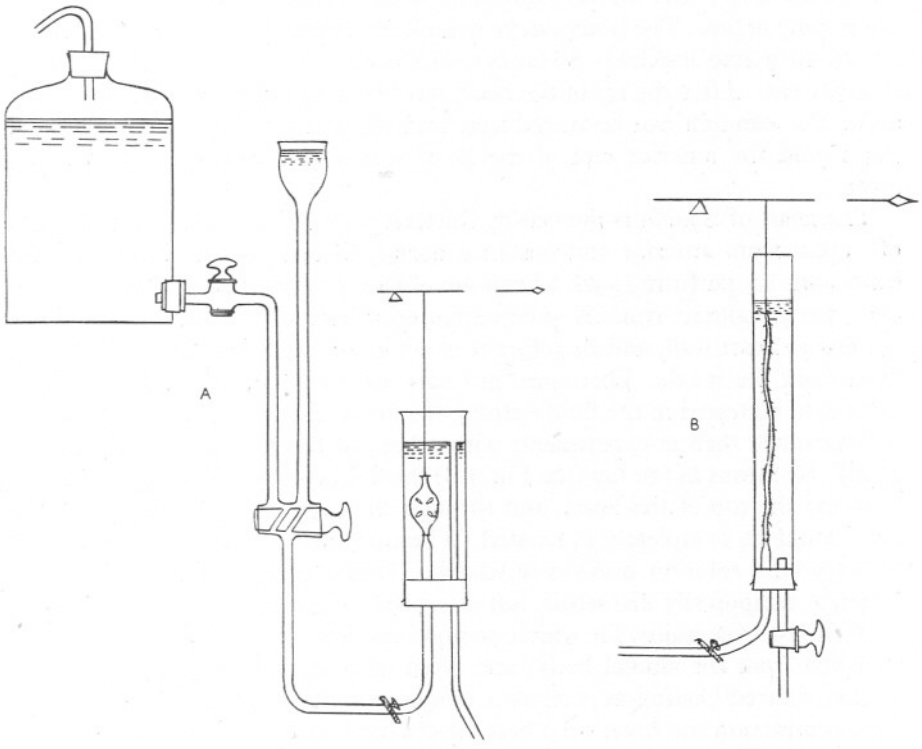

Fig. 1. A, the apparatus used for perfusing the hearts of *Cancer*, *Maia* and *Homarus*. The Mariotte bottle was only used for prolonged washings. Before, during and for a short time after each test perfusion was made from the thistle funnel, with constant addition of fluid. The rate of perfusion was controlled by means of the screw clamp. B, the modification of the heart chamber used for perfusing the heart of *Squilla*, drawn to a larger scale than A.

(vii) Perfusion was begun first from the Mariotte bottle (Fig. 1 A), but, as differences in the level of the perfusion fluid may cause some changes in the heart rhythm, the two-way stopcock was turned before each test and the perfusion was made for some time from the thistle funnel into which later was poured the saline with the substance to be tested. In this way the same conditions of perfusion prevailed before, during and after each test. The Mariotte bottle was only used for prolonged periods of unattended perfusion and for washing out test solutions.

The volume of each test solution, i.e. saline with the substance to be tested added, was as a rule the same, viz. 20 ml.

To expose the heart of *Squilla mantis* the dorsal parts of the terga and of the extensor muscles must be removed as far as is necessary to give free access to the long heart tube. The latter can then be taken out, preferably from the hind end forwards, by successively cutting the arteries and the connexions with the underlying organs. The heart can be completely isolated, but it is safer to leave it with the gonad attached. So far as could be observed this does not influence its reactions. After the tip of the heart has been cut off close to the posterior aorta, the cannula can be introduced and secured by a ligature. A thread put round the anterior end of the heart serves to attach it to the writing lever.

The heart of *Squilla* is pierced by thirteen pairs of ostial orifices and sends off, apart from anterior and posterior aortae, fifteen pairs of arteries. The conditions for perfusing such a heart are obviously very unfavourable. Moreover, the ganglionic trunk is situated not on the inside but on the outside of the dorsal heart wall, and therefore it is not easily accessible to the perfusing fluid from the inside. Therefore, in order not to dilute too much the substances to be tested in the fluid bathing the heart, the tube used for perfusion was narrower than in experiments with other crustaceans and contained only 20 ml. By means of the tap (see Fig. 1 B) the fluid could be kept at a level just covering the top of the heart, and through the same tap the contents of the tube could be completely evacuated. When refilled the tube contained only traces of the solution previously tested. During change of fluid the heart action is temporarily disturbed, but afterwards resumes normally.

With few exceptions, for which no good reason could be found, the heart, removed from the animal body, and often quite motionless during the dissection, started beating as soon as it was immersed and perfusion began. At each contraction the heart tube becomes twisted so that its top moves clockwise round the vertical axis. This movement is obviously due to the spiral course of the muscle fibres; the vertical component of the contractions, however, has sufficient amplitude to give quite satisfactory records.

Action of Extracts of the Pericardial Organs and of Drugs

Cancer pagurus

Action of Pericardial Organ Extracts

The addition of extract of its own pericardial organs to the perfusion fluid has a very distinct effect on the heart beat of *Cancer*, increasing the frequency and amplitude of the contractions. This effect proved to be dependent on the activity of the extracts and on the sensitivity of the heart, both factors being subject to individual variations. The record in Fig. 2 A was obtained with a

concentration of 0·4 ml. of the stock solution diluted to 20 ml. On occasion a distinct reaction was noted with 0·1 ml. of the stock solution of the extract, i.e. using 1/100 of the active substance of the organs of one animal, diluted in

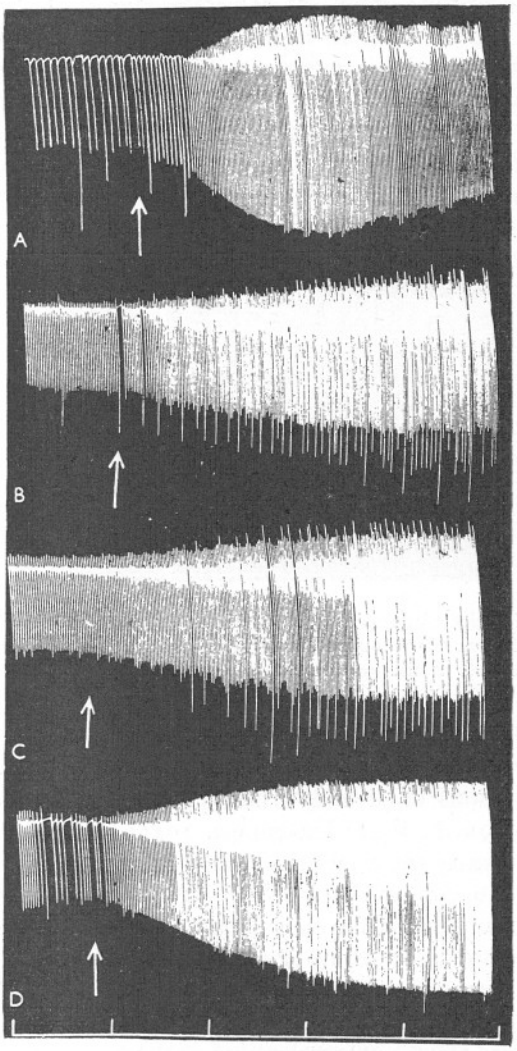

Fig. 2. The successive action on the same heart of *Cancer* of (A) pericardial organ extract of *Cancer*, (B) adrenaline ($1:10^6$), (C) noradrenaline ($1:10^6$) and (D) the same extract as in A. The records read from left to right; the arrows indicate approximately the time at which the reagents reached the heart; time scale in minutes.

20 ml. of saline. In other experiments with other hearts and extracts greater quantities, up to 4 ml. of the stock solution, were needed to produce a similar reaction.

The effect of the extract in exciting the heart beat is not long-lasting and after all the fluid containing the extract has passed through the heart and been replaced by ordinary saline the heart gradually returns to its previous rhythm.

Extracts of pericardial organs of *Maia squinado* (Fig. 3) and of *Portunus puber* produced similar reactions in the *Cancer* heart. To obtain effects equal to the above with the *Maia* extracts a higher average dose was required, indicating that in the organs of this animal there is less accelerating principle. With *Portunus* comparisons are uncertain, not only because of its much smaller size, but also because the dissection of its pericardial organs is difficult and probably loses much of their parts.

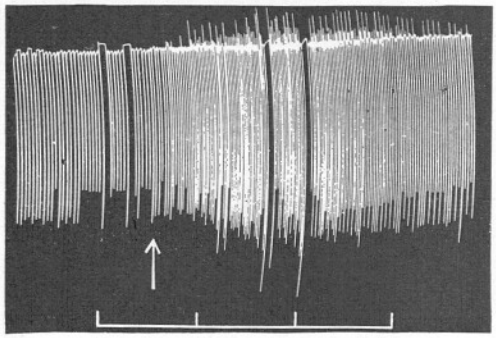

Fig. 3. The effect of pericardial organ extract of *Maia* on the heart of *Cancer*. The record reads from left to right; the arrow indicates approximately the time at which the extract reached the heart; time scale in minutes.

Action of Adrenaline, Noradrenaline and Tyramine

The action of adrenaline and acetylcholine on crustacean hearts is known through investigations of several writers (Hogben & Hobson, 1924; Bain, 1929; Welsh, 1939*a*, *b*; 1942; Davenport, 1941; Ghiretti, 1947), and there was no need to control the established facts. We tried these drugs, however, in order to be able to compare their effects with those produced by the extracts of the pericardial organs on the same hearts and under the same conditions.

Of the drugs tested, adrenaline (Parke, Davis and Co.) and noradrenaline (Levophed, of Bayer), at concentrations of $1:10^6$ or $1:10^7$, showed effects most resembling those of the extracts. These two drugs and the extracts were so much alike in their effect—an acceleration of the beat and a gradual increase in amplitude, sometimes to remarkable maxima, followed by an equally gradual return to normal—that often no differences could be noticed. However, some hearts appeared to react somewhat differently in response to the extracts from their reaction to the drugs. Fig. 2 shows records obtained with the same heart treated (A) with the extract, then (B) with adrenaline, (C) with noradrenaline and (D) again with the same extract, the last trial to eliminate

the possibility of the previous treatment having influenced the response of the heart to that following. It will be noticed that the extract, apart from the increase in frequency and amplitude, has also a steadying effect on the beat, indicated by more even contractions.

The action of acetylcholine is different (Fig. 4). It accelerates the heart rhythm and may also increase slightly its amplitude; at higher concentrations, however, the amplitude not only does not become greater, but markedly decreases, becoming even smaller than during the previous 'normal' beat till the heart stops in diastole or nearly in this condition. On the latter point our observations on *Cancer* and *Maia* differ from those of Welsh (1939*a*, *b*), Davenport (1941) and Ghiretti (1947), who recorded stoppage not in diastole but in systole. In conversation with Dr J. H. Welsh we have learned that in his experiments he used a counterbalanced writing lever, whereas in the experiments reported in this paper we used a spring-loaded lever, so that the heart was always pulling against the tension of a spring. This may explain the different conditions of stoppage after acetylcholine in the two sets of experiments.

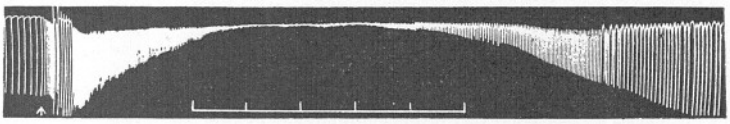

Fig. 4. The effect of acetylcholine ($1:10^3$) on the heart of *Cancer*. As in all the kymograph records reproduced here, contractions are downwards so that the stoppage of the heart has taken place in diastole. The record reads from left to right; the arrow indicates approximately the time at which the drug reached the heart; time scale in minutes.

The experiments with tyramine-hydrochloride (L. Light and Co.) gave uncertain results. In some a slowing action of this drug at concentrations of $1:10^7$, $1:10^6$ or $1:10^5$ was recorded; in others it was ineffective at concentrations of $1:10^6$ and slightly accelerating at $1:10^5$. Since the effects obtained do not resemble those of the extracts, this substance is not likely to prove the active principle of the organs in question.

The assumption that the active principle may be adrenaline-like was corroborated by the fact that pericardial-organ extract from *Cancer*, after addition of sodium hydroxide, gave a distinct fluorescence reaction of the same green colour as adrenaline. The intensity of the reaction shown by the extracts of organs of a single specimen prepared with 10 ml. saline corresponded approximately to a $1:10^6$ solution of adrenaline. On the assumption that this principle would be released by the organs into the pericardial cavity, we tested blood taken from this cavity and found it to give a fluorescence of the same kind. This reaction, however, was not shown by blood of the same animal taken from the leg arteries. The extracts of the organs and the blood of *Maia*

either did not give the fluorescence reaction at all or at the most very slightly. The extracts from the organs of *Portunus* gave a distinct positive fluorescence reaction.

It follows from these experiments that the pericardial organs in decapod crustaceans release some adrenaline-like principle which has an excitatory effect on the heart beat. Experiments with another crab, *Maia squinado*, show, however, that this is not a complete statement of the action of these organs.

Maia squinado

Action of Pericardial Organ Extracts

The action of the pericardial-organ extract on the heart of *Maia* is strikingly different from that in *Cancer* for it decreases the frequency of the

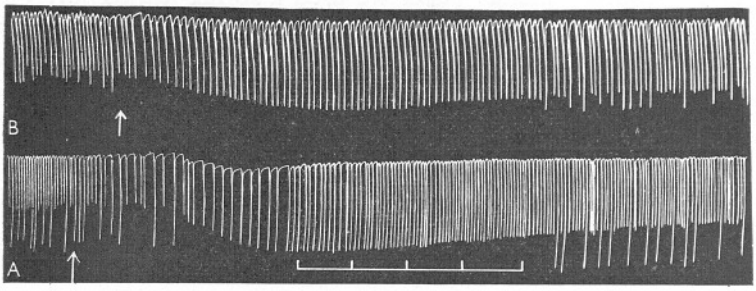

Fig. 5. The effect on the heart of *Maia* of its own pericardial organ extract. A shows the fourfold action—decrease in frequency and increase in amplitude of the beat, increase in tonus and the greater regularity of the beat, with a return to its previous condition as the extract is washed out of the heart; B shows more clearly the steadying effect of the extract, and in this heart there was no increase in tonus. The records read from left to right; the arrows indicate approximately the time at which the extracts reached the hearts; time scale in minutes.

beat. The amplitude becomes only a little greater and never reaches such a height as in *Cancer*, and the higher concentrations do not bring about a further increase.

Other effects, not always noticeable, are the steadying of the beat and an increase in tonus. The fourfold action of the extracts may be seen in Fig. 5A. The steadying effect is also well illustrated by Fig. 5B showing how the uneven beat of the heart became more regular after treatment with the extract and, after cessation of the action of the extract, returned to its previous rhythm with the same irregularities.

The heart in *Maia* proved to be less sensitive than in *Cancer*, and for recording the action of the extracts 2–5 ml. of the stock solution were made up to 20 ml. of the perfusion fluid.

The result of the perfusion of *Maia* heart with the extract of *Cancer* is

shown in Fig. 6. The heart responded by an increase in amplitude and by a slight decrease in frequency of beat. This shows, therefore, that the hearts of the two species react differently from each other to their own respective pericardial extracts, and these differences are maintained when the hearts are subjected to treatment with extracts from the opposite species.

Action of Drugs

The heart of *Maia* responds to adrenaline by an increase in frequency and amplitude of beat. It will, however, be noted that *Maia* heart is less sensitive than *Cancer* heart to this principle in reacting with less vigorous contractions; the effect shown in Fig. 7 is rather exceptional and different sensitivities of the hearts could often be observed. Thus the reaction in one heart was just

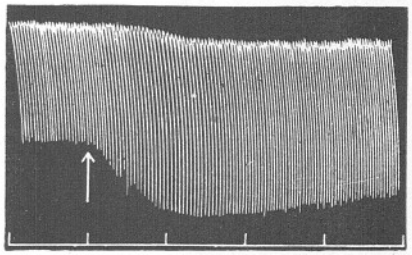

Fig. 6.

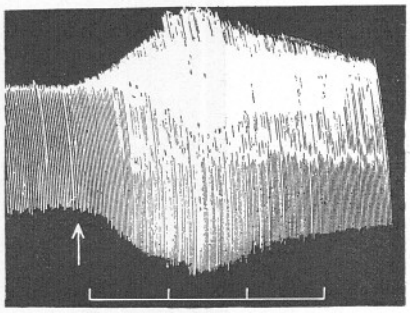

Fig. 7.

The records read from left to right; the arrow indicates approximately the time at which the active principle reached the heart; time scale in minutes.

Fig. 6. The effect of pericardial organ extract of *Cancer* on the heart of *Maia*.
Fig. 7. The effect of adrenaline ($1:10^6$) on the same heart of *Maia*.

distinct with a concentration of $1:10^8$, whereas in another no effect was noted with this concentration. The action of noradrenaline is similar to that of adrenaline, but sometimes somewhat stronger contractions were observed after treatment with noradrenaline than with adrenaline of the same concentration.

The action of acetylcholine does not show marked differences from that on the heart of *Cancer*.

Tyramine in higher concentrations, such as $1:10^5$, first slows the beat and decreases the amplitude and then accelerates the beat and increases the amplitude. In lower concentrations, such as $1:10^7$, a slowing of the beat was recorded. The regular heart rhythm seems to be affected by this drug and occasionally the heart stops for a while. Tyramine thus seems unable to play the main role in the action of the pericardial organs.

Homarus

Because of the arrangement of the pericardial organs in *Homarus* only a small part of them could be cut out, and the extracts contained, at best, only a small fraction of the active substance presumably present in the whole of the organs. Applied to the heart of *Homarus*, which was perfused in the same way

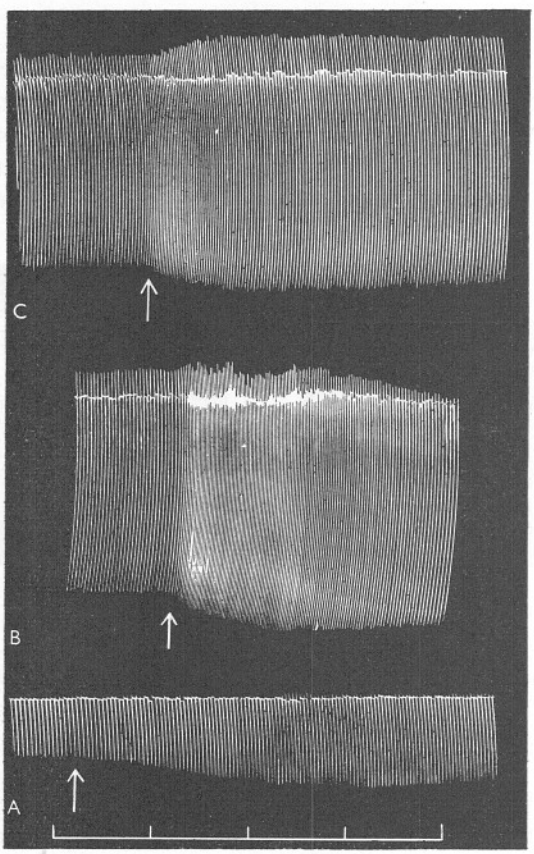

Fig. 8. The effect on the heart of *Homarus* of (A) pericardial organ extract of *Homarus*, (B) pericardial organ extract of *Cancer*, and (C) pericardial organ extract of *Maia*. Records read from left to right; the arrows indicate approximately the time at which the extracts reached the heart; time scale in minutes.

as those of crabs, the extracts increased the frequency and amplitude of beat (Fig. 8A); both effects however being less pronounced than in *Cancer*. Extracts from *Maia* and from *Cancer* both have an excitatory effect and no marked difference in the response to either was noticed (Fig. 8B, C). Even when a certain *Homarus* heart showed a peculiar reaction by first increasing the frequency and then slowing it abruptly the records were practically identical with *Maia* and with *Cancer* extracts. In general the heart of the lobster

appeared to be less sensitive to the extracts and to adrenaline and noradrenaline as well. The threshold concentration of both these compounds was as high as $1:10^5$.

Squilla mantis

Obviously, owing to its anatomical features, only a part of the inside of the heart of *Squilla* can come into direct contact with the undiluted stream of perfusion fluid, and therefore, if some substances are added to this fluid, they can act on remaining parts of the heart elements only after being diluted by the fluid already filling the container. Therefore even negative results would not be decisive. Fortunately, it was found that extract made from the pericardial organs of a single specimen (five lamellae) was sufficient to cause a distinct increase in frequency and amplitude of beat (Fig. 9A). Similar effects were brought about by adrenaline and noradrenaline (Fig. 9B). The concentration of these substances in the perfusion fluid was $1:10^6$, but, as just pointed out, the concentration at the moment of their action on the receptive elements of the heart was uncertain.

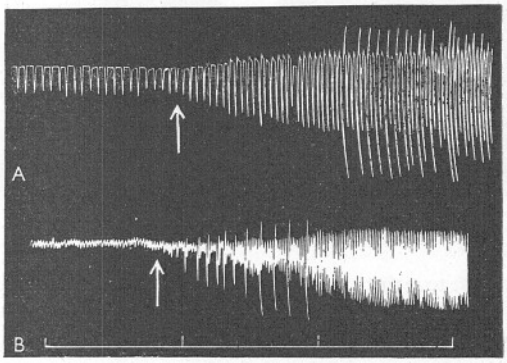

Fig. 9. The effect on the heart of *Squilla* of (A) pericardial organ extract of *Squilla*, and (B) adrenaline ($1:10^6$). The records read from left to right; the arrows indicate approximately the time at which the reagents reached the heart; time scale in minutes.

The action of the extracts from crabs on *Squilla* heart, and vice versa, could not be determined, since all these animals were not available at one and the same place, and we had not yet worked out a reliable method of preserving the active extracts for future use.

The fluorescence test with the pericardial organs of *Squilla* gave uncertain results. At this stage of the work it could not be determined whether the active principle in *Squilla* is different from that in *Cancer* or too small in amount to give the reaction.

Tests on Release of Secretion into the Blood Stream

In order to test the supposition that the pericardial organs release the active substance into the blood the following experiments on crabs were performed.

Perfusion of the heart with blood. Blood was taken, one sample from the leg arteries and another from the pericardial cavity of the same animal. Each sample was filtered and, after dilution with an equal amount of saline, tested on the heart. The result of this experiment in *Cancer* (Fig. 10) shows that the blood from the pericardial cavity has a similar stimulating effect to the extracts of the pericardial organs, whereas that from the leg arteries slows the heart beat and decreases its amplitude.[1]

Perfusion of the pericardial organs in situ. An animal was bled through the leg arteries and then from the pericardial cavity through a small hole bored into the carapace above the heart. After the animal had been fixed by means

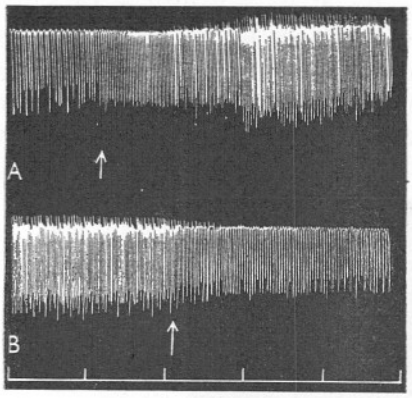

Fig. 10.

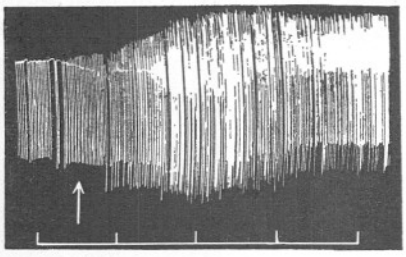

Fig. 11.

The records read from left to right; the arrow indicates approximately the time at which the active material reached the heart; time scale in minutes.

Fig. 10. The effect of blood on the heart of *Cancer*. The blood was filtered after clotting and the filtrate diluted with an equal volume of saline. A, blood taken from the pericardial cavity stimulates the heart; B, blood taken from the leg arteries depresses it.

Fig. 11. The effect on the heart of *Cancer*, of saline which had been perfused through the pericardial cavity *in situ*, passing over the pericardial organs.

of a clamp with the ventral side upwards, the pericardium was perfused through three tubes introduced into the branchio-cardiac veins. The fluid flowing out through the hole in the carapace was collected and tested on the heart of a second animal perfused in the ordinary way. In experiments made with *Cancer* this fluid showed a distinct stimulating effect (Fig. 11) and gave a positive fluorescence reaction.

With *Maia* the results were uncertain. As the active substance of the pericardial organs and the blood both slow the beat, the differences in their action

[1] Blood of crustaceans taken out from the animal body ceases to be a proper medium for the tissues: it affects the heart action, decreasing the amplitude of the beat, and often causing an irregular rhythm and sometimes even stoppage of the heart. This is obviously due to the quickly occurring changes in the composition of the blood.

(an increasing of amplitude by the former and decreasing by the blood) are hardly distinguishable. Moreover, the exactness of the experiments with perfusion *in situ* is somewhat impaired by the fact that, even after a rinsing of the pericardial cavity prior to collecting the fluid for the tests, some of the blood remains there and passes into the perfusion fluid, revealing its presence by an opalescence. The presence of blood may influence the reaction of the heart on test, but the preliminary washing of the pericardial cavity cannot be prolonged lest the pericardial organs should be exhausted.

Possible Errors

As noted by other workers in this field, allowance must be made for the hearts taken out of various specimens behaving in quite different ways. Some hearts start to contract strongly and in fairly regular rhythm immediately on being immersed in the saline and after the stream of perfusion fluid passes the cavity of the heart; others need some time to adjust themselves to these conditions. Several did not contract at all for a long time, but did so after addition to the perfusion fluid of a small amount of the pericardial extracts or of adrenaline, and then continued to beat regularly for several hours. The degree of stretching of the heart and the rate of perfusion must be found empirically. We tried at first, in order to compare the action of various substances under conditions as similar as possible, to maintain a constant rate of perfusion, viz. 20 ml. of the solution through the heart in 3 min. This was approximately the rate of flow in most of the experiments, but various hearts show individual predilections, and even the same heart, after beating for some time at a certain speed of perfusion, may require later a faster one. Usually the hearts respond to the accelerated perfusion with stronger contractions. However, even this rule is not without exception: sometimes the heart worked definitely worse at the flow-rate usually used and needed one much slower.

This different behaviour may be partly due to blood clots forming in the heart and thus creating different conditions for passage of the perfusion fluid. It may be, too, that individual variations in the situation of the ganglionic trunk of the heart play some role. For instance, since this trunk lies on the inside of the dorsal heart wall and is partly covered by the muscle bundles, it is possible that, because of variations both in the thickness of these bundles and in the position of the ganglion cells, this pacemaker may be more or less accessible to the action of the mediators. The thickness of the muscle bundles is perhaps responsible for less satisfactory results with the hearts of the largest specimens of *Maia*. We preferred, therefore, to use medium-sized animals (carapace measuring about 13×17 cm.), rather than large ones.

The results of the experiments depend not only on the variable sensitivity of the hearts but also on an even more unstable factor, the activity of extracts, which appear to vary greatly, presumably in relation to the condition of the animals.

All the experiments recorded above were made during the summer months. When we tried to continue this work in January, however, the results obtained were unexpectedly confusing. A concentration of *Maia* organ extracts several times higher than that always found previously to be effective did not produce any reaction and even in one specimen slightly accelerated the beat.

It was then found that the hearts responded to treatment with adrenaline and noradrenaline in a way never previously observed by us and, moreover, in successive experiments conducted under identical conditions the results obtained were very different. Thus one heart did not react at all to a concentration of 1:5000; another reacted to a concentration of 1:10,000 by a slight acceleration without an accompanying increase in amplitude; again another, treated with a concentration of 1:1,000,000, responded by a spectacular increase in tonus, acceleration, and a notable decrease in amplitude. This was the same heart which accelerated in response to a highly concentrated pericardial organ extract. The reason for this abnormal behaviour of the hearts was possibly the low temperature at which the animals were kept in the outdoor storage tanks.

Although the experimental evidence speaks in favour of a hormonal nature of the substance released by the pericardial organs it can be objected that the active substances might be compounds of the tissues constituting the organs or of blood which pass into the extracts. It is known from experiments of Welsh (1939*a*) that nervous tissue from both the central nervous system and from peripheral nerves has an effect on the heart, and, as this writer concluded, 'the active principle in question is, with little doubt, acetylcholine'. We tested the effects of an extract of leg nerves of *Maia* and found that these were the same as in Welsh's experiments, and certainly different from those produced by the extract of the pericardial organs. Pieces of pericardial wall and blood clots even much larger than those which often adhere to the pericardial organs do not produce any effect.

Since on dissection of the pericardial organs in *Homarus* the removal of a certain amount of connective tissue with the organs was unavoidable, extracts from pieces of this tissue were made and tested but did not produce any apparent effect.

Discussion

The recorded results favour the assumption that the pericardial organs of the Crustacea contain some specific substances influencing the heart action. It must be emphasized that very small amounts of these substances are able to produce distinct effects, as may be inferred from the following estimation. The weight of the pericardial organs in a large specimen of *Maia* (carapace 17 × 20 cm., weight 1560 g.) was found to be 5 mg. and in *Cancer* (carapace 21 × 18 cm., weight 1810 g.)—8 mg. (Both organs were rinsed in saline and some of this fluid adhered to them during the weighing operation.) As stated

before, 1/100 of the active substance of these organs diluted in 20 ml. was sufficient to show its action on the *Cancer* heart. Assuming that this substance made up as much as 5% of the total wet weight of the organs, which is certainly well on the high side, it is evidently efficient at a concentration of 1 : 5,000,000 or less.

In *Squilla* the weight of pericardial organs was not measured directly but from the dimensions of a lamella (about $1000 \times 300 \times 50\mu$) the weight of five of them can be supposed not to exceed 0·1 mg.; then the concentration of the active substance in 10 ml. of the perfusion fluid assuming the same 5% of total wet weight would be 1 : 2,000,000. As has been said above, this concentration was lowered by the fluid bathing the heart. Clearly then, in the investigations aiming at identifying the active principle, only such compounds as are capable of giving similar effects at very low concentrations ought to be taken into account.

There is one feature in the behaviour of the hearts in the two species of the Brachyura which requires particular consideration. Were the experiments performed with the heart of *Cancer* only, the results could be summed up in the statement that the pericardial organs in crabs (*Cancer*, *Maia*, *Portunus*) release a substance increasing the frequency and amplitude of the heart beat. Furthermore, the resemblance of the action of this substance to that of adrenaline and noradrenaline and the presence of a positive fluorescence reaction might lead to a conclusion that the function of the pericardial organs consists in liberating an adrenaline-like principle into the blood. The much weaker fluorescence reaction or its absence in the extracts from *Maia* would then show that the amount of this principle varies in different species of the Brachyura. From the experiments with the perfusion of the hearts in *Homarus* and *Squilla* the same or a similar principle would be admitted in the Macrura and the Stomatopoda.

The reactions of the *Maia* heart are similar in that the amplitude of the beat is increased and a stabilizing effect can sometimes be observed in both species of crabs. There is, however, a remarkable discrepancy in the chronotropic action, for the extracts from both *Cancer* and *Maia* accelerate the heart of *Cancer* and slow that of *Maia*. As the heart of *Maia* is accelerated by adrenaline it must be assumed that either (i) the substance in the extracts which stimulates the heart of *Cancer* is not adrenaline-like, but an unknown principle of such properties that it excites the heart in one species and inhibits it in the other, or (ii) the same stimulating principle is present in both extracts but its effect on *Maia* heart is inhibited by the presence of some other factor to which this heart is more sensitive. If the second alternative is regarded more probable, two possibilities have to be considered: this second factor could be either some non-specific substance of the tissues of the pericardial organs extracted together with the stimulating principle, or it is another hormone which, in the living animal, is also released into the blood of the pericardial

cavity. The negative results of the experiments with the extracts of various tissues do not favour the first alternative. On the other hand, the inhibiting effects on *Maia* heart of small amounts of this substance seem to point to a hormonal nature. If this is so we reach the conclusion that the pericardial organs in the Crustacea liberate two hormones, one stimulating and the other inhibiting the heart rhythm. To the latter can perhaps be ascribed the stabilizing effect recorded sometimes in *Maia* and *Cancer*. The different reactions exhibited by the hearts of the two species of Brachyura could be explained in this way: that the heart of *Cancer* is more sensitive to the action of the accelerator and that of *Maia* to the inhibitor; and, when treated by the artificial mixture of the two, each heart responds according to its particular sensitivity.

In the other crustaceans investigated (*Homarus* and *Squilla*) the pericardial organs can be assumed to release a stimulating hormone, but the evidence so far available does not permit us to draw any conclusion as to the presence or absence of the inhibitory hormone.

Although the idea that the pericardial organs may have a neurosecretory function was based on the histological evidence alone, the latter did not help much in the solution of the problem whether these organs produce two different substances. The fine neuropile-like terminations of the nerve fibres, by which presumably the substances are given off into the blood, do not show any differences. It is, however, worth noting that in *Squilla* the nerves forming the pericardial organs are without doubt made up of two different sets of fibres and, as has been suggested in the description of these organs (Alexandrowicz, 1953*a*), their structure in the Decapoda may be the same, although here the diversity of origin of the nerve elements is not so easily discernible as in the Stomatopoda. If, therefore, it were proved that the pericardial organs in fact produced two hormones, the existence of two kinds of nerve fibres offers a possible explanation of their separate elaboration.

It appears from this work that in future physiological investigations of the crustacean heart the role of the pericardial organs must be taken into account. The experiments hitherto made on stimulation of the regulator cardiac nerves (for bibliography see Krijgsman, 1952) were as a rule performed after the pericardium had been opened and the possibility of noting the action of the pericardial organs thus eliminated. Only Parrot (1941) set up experiments in such a way that these organs could have produced their effect. This writer stimulated the inhibitory cardiac nerves in *Maia* in which the circulation was maintained, and took samples of the blood for testing on the intestine of the same animal. He found that such blood had a stimulating effect on the intestine and believed this to be caused by a substance released by the inhibitory nerves. This might have been so, but it seems more likely that in his experiments not only the inhibitory cardiac nerves but also the nerves of the pericardial organs were stimulated to release their hormones into the blood.

Possibly also in the experiments of Wiersma & Novitski (1942), in which the perfusion fluid was poured into the pericardial cavity, the pericardial organs had a chance to come into play; and conceivably their hormone was responsible for after-effects of inhibition supposed to be caused, as they wrote, by a 'liberation of a substance which can exert its influence at a later time'.

There seems to be no other instance when the action of the pericardial organs could be suspected of having affected the results of experiments (see the survey by Fredericq, 1947).

Summary

Experiments with isolated hearts of various Crustacea (*Cancer pagurus*, *Maia squinado*, *Homarus vulgaris*, *Squilla mantis*) were performed in order to test the postulated secretory function of the pericardial organs (nerve trunks of unusual structure lying in the pericardial cavity). Tests were made with extracts of these elements added to the fluid perfusing isolated hearts.

In *Cancer*, *Homarus* and *Squilla* the extracts caused a distinct increase in amplitude and frequency of the heart beat, and their effect proved to be very similar to that produced by adrenaline and noradrenaline tested on the same hearts. In *Maia* the extracts produced an increase in amplitude and decrease in frequency of the heart beat.

Extracts of the pericardial organs of *Cancer pagurus* gave the fluorescence reaction characteristic of adrenaline and related compounds. Blood taken from the pericardial cavity gave the same reaction, but that taken from the leg arteries did not.

It is assumed that the function of the pericardial organs in the Crustacea consists in liberating, through fine neuropile-like terminations of the nerve fibres, some hormone passing with the blood into the heart and producing on it a stimulating effect. There is certain evidence that the pericardial organs might release a second hormone having an inhibitory and perhaps also a stabilizing effect on the heart rhythm.

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A NEW DREDGE FOR COLLECTING BURROWING ANIMALS

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(Text-figs. 1–2)

At Plymouth the most successful dredgings are those from soft mud or shell gravel, grounds which offer little resistance to a heavy dredge of the normal pattern. From a sandy bottom reasonable samples cannot be collected because the ordinary rectangular dredge will not dig in more than, at the most, two inches. This fact, although it had long been inferred from the poor catches, has recently been proved by direct underwater observations.

A dredge of new design has been developed which will dig into the bottom, whether sand or mud, very rapidly and continue to dig the harder it is pulled. The name 'anchor-dredge' is proposed for this instrument. The basis of the design is a sharpened steel plate which is dragged at an acute angle to the sea-bed; this reduces the tendency for the sand to be broken up, as it is with most dredges, which are therefore left with nothing resistant to bite into. Once the anchor-dredge has dug in a little way, the broad plate offers considerable resistance to the sand, forcing it to go on digging instead of moving horizontally through the soil. The construction of the anchor-dredge with a single digging side leaves it clear from the obstruction of arms or chains and free to dig to its whole depth. The long single arm prevents the warp from disturbing any fast-moving burrowers which are about to be dug up, besides controlling the 'angle of attack' of the dredge. To keep the dredge the right way up, it was at first lowered on a double bridle one part of which fell off as soon as it reached the bottom. A much simpler method was subsequently devised. The short upright C (Fig. 1) was removed and a trawl-float lashed in its place. The float has sufficient buoyancy to keep the dredge the right way up as it is being lowered.

The first time the new dredge was tested in moderately deep water, on a ground of fine sand near the Eddystone, it collected a terrebellid polychaete, *Lysilla loveni* Malmgren, new to the Plymouth fauna. This result seemed to justify the belief that the dredge was more efficient on that type of ground than those which had previously been used.

The anchor-dredge is primarily intended simply as a collecting instrument, and not as a quantitative bottom-sampler. It might sometimes be used in

conjunction with a bottom-sampler to ensure that the more deeply burrowing animals are not overlooked.

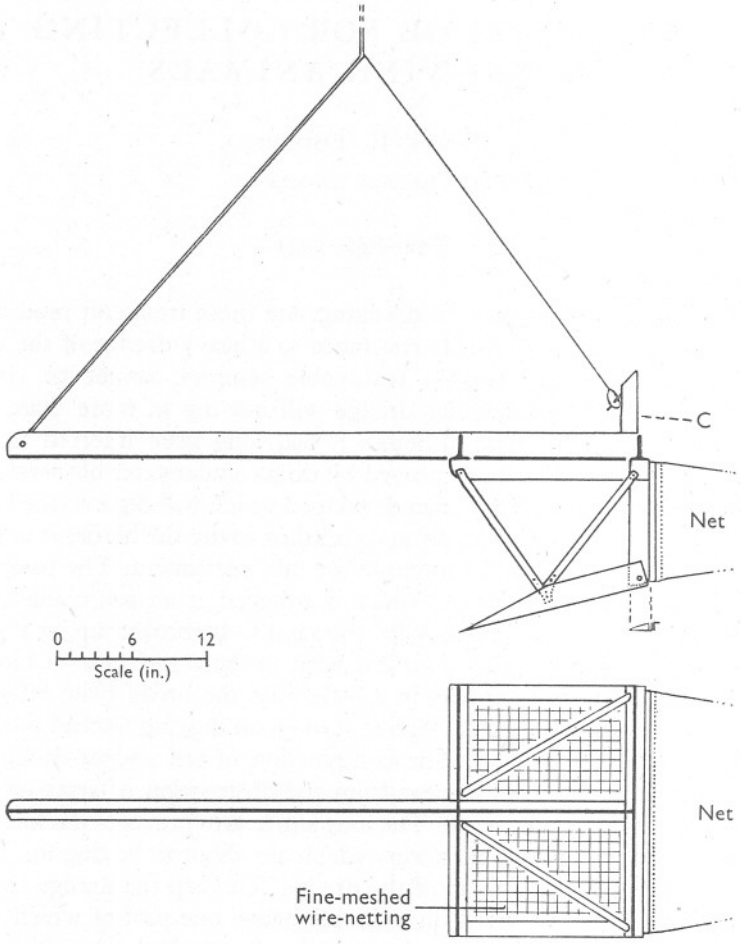

Fig. 1. Plan and side-view of the 'prototype' anchor-dredge.

Structural Details and Methods of Operation

Fig. 1 shows the structural details of the first model of the anchor-dredge. It was built very cheaply from scrap material, but has proved satisfactory for testing the design. Although originally intended for the motor-launch *Gammarus*, it has also been worked from the M.F.V. *Sula*. Experience has shown that a slightly smaller model would be more suitable for the *Gammarus* because, without a power capstan, it is hard work for three men to haul the present dredge when the net is full. The weight of the dredge empty is

42 lb. (19 kg.) and it has dug up as much as three bathfuls of sand in one haul. The net may be of stramin or fine netting. For general purposes cotton 'lant' net of $\frac{1}{2}$-in. mesh was found successful.

The best position for working the dredge from a trawler is through the forward gallows. While the ship is moving or drifting very slowly astern the warp is paid out as fast as possible. If the trawl-winch will not 'steam out' the warp, the use of rope is preferable, to avoid pulling the warp off the winch drum by hand. When the length of warp released is about twice the depth of the water the dredge will bite sufficiently to pull the warp off the winch by itself. The ship is then given a touch astern on the engine, and the warp allowed to run out freely until its length is at least three times the depth of the water. At this point a gradual application of the winch brake causes an increasing strain on the dredge, and eventually brings the ship to a stop. One such tug is all that is necessary to secure a good sample, and hauling may begin immediately the ship has lost sternway. As far as possible the dredge should be shot and hauled in a straight line; for, should the ship drift off this line, the lateral strain imposed on the long arm of the dredge might be too strong for it. This effect is more readily avoided by the use of the forward gallows, for then, as the dredge is hauled, the ship pulls herself ahead and can be readily controlled; whereas, if the dredge is worked from the stern gallows, the ship moves slowly astern during hauling and is consequently very difficult to steer. From a motor-launch a rope warp is normally used, and shooting the dredge is simplified. The boat can now be allowed to drift during hauling as the strain on the dredge is much less.

Results

Both from inside and outside the Sound the anchor-dredge has taken a number of animals which had previously only been caught very rarely by other dredges or are normally only taken by digging on the shore, e.g. *Cerianthus lloydi* Gosse, *Myxicola infundibulum* (Renier), *Ensis siliqua* (L.), *Lutraria lutraria* (L.), *Acronida brachiata* (Montagu).

Comparison with other Dredges

In Whitsand Bay on a sandy bottom the *Sula's* heavy dredge, which has a slightly oval frame, could not catch any of the in-fauna except the small whelk *Nassarius reticulatus* (L.), which lies just buried, half an inch to one inch below the surface. Two hauls of the anchor-dredge, however, took five large lamellibranchs (two *Lutraria lutraria* and three *Callista chione* (L.)), four *Echinocardium cordatum* (Pennant) and several polychaetes.

The conical dredge is normally recommended for sampling the in-fauna (E. J. Allen and S. W. Kemp in Fowler & Allen, 1928, p. 329); but one underwater observation showed that, although the bag filled up when the

dredge was towed some distance, it did not dig in more than approximately three inches. Moreover, if the towing speed was too great, the dredge turned on its cutting edge until the net pointed upwards and no digging at all was possible. The anchor-dredge dug to six inches with the *Gammarus*, and probably to about eight or nine inches with the *Sula*.

The anchor and conical dredges were compared with one another in the following test from the motor-launch *Gammarus*. At a depth of about 25 ft. of water in Cawsand Bay the dredges were each shot twice on either side of a marker buoy. The anchor-dredge in four hauls took eight complete and six damaged razor shells (*Ensis* spp.). The conical dredge in its four hauls also took six damaged *Ensis*, usually the top third of the shell, but not a single entire specimen. The conical dredge caught fifteen *Nassarius* sp. compared to the anchor-dredge's six, indicating that it had dragged for some distance before digging. This dragging could often be felt on the warp. To make the conical dredge dig it was necessary to stop the engine until the dredge had checked the boat's way, and then very gradually increase the speed of the engine. If the warp was slack as the speed of the engine was increased, giving the dredge a strong tug, it broke out of the ground immediately; unlike the anchor-dredge which breaks out only when the warp makes an angle of rather less than 45° to the vertical.

In a further test with the *Sula* in Whitsand Bay the following catches of large burrowing animals were made; as shown in Table I. The results are based on twelve hauls of the conical dredge, of which the two poorest have been omitted, and ten hauls of the anchor dredge including one in which it came up partly broken. As the conical dredge did not dig in sufficiently to stop the ship, it was dragged over a distance of at least 50 yards. The depth was approximately 6 fathoms and about 30 fathoms of warp were used for each dredge.

TABLE I

	Conical dredge	Anchor-dredge
Ensis spp. either whole or in part, but longer than 1½ in.	2	8, including one 7-in. *Ensis siliqua*
Callista chione (large bivalve)	3	14
Echinocardium spp.	5	34

Another dredge which also digs deeply into sand or mud has been described by G. Gustafson (1934). It was a circular or ring type with arms. A similar dredge was tried out at Plymouth before the war but was found too heavy for general use.

IMPROVEMENTS

Fig. 2 shows a design for a slightly enlarged and strengthened model of the anchor-dredge, which it is hoped will be suitable for working from either the *Sula* (length 60 ft.) or the *Sabella* (length 90 ft.). The base plate is

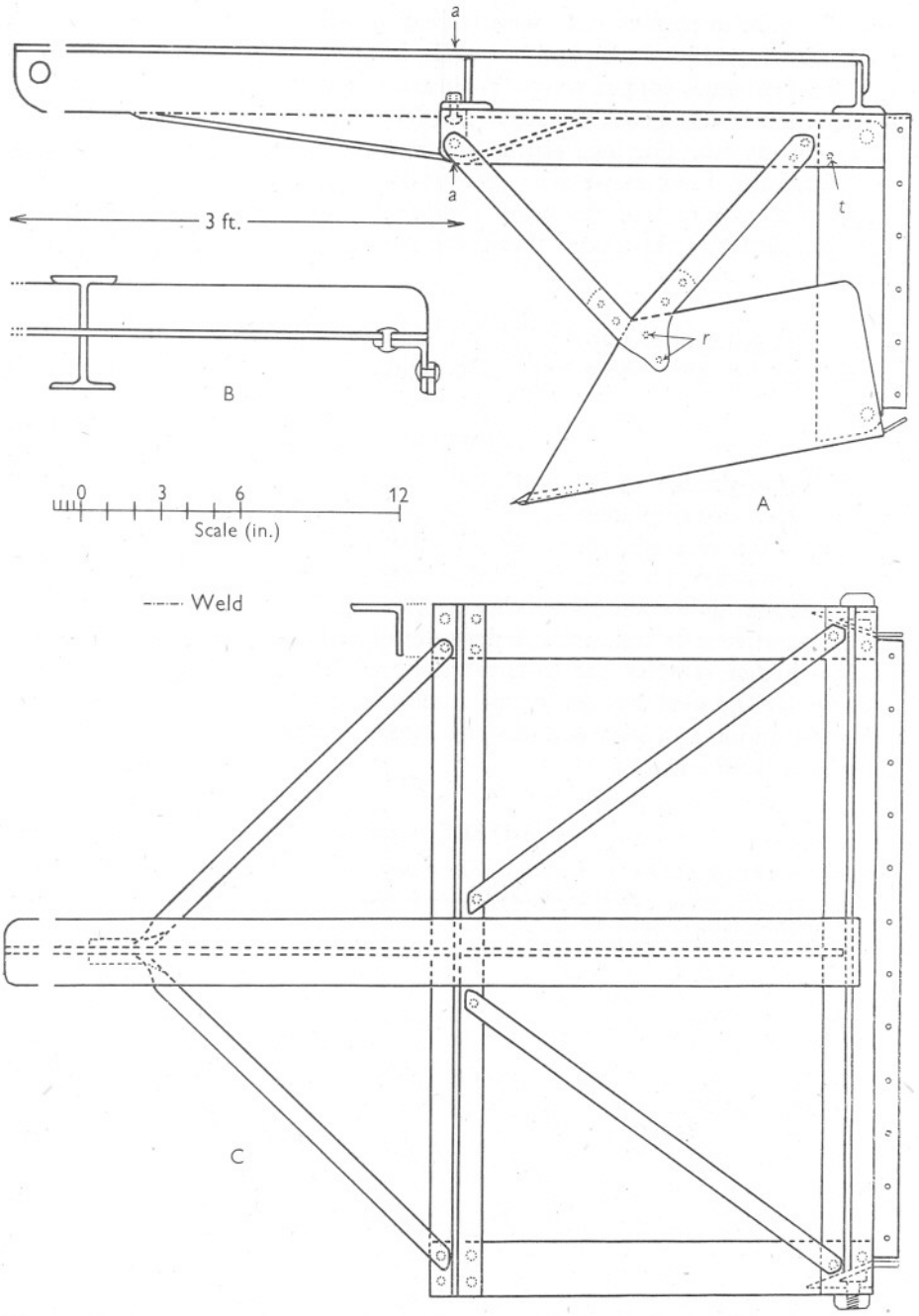

Fig. 2. Plans of the enlarged and strengthened anchor-dredge. For clarity some details have been omitted. A, view from the side. B, section through *a*...*a*. C, view from above. The function of *r* (small copper rivets) and *t* (a small bolt) is explained on p. 198.

thicker, $\frac{3}{16}$ in. instead of $\frac{1}{8}$ in., with large $\frac{1}{8}$ in. side plates to prevent loss of material. Should the base plate be caught under a boulder or other obstruction the two small copper rivets (*r*) shear, leaving the plate free to swivel. If the strain is very great, the small bolt (*t*) can also shear so that the whole dredge opens out. The long arm is heavier than in the first model, and has two additional T-pieces welded on to provide extra strength at its junction (*a*...*a*) with the first of the cross T-pieces. These change the arm to an I-section girder at this point, where the greater part of the lifting strain is carried.

I am most grateful to Captain W. J. Creese and the crews of the *Sula* and *Gammarus* for their help and cooperation in testing and working the dredge.

Summary

The 'anchor-dredge' is a lightly built one-sided instrument designed to catch deep-burrowing animals. The depth to which it digs is dependent on the pull of the warp and not on the weight of the dredge.

From a trawler, it is best to work the dredge from the forward gallows, and give it a 'tug' as the ship is moving astern.

Comparative tests and direct underwater observations have shown that the anchor-dredge samples the in-fauna more efficiently than do either large rectangular and oval dredges, or the conical dredge.

A description and plan is given of a larger model, designed for use from a 60-ft. or 90-ft. ship.

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NOTES FROM THE PLYMOUTH AQUARIUM. II

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CONTENTS

The purpose of these occasional notes has already been explained (Wilson, 1949). The present notes comprise a number of observations on the habits of fishes.

THE FEEDING OF *TORPEDO NOBILIANA* BONAPARTE

As known from ancient times, Electric Rays (*Torpedo*) have the power of giving severe numbing shocks, and it has repeatedly been stated that this power, which is electrical, is used both as a means of protection and for paralysing prey. That the shock is used in the capture of prey seems never to have been definitely proved. Indeed Roule (1935, p. 161) expresses the opinion that the electric shock is not used for securing food and even doubts that it is normally and effectively used as a means of defence. There appears to be no detailed account of the manner in which *Torpedo* feeds, but the ninth edition (1925) of the *Guide to the Aquarium of the Zoological Station at Naples* mentions (p. 106) that the Electric Ray rises from the mud to throw itself against approaching mullet (*Mugil*), and that the mullet, overcome by fright and electric shock, fall to the ground to be eaten. This very short and incomplete account was not seen until after the following paragraphs had been written.

Both *Torpedo nobiliana* Bonaparte and *T. torpedo* L. occur from time to time off Plymouth, the former being by far the commoner, and both have been kept in our aquarium tanks. They are sluggish fishes, spending most of the time lying still on the bottom, occasionally swimming for short spells. They swim by sculling with the tail, which has a large caudal and two dorsal fins. This mode of swimming differs strikingly from that of normal rays, which undulate the pectoral fins and trail their tails; it resembles the swimming movements of *Rhina squatina* (L.), another fish with a sluggish disposition and, as noted below, some other resemblances to *Torpedo*.

In the 1930's some time was spent observing *Torpedo* in the hope of discovering the manner in which the prey is captured. The rays observed never attempted to secure squid or dead fish thrown into the tank; but round fishes,

such as cod (*Gadus morrhua* L.) and pollack (*G. pollachius* L.), if kept in the same tank, gradually disappeared one by one. In March 1935 a *Torpedo nobiliana*, 2½ to 3 ft. long, was seen with the tail of a cod, about 20 in. long, sticking out of its mouth. The cod was really too big for the ray and took over an hour to disappear. For several days afterwards the ray had a bloated appearance. Later in the same year I arrived at the tank in time to see a pollack disappearing into the mouth of the same or a similar ray. Late in 1937 another *T. nobiliana* almost certainly killed a pollack much too big for it to swallow. The pollack was found lying on the bottom of the tank still twitching. Round its middle was a dark mark, and dissection revealed that the vertebral column was broken. However, in spite of much time spent in observation, the manner of attack was never seen.

When the aquarium was reopened after the war further specimens of *Torpedo* were obtained, but in general they were unwelcome owing to the apparent impossibility of feeding them except with living fish. The same difficulty had occurred with *Rhina squatina*, but had been overcome by drawing dead fish through the water on the end of a wire. When the fish arrived near its mouth, the *Rhina* gave a very quick upward thrust of the head and rose from the ground to seize it. In *Rhina* the large mouth is at the extreme anterior end and is well suited to this habit. It was thought that *Torpedo* might similarly react to a dead fish drawn smartly through the water to within striking distance, though how it would attack it was a matter for speculation. The mouth of *Torpedo* is relatively much smaller than that of *Rhina* and is situated underneath the head well behind the anterior end, a poor position, apparently, for seizing actively swimming prey.

In the autumn of 1952, when two medium-sized *Torpedo nobiliana* (each about 2 ft. long, one a male, the other a female) had settled down in the largest tank, the feeding technique used for *Rhina* was tried and at once proved successful. A dead pout, *Gadus luscus* L., on the end of a wire fixed to a bamboo pole, was pounced upon by the male ray as soon as it was drawn to a position a few inches above the bottom in front of the ray. With a very quick movement the ray sprang forwards and upwards and made to envelop the fish, its wings (pectoral fins) and snout (the straight anterior border of the head) being curled around ventrally. The stiff wire prevented the completion of the manoeuvre, and as the pout was pulled away and upwards the ray adopted first a vertical position, with the wings surrounding the prey, and finally turned over upside-down in mid-water. It could then be seen that the pout was between the jaws of the ray, whose wings, snout, pelvics and tail were all bent or bunched upwards (that is ventrally) as if to surround it. The pout was unhooked from the wire, and the ray righted itself and flattened itself out on the bottom, by which time the meal had been swallowed. Much the same performance was repeated with another pout, after which the ray lost interest and on that day gave no further display.

The female was then tempted. It pounced in a similar manner, but, encountering obstructing rockwork, failed to secure the fish. In spite of repeated tempting no further interest in trailed fish was shown that day.

Subsequently many more pouncing responses were elicited and the manner in which *Torpedo* normally captures its prey became clear. The ray lies perfectly still on the bottom until a fish swims within reach, that is into a position just in front of the ray and not too far above the bottom. For a ray 2 ft. long the maximum distance above bottom seems to be about 6 in. The fish can approach from any direction. On one occasion a ray pounced on a bamboo pole as it was pushed quickly downwards to retrieve a detached bait lying motionless on the bottom. The bait must not be moved too slowly, otherwise a response does not seem to be forthcoming. The experience with the bamboo pole suggests, too, that detailed shape is unimportant. The bait need not touch the ray. Baits used were pout, hake and horse mackerel. The baits were attached directly to a wire, or to a nylon thread attached to the end of the wire. Baits on nylon threads were completely covered over and pinned to the bottom by the pouncing ray, the wings and anterior border of the head being bent downwards to entrap it. This pinning of the prey to the bottom is a normal procedure for ordinary rays; those which feed on crustaceans such as small crabs swim over them and pin them down until the jaws are able to take hold. Chewing movements are then visible on the upper surface above the gill arch region, and such chewing movements were sometimes seen when *Torpedo* had a fish below it. Strangely enough, *Torpedo* never succeeded in taking hold of a dead fish lying motionless under it, however long it was left there, and the fish was invariably easily pulled out from under the ray. The only fish which were secured and eaten were on wires. A possible explanation is that the *Torpedo* missed the struggles or other movements of living prey, and that the slight prodding which must occur with a dead fish on a stiff wire held in the hand simulated those movements and enabled the ray to find it by the feel. Even so, although the rays readily pounced, sometimes repeatedly, on a fish trailed on a wire it was not often that they succeeded in getting it into the mouth and eating it.

The feeding reactions of *Torpedo*, so far as they appear to the eye, are not very different from those of ordinary rays in the Plymouth tanks, except that they are quicker and appear to depend less on the olfactory sense. Large *Raia clavata* are known to feed on fish such as herrings and sprats (Steven, 1947), but on the whole the food of ordinary rays consists of crustaceans, smaller and less able to escape quickly. *Torpedo* catches relatively large active fishes, and it seems a reasonable conclusion that it is able to do so because it can numb and quieten them with its electric organs. But before this conclusion can be accepted the giving of the shock during capture needs to be demonstrated.

A dead horse mackerel, *Caranx trachurus* (L.), about 9 in. long, was fixed to the end of a waterproof electric flex. Each wire of the double flex was

soldered to an electrode and the electrodes were inserted into the muscular tissues of the fish, one at each end. The flex was connected to a Pye Scalamp Galvanometer. The fish was now trailed in the usual way. Several times the fish touched the back of the ray but no deflexion of the galvanometer occurred. Eventually the ray (the female) pounced, and at that instant when it folded its wings and head on to the prey a strong shock was registered. The ray failed to secure the fish. After several more attempts without any response the female was abandoned and the male was tried. As soon as the bait arrived in the proper position the ray pounced and again a strong shock was registered. This time the bait was secured and the ray began to swallow it with the flex still firmly attached. To release the flex by pulling out the electrodes the ray had to be drawn vertically up in the water and almost over on to its back, and up towards the surface, its wings and snout being folded ventrally as usual. It was some little time before the electrodes were tugged away, just as the tail of the horse mackerel was disappearing into the mouth of the ray, but no further shock was registered. The shocks given by both rays were probably considerable. The galvanometer, on lowest sensitivity, gave far more than full-scale deflexion, indicating that the shock was of the order of volts. With the instrument used it was not possible to obtain a better measurement; the purpose had been only to demonstrate that a shock is given at the moment the ray touches its prey.

The effect on a living fish may perhaps be surmised from an occasion when a turbot attempted to seize the bait at the moment the *Torpedo* pounced. The *Torpedo* missed the bait and partially enveloped the turbot in a vigorous manner, pinning it to the ground. Convulsive contractions of the electric-organ region (these are mentioned by Day, 1880–84) and strong chewing movements were seen. The tail of the turbot projected from under the ray at one side and curled stiffly upwards, and was not flapping about as might be expected of a trapped but otherwise unharmed turbot. After a time the chewing motions ceased but the ray still lay over the turbot. Eventually the ray was pushed aside with a pole, revealing the turbot, its tail now back on the ground, alive and undamaged but breathing perhaps a little slowly. After a few minutes it swam away, and it is still alive. The *Torpedo* gave no further responses that day. It is well known that after use the power of the electric organs is weakened and time is needed for recuperation.

During these observations the use by *Torpedo* of its electric powers in a defensive manner was also seen. On several occasions a dogfish or nurse-hound nosing over the ground for food came into contact with a *Torpedo* and on doing so gave a sudden start and swam away. Contractions of the electric-organ region were not then specially noticed. Conger eels nosing a ray have suddenly come to an abrupt stop and then quickly retreated backwards a long way. On two separate occasions large lobsters walking over the ray towards a piece of food suddenly jumped backwards with a vigorous flap of the tail.

At other times lobsters have walked unharmed over the rays. Once a *Torpedo* and a large *Rhina* lay facing one another a short distance apart. On a fish being trailed between them both attempted to seize it at the same instant, with the result that they collided head-on in mid-water. At that moment the *Rhina* gave a sudden start and swam away, displaying unusual energy for its species. To the onlookers there was no doubt that it had received a shock, probably the one intended for the prey.

The observations recorded throw little or no light on the manner in which the prey is perceived. The eyes of *Torpedo*, like those of *Rhina*, are small, but they may be sufficiently sensitive to detect the movement of an object of approximately the size and shape of a swimming fish suitable for food, and to fix its position. In nature all such objects are likely to be suitable for food and not a source of danger. Bateson (1890) figures the eyes of both *Torpedo* and *Rhina* and shows that while by day their pupils are only narrow slits, by night they are circular and widely open. However, the prey may be perceived by other senses, by the disturbance it makes in the water, or in other ways. The point demands investigation.

In these observations I have been much assisted by Mr G. R. Forster, and by the attendants Messrs W. H. Gladwell and A. N. Bennett. I am indebted to Mr B. C. Abbott for lending the galvanometer, and Messrs F. J. Warren and A. E. Stoate for working it.

An Alga Disliked by *Mugil chelo* Cuvier

In a tank containing grey mullet (*Mugil chelo* Cuvier) any algal growth on sides and rock is usually kept very short by their browsing activities. Consequently, when in 1950 an isolated growth of a small red seaweed on the top of a rock arch stood out conspicuously amid the eaten-down growths all around, the event was specially noted. Soon patches appeared in other parts of the tank and were obviously being left alone by the mullet. In April 1951 the alga covered fair areas of rockwork and of the sides of the tank and overflow pipe. In the same month the alga was identified by Dr M. W. Parke as *Polysiphonia urceolata* (Dillw.) Grev. in fruiting condition bearing tetrasporangia. A year later this small red seaweed covered almost the whole of the rockwork, and most of the sides of the tank and the overflow pipe. Several samples were taken and the previous identification checked. On this occasion the plants had vorticellids and other organisms growing on them, but the main bulk of the samples consisted of *Polysiphonia urceolata*, which can therefore be retarded as being distasteful to grey mullet.

Behaviour of *Gadus morrhua* L.

In December 1947 several evenings were spent photographing three large cod (*Gadus morrhua* L.) in the second-biggest tank in the aquarium ($15\frac{3}{4} \times 9 \times 4\frac{1}{4}$ ft. deep). One of the cod was blind in the right eye and it

invariably took up a position in the top left-hand corner of the tank with its blind side close to the glass; from this position the good eye could survey the whole interior of the tank. The other two fish generally swam about together, keeping mainly to the other half of the tank. If together or singly they reached its neighbourhood, the blind-eyed fish immediately rushed at them and chased them away, returning to its corner afterwards. This blind-eyed cod died in March 1949 and proved to be female with maturing but not ripe ovaries. It was 71 cm. long.

The two companionable cod rarely separated for long and one would frequently rub its side against the other, often retiring a short distance to swim quickly back again and, swerving laterally, graze the lower middle region of its body against the other fish as it rushed past. The behaviour of these two strongly suggested courtship. It was always the same fish which followed the other about and took the active part in these proceedings. This fish died in October 1949; it was a male, 72 cm. long, with, at the time of death, immature testes. Its companion, which was so attractive to it, expired some weeks after the evening observations here recorded. It had very swollen ovaries and may have died from egg-binding. The length was not recorded but was close to that of the male.

Attempts to photograph the courtship were unsuccessful. The actions took place quickly at intervals, always well back in the tank almost out of range of the flashlights.

Cepola rubescens L. in captivity

Day (1880–84) and Fage (1918) express the opinion that *Cepola rubescens* L., commonly called the Red Bandfish, prefers rocky situations at moderate depths, but at Plymouth this is not so. *Cepola* is most frequently taken in the trawl on, or near, the Rame Mud, a few miles seawards from Rame Head, at depths round about 25 fathoms, and it is rarely taken on other grounds. Specimens rarely survive capture; distension of the air-bladder caused the fishes to float at the surface when placed in a tank and death usually followed within a few hours. Since November 1950, however, a few individuals, taken in trawl hauls of short duration and raised more slowly than usual to the sea surface, have survived. A few have lived for some months in the aquarium, though not all together at the same time. The first of these, a *Cepola* 43 cm. long, was brought in on 23 November 1950 and lived until 26 June 1951, when it was attacked and partly swallowed by a very hungry three-bearded rockling (*Onos tricirratus* (Bloch)), which had been living in the same tank for the whole of that period. Other specimens did not survive so long, dying from various causes. One or two kept for 9 or 10 weeks were never seen to feed. Another fish got itself jammed between the edge of the glass and the wall of the tank; another was killed by a lemon dab (*Pleuronectes microcephalus* Donovan) which pinned it down, perhaps accidentally, under its body; some

died without obvious cause. Those which survived for more than a few days were a small proportion of those brought in alive. The species thus seems to be readily injured, both in the trawl and in simple accidents during captivity.

In captivity a red bandfish usually takes some time to learn to feed. At first it may catch a few *Hemimysis lamornae* (Crouch), which breed in the tanks, but sooner or later it generally learns to take small pieces of squid (*Loligo*) or worms (*Nereis*) sinking down towards it from above, though for many days it may 'blow' such pieces out of its way. Once it is feeding it will compete for food with other fishes, and some individuals have even learnt to take squid from the hand. Towards other fishes *Cepola* can be fierce, biting viciously with long sharp teeth at small pollack and soles. A large *Cepola* was seen to snap at a smaller one.

Cepola swims slowly, with very pronounced body waves. The tank in which the specimens were kept was probably too small to encourage speedy swimming, but forward darts after food, followed by rapid backward retreats, were often seen at feeding time. While a few individuals have swum, or rested, horizontally close to the bottom the majority have maintained themselves vertically head uppermost, swimming with slow body waves, with the ventral surface close up against the slate sides or back of the tank, but never against the glass. This position may have been adopted to avoid the soles living on the bottom in the same tank; but it seems more probable that the vertical posture is normal. However, the individuals which by day always adopted such a position were occasionally seen, especially at night, after sudden switching on of the light, to be swimming slowly over, or resting horizontally on, the bottom. A variation in posture sometimes adopted for short periods has been to curl the posterior third, or half, of the body into a loop, and to rest with the loop on the ground, the anterior part of the body being upright. Sometimes a length of tail has been laid on its side flat along the ground with the rest of the body raised up off the bottom at an angle of about 30°, but bent or twisted to bring the front portion and head into the normal dorsum-upper-most position.

The vertical swimming habit in such an elongated tapering fish suggested to Mr P. G. Corbin that the fish may normally inhabit a vertical burrow, either of its own construction or one occupied, or abandoned, by some burrowing animal such as *Upogebia*, which is numerous where *Cepola* commonly occurs. Indeed, Mr Corbin informs me that more than once a *Cepola* has been brought up in a dredge full of mud and burrowing bottom fauna. With this possibility in mind vulcanite pipes of various diameters and lengths were put into the tank, horizontally, vertically and at various angles, to see if the *Cepola* would enter them, but none did so.[1]

[1] On 24 February 1953, while this paper was in proof, a *Cepola*, length about 20 in., was obtained in unusually good condition. It swam vertically, as usual, and four days later was found to be inside a long vulcanite pipe of about 3½ in. internal diameter. This pipe (which was suspended vertically with its lower end just above the tank floor in one corner, its upper end above water level) was receiving water from an inflow nozzle.

The surmise that *Cepola* normally lives in a burrow received support when one was several times seen, in July 1951, to take up a mouthful of the shell gravel covering the floor of its tank and blow it out again. Later, in February 1952, another specimen made determined efforts to excavate a hole and it got as far as making a well-defined depression in the gravel. Had the gravel been deeper, and had the sides of the hole not slid in as the bottom was excavated, the hole would undoubtedly have been dug out to a fair depth. Twenty or thirty times, after this fish was first observed to be busy, a mouthful of gravel was removed from the hole and deposited well to one side. On each occasion the *Cepola* approached the hole swimming horizontally over the ground, bent down its head vertically into the hole, seized a mouthful of gravel, straightened up and swam a short distance to where the gravel was dropped out of its mouth, and returned for another load. Now and again it rested upright on coiled tail, the loop of the tail within the shallow depression which was the hole it had made. In a stiff mud, such as that of the region where most *Cepola* are caught, the actions just described would excavate a vertical burrow which if carried deep enough would accommodate the fish. If it does live in such a burrow it may have its head near the entrance ready to dart out at passing prey. The fact that specimens which swam vertically by day were sometimes seen in a horizontal position at night may indicate that during the hours of darkness they naturally come out of their burrows to feed. Those that are caught in the trawl by day may also be out hunting for food. However, it is not yet established that *Cepola* ever lives in a burrow, and further observations are needed to confirm or refute this idea. Another possibility is that the excavating activity is connected with nest-building, but this seems unlikely, as the available evidence, although incomplete, indicates that the egg is pelagic (Holt, 1891). Moreover, Clark (1920) states that the spawning period is in August, whereas the most intense excavating activity was observed in February.

Since the above account was written further observations have been made. On 7 January 1953 several young *Cepola*, 5–10 cm. long, were caught in an Agassiz trawl 6½ miles south of the Breakwater, on a bottom of slightly muddy sand. When brought in they were floating with distended air-bladders, but the most lively of them were placed in one of the central table tanks in the aquarium and by the next morning a few of these were swimming normally. About noon Mr P. G. Corbin called my attention to one which was burrowing in sand beside a perforated vulcanite partition whose lower edge rested on the sand. The burrow was being constructed partially under this partition, the fish picking up in its mouth sand from the bottom of its hole, turning round and blowing it away well to one side. Relative to the size of the fish the depth of sand was sufficient for the excavation of a hole, as wide as it was deep, into which the fish was able to retire with its tail coiled around inside and its head out of the entrance. In a corner of the tank another small *Cepola* had

excavated a shallower depression, but it and several others were swimming vertically in the corners or against the slate sides of the tank. Throughout the day they so continued, and the fish in its hole remained there. The following morning the latter and all but two of the others were missing. A search discovered a small *Cottus bubalis* Euphrasén hiding under a rock. When killed and opened the partially digested remains of at least two *Cepola* were found in its stomach.

The burrowing of these very young *Cepola* disposes of the idea that only breeding individuals do so, in order to make nests, and short of final proof strongly supports the supposition that *Cepola* normally lives in a hole or a burrow which it makes for itself. The form of the burrow has still to be determined, whether long and deep to take a fish stretched at full length, or shallow and wide in which it could lie coiled up. Perhaps both types are made, according to the depth and consistency of the bottom deposit. It may be that they are often excavated to pass beneath a stone or other solid object lying on or embedded in the surface of the deposit, in a similar way to the hole made under the partition.

Polyprion americanum (Schneider) in captivity

A fine Wreck-fish or Stone-bass, *Polyprion americanum* (Schneider), about 50 cm. long, was caught, on 7 September 1951, about 5 miles south-west of Bolt Tail by Mr F. Jarvis, Hope Cove, Kingsbridge, and brought to the Laboratory, where it was placed in a tank containing various species of Labridae. The fish had been found inside a tea-chest which was floating on its side; there were no barnacles or other obvious growths on the tea-chest, according to information obtained from Mr Jarvis. Couch (1862) states that the species is especially prone to gather under masses of floating wreckage where it feeds on smaller fishes attracted to the shelter of suspended barnacles or weeds.

This specimen, probably the first of its species to be kept alive at Plymouth, soon settled down and since its arrival has grown about 5 cm. It has fed mainly on fresh fish, readily taking whole pout, *Gadus luscus* L., and small whiting, *Gadus merlangus* L. It will not eat squid—which most of the other fishes take so eagerly—unless it is very hungry. It has a fierce disposition which it wreaks principally on the largest ballan wrasses (*Labrus bergylta* Ascanius), leaving alone the smaller species, the gaudily coloured Cuckoo Wrasses (*Labrus mixtus* Kröyer), and even the smaller ballans. Almost whenever the large ballan wrasses, 30–40 cm. long, appear out of hiding amid the rocks, they are chased and often bitten. In August 1952, Mr W. H. Gladwell, the aquarium attendant, saw the *Polyprion* kill a large ballan wrasse by repeatedly pushing or beating it against the slate wall of the tank, the wrasse being held by the back. These large wrasses are too big to be

eaten; and the *Polyprion* is scarcely likely to attack them because it is hungry, for it could more easily catch and eat the smaller wrasses. Indeed, the *Polyprion* attacks the large fishes as readily after a full meal as before. It is apparently the sight of another large fish which arouses the animosity of the *Polyprion*, which may well have a habit of attacking other fish near its own size, or larger, which appear to be encroaching on its territory. As a fish-eater it would tolerate the presence of fishes of a size suitable for food.

When not attacking another fish the *Polyprion* swims slowly up and down its tank in mid-water and does not frequent the rocks as do the wrasses. Forward propulsion is aided by movements of the enlarged lobes of posterior dorsal and anal fins; the spiny anterior or dorsal is erected as the fish turns. It is often attracted by and watches closely anyone looking into its tank. The overall colour is a fine steely-blue with a velvety plum-like bloom, lighter on the underparts. The caudal fin is bordered by a dark band, with a tendency to a light outer edge. The iris is golden and black and the tip of the protuberant lower jaw has a dark patch. Altogether it is a strikingly handsome fish.

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THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY LARVAE

THE 1952 EXPERIMENTS

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Introduction

In respect of their influence on the settlement reactions of *Ophelia bicornis* larvae, sands can be classed as attractive, neutral, or repellent (Wilson, 1953: mainly on the basis of the 1951 experiments). There are varying degrees of attractiveness and repellence, and the relative effect of sands on larval reactions can be tabulated. From the tables 'it should be possible to forecast with fair accuracy the settlements which will be obtained in mixtures of various sands' (Wilson, 1953, p. 427). Part of the 1952 experiments were devoted to investigating this possibility.

Earlier experiments had indicated, though not finally proved, that some of the repellent property of Salthouse Lake sand could be transferred, by contact under water, to Bullhill Bank sand. This was demonstrated by mixing large grains of the latter with small grains of the former and separating by sieving. In the first experiments along these lines (Wilson, 1952) the sands were not fresh and were sometimes dried for sieving, and all were subsequently sterilized. In 1951 a similar experiment (43 A) with fresh sands, mixed in sea water, again seemed to show partial transference of a repellent factor, presumably organic in nature. The technique used, however, was not entirely satisfactory, for it involved interference with the natural grade of the sand. Also the sand whose properties it was intended to alter was not itself completely free from organic impurities. In the 1952 experiments these objections were met by using acid-cleaned sands of normal grade sewn into fine-meshed bolting-silk envelopes. The envelopes were partially buried in the fresh natural sands from which it was hoped to abstract the repellent factor, and possibly also an attractive one.

A suspicion that these factors included one or more substances of organic nature led to some tests with sands incubated in nutritive media. In this connexion I am much indebted to Dr C. P. Spencer, not only for practical assistance with the incubation but also for much stimulating discussion on the whole problem involved. The incubation experiments in the present series are largely the result of his suggestions.

The 1952 experiments cover also a number of other points, as will be apparent below. It was not practical during the short breeding season to

investigate only one aspect at a time, and each experiment was compounded, as usual, with more than one object in view. To enable a larger number of tests than before to be dealt with in one day, actual counts of the settled and settling larvae were avoided as much as possible. The assessment of the relative attractiveness of the sand samples had to be speeded up, for it had become necessary to test most sands both by the free-choice method and by the conical vessel method. The former method alone does not distinguish between neutral and repellent sands; the latter distinguishes imperfectly between neutral and attractive sands. Therefore both methods are required. The maximum number of samples which can be dealt with in one day if the larvae be counted, as hitherto, is about twelve. For many results, however, exact counts are not necessary, obvious differences being apparent on inspection. In a few minutes such differences can be recorded in words, when to obtain figures would take much longer. Only when the differences are not great are words unsatisfactory, but in the type of experiments used in this work close figures are also of little significance. By drawing up a scale of impressions of increasing number, expressed in words, three times as many sand samples could be disposed of in one day as when counts were made. The scale of words is shown in Table II and is used throughout Tables III–XI. When two samples are shown to differ one from another only by adjacent words in Table II, then the difference between them is scarcely significant, but if the words are far apart then the difference is real. By spreading out all the sand samples, of any one experiment, in dishes alongside one another it was relatively simple to compare them for larval content, and grade them accordingly.

Notes on Technique

General techniques differed little from those of previous years (Wilson, 1952, 1953). The flat-bottomed Pyrex crystallizing dishes (about 7 cm. diam.), in which little heaps of the sands were tested under free-choice conditions, were all cleaned in hot strong sulphuric acid. The conical vessels were cleaned only in hot water containing Lissapol C and scrubbed with a bottle brush, as were also the finger bowls in which the fertilizations were made. The earlier experiments (Exps. 52–56) were in water from off Millport in the Firth of Clyde, the later ones (Exps. 57–59) in a mixture of the Clyde water and water from the English Channel near the international hydrographical station E 1, or in E 1 water only (Exp. 60). All sea water was passed through a Berkefeld filter before use.

Sands which are stated to be acid-cleaned were first washed in fresh water and then heated in fuming concentrated sulphuric acid for at least 30 min., cooled and washed in glass-distilled water and stored in acid-cleaned bottles in distilled water. Bolting silks were cleaned in acetone. New pieces were always used and different pieces for each kind of sand. Bolting-silk envelopes

were sewn with white cotton, also cleaned in acetone. These envelopes were made by folding the silk and sewing bent-over edges along three sides, sand being inserted before the last side was sewn up. They were flat and measured roughly 15 × 10 mm. The sand inside the envelopes spread out into a thin layer and was in close proximity to the sand on which the envelope lay and which was sprinkled over it. The dishes containing these envelopes were kept in a good light but shielded from the sun. Daily, as a rule, the envelopes were turned over with forceps sterilized in a bunsen flame, fresh sand being shifted over on top of them. To remove the sand inside they were cut open with sterilized scissors after all grains on the outside had been carefully shaken off. The silk of envelopes exposed to Salthouse Lake sand was often blackened, but not so that exposed to Bullhill Bank sand. Occasionally, in all dishes, the silks showed pink patches after several days.

The flat-bottomed dishes in which larvae had a free choice of the various sand offered to them in little heaps were, as usual, kept in the dark undisturbed during the two days allowed for settlement. The conical vessels were, as usual, surrounded by black paper so as to be lit only from above, and they were decanted after about 24 hr. to wash away all larvae which were not settling. All larvae washed away by the decanting and re-filling process were examined to make sure that none were metamorphosing. It was confirmed that sands could be much disturbed without washing away metamorphosed or metamorphosing larvae, which always clung tightly to the sand grains.

The Experiments

The 1952 results (Tables III–XI) are expressed as grades of abundance (Table II), with few exceptions. Only larvae actually in the sand samples are mentioned in these tables. The unsettled larvae decanted from the conical vessels are not of special interest; neither are those in the free-choice dishes which were still swimming, or were lightly attached to the surface film or to the clear glass bottom between the sand heaps. There were always many such larvae and they were specially numerous when all the heaps were of neutral or repellent sands. They were always unmetamorphosed, except for a few metamorphosed worms that occasionally crawled out of overcrowded heaps on to the glass between them. Sometimes in heaps of fresh Bullhill Bank sand so many larvae had settled and metamorphosed that their activities caused a spreading of the sand until the base of the heap was extended to almost twice its proper diameter.

Each experiment had a control not recorded in the tables. This was a small flat-bottomed dish, the bottom completely covered with fresh Bullhill Bank sand, placed in the dark with the free-choice dishes. Invariably most of the larvae put into this dish were found to have metamorphosed, few or none being on the surface film, or still swimming.

Unless stated to the contrary, the sands mentioned in the tables were fresh, unwashed and unsterilized. They had been collected on the dates mentioned and had been kept since collection in clean stoppered jars. The Bullhill Bank sands were all from the surface of the middle of the Bank at places where *O. bicornis* adults were abundant. The Salthouse Lake sands were all from Station II (see Wilson, 1952, pp. 59 and 61), where *Arenicola marina* and other species were plentiful (for list of species see Holme, 1949) but *Ophelia bicornis* absent. Table I gives the percentages by weight of the various mesh sizes of these two sands, for comparison with the sands of previous years. The washings, normal sterilization (heating in a glass vessel immersed in a bath of boiling water for a few minutes) and treatments with activated charcoal mentioned were all carried out in tap water, as had been usual. The mixtures were all made by measuring, in the stem of a pipette, equal volumes in water of the two sands used.

The experiments are numbered consecutively with those already published.

Experiment 52 (Table III)

Series A consists of tests of sands put into medium (0·5 % peptone and 0·2 % glucose in sea water) 3–4 hr. after collection and incubated in a dark oven at 22° C. for 6 days. One sample of Bullhill Bank sand was inoculated with a very few grains of Salthouse Lake sand. At the end of this period the medium, which had become either yellow (Bullhill Bank sand) or greenish grey (Salthouse Lake sand) with a strong odour, was poured off and the sands well washed with clean sea water before testing. Microscopic examination of portions of the washed sands showed large numbers of minute organisms sticking to the sand grains, or free and in motion in the interstices.

Table III shows that incubation of the sands lessened their attractiveness for the larvae, compared with the unincubated controls, both under free-choice conditions and in the conical vessels. Salthouse Lake sand, both without and with incubation, was less attractive than Bullhill Bank sand correspondingly treated. Inoculation of Bullhill Bank sand with grains from Salthouse Lake, thereby introducing bacteria and micro-organisms from the latter place, had no noticeable effect. The hypothesis that Salthouse Lake contains species, culturable in the medium, which are repulsive to the larvae and which are absent from the Bullhill Bank is unsupported by this inoculation experiment. The culturable species, or their metabolic products, normally present in the Bullhill Bank sand, are attractive, or at least not repulsive, in normal abundance. When more abundant they may possibly be offensive to the larvae.

Series B and *Series C* were tests of mixtures. In the first the mixing in equal proportions of a fully attractive sand with one that is neutral (see Wilson, 1953, pp. 425–7 and Table XIII) induced, as was expected, a good settlement under free-choice conditions and an even better settlement in a conical vessel.

The second mixture, of a fully attractive with a fully repellent sand (Wilson, 1953, Table XIV), produced very little settlement under either condition, this result again being the one anticipated. The repellent property of the sterilized Salthouse Lake sand had a stronger influence on larval reactions than the attractive property associated with Bullhill Bank sand.

Experiment 53 (Table IV)

For *Series A* small portions of the acid-cleaned sands removed from bolting-silk envelopes after 5 days in sea water, with or without contact with fresh unwashed sands, were examined microscopically. That which had been in sea water only showed some very minute organisms, which may have been bacteria, and was otherwise very clean. The other sands were also relatively clean but, in addition to what may have been bacteria, a number of ciliates, minute diatoms and other living plant cells were seen. They were more abundant in the sand which had been in contact with the Salthouse Lake sand than with that from the Bullhill Bank.

The result (Table IV), compared with the two controls, shows a marked increase in attractiveness of the acid-cleaned sand which had been amid fresh Bullhill Bank sand. That which had been with Salthouse Lake sand showed no such increase; instead there appears to have been a slight decrease in attractiveness, this being most marked in the conical vessel test.

Series B, *C* and *D* are further tests of mixtures. In the first the formalin-treated sand appears to have been a little repellent and not neutral as a similarly treated sample mentioned in a previous paper (Wilson, 1953, Table XIII). No doubt different samples vary a little in their properties. The mixture in this instance induced somewhat smaller settlements than had been expected on the basis of formalin-soaked sand being neutral, but this result is in agreement with one component of the mixture having a slightly repellent action. The second mixture was to test a suspicion, based on earlier results (Wilson, 1952, pp. 103 and 155), that boiling Bullhill Bank sand for a prolonged period in distilled water makes it a little less favourable than normal sterilization, and therefore on the repellent side of neutral. In this test the boiled sand acted as a neutral sand and the result is closely similar to that of Exp. 52A with normally sterilized sand. Again, different samples could vary according to their condition before boiling. The third mixture of a fully attractive sand with that of a repellent sand converted to attractiveness by the use of activated charcoal gave, under both conditions, the heavy settlements which had been anticipated.

Experiment 54 (Table V)

This was devoted to testing a number of mixtures of sands whose relative powers to attract or repel were fairly well known. The settlements obtained in all four mixtures were much as expected. In *Series A* and *B* the mixing of neutral with fully repellent sands produced strongly repellent mixtures. If

boiling the Salthouse Lake sand made it more repellent than normal sterilization the fact is not shown by these fifty-fifty mixtures. In *Series C* the mixture of a fully attractive sand with a neutral or nearly neutral sand (in Wilson, 1953, Table XV, the oolitic sand is perhaps placed too far towards the repellent side of neutral) was favourable to settlement, but was not fully attractive. In *Series* D a fully repellent with a fairly attractive sand gave a mixture which was repellent, though not fully repellent.

Experiment 55 (Table VI)

Part of this experiment was devoted to tests of acid-cleaned Bullhill Bank sand inoculated with a few grains of fresh Bullhill Bank sand and fresh Salthouse Lake sand and incubated at 22° C. in a series of media containing less nutrients than the medium used for Exp. 52A. Sea water was enriched with 2·5 mg. per litre ammonium/nitrogen and this was further enriched with (*a*) 2·5, (*b*) 5, and (*c*) 10 mg. per litre of glucose to give a series of increasing concentrations. The results, though interesting, are too indefinite to be worthy of detailed consideration. So far as they go they suggest that inoculation of the acid-cleaned sand by either of the fresh sands, followed by incubation, slightly increases its attractiveness, and this may vary a little according to the concentration of the medium and hence probably of the number of micro-organisms present.

The remainder of this experiment, here designated *Series A* and *B* in Table VI gave definite results. *Series A* followed closely on the lines of Exp. 53A, using this time acid-cleaned Salthouse Lake sand which was of finer grade than the acid-cleaned Bullhill Bank sand used before (see Table I). Once again the sand which had been amid fresh Bullhill Bank sand became distinctly attractive, whereas that which had lain amid fresh Salthouse Lake sand did not, showing little difference from the controls.

Series B is a test of another mixture. In a previous paper (Wilson, 1953) it is shown that soaking fresh Bullhill Bank sand in distilled water can destroy its attractiveness under free-choice conditions, though a good settlement can be obtained when it completely covers the bottom of the vessel containing the larvae. It was rated as a neutral sand. In the present test there was a small settlement in the free-choice dish, although the sand had soaked for 3 days in distilled water. This suggests that this particular sample was a little on the attractive side of neutral, but the settlements obtained in the mixture with fully attractive fresh Bullhill Bank sand were of the type associated with a mixture of the latter with a neutral, or nearly neutral sand. They were, indeed, what had been expected.

Experiment 56 (Table VII)

For *Series A* three conical flasks each containing a small quantity (barely sufficient to cover the bottom if spread out) of acid-cleaned Bullhill Bank sand

in filtered sea water were exposed to light on a window bench shielded from the sun. To one of these flasks a few grains (less than 1 %) of fresh Bullhill Bank sand were added, and to another a similar quantity of fresh Salthouse Lake sand. The flasks were gently shaken almost every day for 10 days, the sand in each flask piling up into a small heap in the middle. On the tenth day small samples from each were removed to be tested with larvae in the usual manner. In addition, a few grains of fresh Bullhill Bank sand and of fresh Salthouse Lake sand were mixed with small samples of the control sand to which nothing had been added previously. These mixed samples served as additional controls intended to distinguish between the effect of adding a few grains of the fresh sands just before the test and adding a few grains several days previously.

The settlements obtained with these various sands are set out in Table VII. The addition of a few grains of fresh sand just before testing with larvae seems to have made but little difference, except perhaps that the Salthouse Lake grains may have improved it slightly. It seems certain, however, that sands to which fresh grains had been added 10 days before testing were more attractive than any of the controls, and that the Salthouse Lake grains had had the greater effect.

The original sands in their flasks on the window bench were kept for nearly another 2 weeks, but some more acid-cleaned sand was added (on 1 July 1952) to the control flask because insufficient for another experiment remained. The tests were then repeated in an identical manner as part of Exp. 57 but only in a free-choice dish. The results were similar to those of the present experiment. There was no further increase in attractiveness of those sands to which grains of fresh sand had originally been added but these sands were still the most attractive.

Series B duplicates *Series A* except that the flasks were kept in a dark cupboard instead of on a window bench, and were exposed to light only for a few moments daily while being shaken. Table VII shows that the resulting settlements obtained were closely similar to those in sands exposed to the light. This *Series B* was also repeated (with the same sands kept longer) at a later date (as part of Exp. 58—free-choice dish only) and like the repetition of *Series A* gave settlements which differed hardly at all from the earlier ones.

It is not clear what these results indicate. If the improvement, with time, after inoculation is due to multiplication or metabolism of micro-organisms, then the species involved do not depend on light. The failure of the attractive factor to increase beyond a certain point, even with further time, might be due to early exhaustion of necessary nutrient materials from the filtered sea water used. Other explanations are possible. For instance organic matter on the fresh grains which were added may take time to transfer to the acid-cleaned sand.

Exp. 56 included tests of two more mixtures. The first (*Series C*) is a mixture

of two neutral, or almost neutral sands, and gave, as anticipated, a small settlement under free-choice conditions and a good one in a conical vessel. It should be compared with Exp. 54C where the same oolitic sand was mixed with an attractive sand. The second mixture (*Series D*) is similar to that tested in Exp. 52C, except that here the Salthouse Lake sand was dried after washing and before normal sterilization. There seems to be little difference, both are strongly repellent, but in this experiment the larvae, which were a day older than usual, were a little more ready to settle than they generally are. The result, however, is only slightly different from that expected. Settlements in a fifty-fifty mixture of a fully attractive with a fully repellent sand are likely to vary around the neutral point according to the condition and age of the larvae. The 5-day-old larvae of Exp. 52C found such a mixture rather repellent, the 6-day-old larvae of the present series found it somewhat attractive. The antagonism of the two factors, one attractive, the other repellent, is clearly demonstrated.

The larvae in the clean finger bowl which supplied the experiment showed a precocious tendency to metamorphose without sand of any sort. Thus they displayed an unusual early elongation of the body, loss of prototrochal cilia and elongation of the bristles of the third setiger. Some larvae metamorphosed on the clean glass unusually early. These larvae were markedly more advanced in their development than were those of the same age in the bowl which had supplied larvae to Exp. 55. Account should be taken of this feature in assessing the results of the experiment.

Experiment 57 (Table VIII)

On two previous occasions (Exps. 53 and 55) an attractive factor from fresh Bullhill Bank sand was transferred to acid-cleaned sands consisting largely of quartz particles. In *Series A* of this experiment oolitic sand, which is almost entirely calcareous with but very few quartz particles, was used in place of an acid-cleaned sand. No attempt was made to clean it. Three samples were sewn up in 100-mesh bolting-silk envelopes. One, the control, was placed in sea water only, the other two in sea water amid fresh Bullhill Bank and fresh Salthouse Lake sands respectively. These fresh sands were changed after 11 days when fresher samples were available. After a total of 16 days the sands were removed from the envelopes and agitated in sea water on sieves of 86-mesh bolting silk. This was to ensure the removal of any particles of the fresh sands which might have worked their way through the 100-mesh silk envelopes to become mixed with the oolitic sands inside. The control sand was, of course, similarly agitated on 86-mesh silk: it was then divided into three portions, to two of which a few grains of the Bullhill Bank and Salthouse Lake sands were added. The oolitic sand which had been amid Salthouse Lake sand had a grey tinge and its silk envelope was blackish, as was much of the Salthouse Lake sand itself.

The result is shown in Table VIII. The settlements in the free-choice dish are considered first. There was little difference between any of the controls; the addition of a few fresh grains had had little or no effect and in all three the settlement was small. There was a significantly better settlement in the sand which had been with the Bullhill Bank sand, though the numbers of larvae settling were not large. Most of the larvae in the dish had gathered on the surface film or were still swimming freely, or were on the glass of the bottom, unmetamorphosed. The oolitic sand had apparently been made only a little more attractive by contact with Bullhill Bank sand, but none the less this increase in attractiveness was definite. On the other hand, the oolitic grains which had been amid Salthouse Lake sand had failed to induce any larva to metamorphose.

In the results with conical vessels there is no distinction between the controls and the oolitic sand which had been with Bullhill Bank sand. All gave settlements characteristic of neutral, or near neutral, sands. Only in the oolitic sand which had been with Salthouse Lake sand was there a difference to be noted. Here there were markedly fewer metamorphosing or metamorphosed larvae than in any of the other sands and there were rather more unmetamorphosed larvae present: the sand, in fact, appears to have been slightly repellent.

In *Series B* a new type of experiment was undertaken. A quantity of fresh Bullhill Bank sand was thoroughly shaken up with about three times its own volume of filtered sea water. After allowing all heavy particles to settle some of the water was carefully poured off into a clean dish. This was then examined to make sure that no sand grains, not even the smallest, were present. Only a little silt was to be seen. A small quantity of acid-cleaned Bullhill Bank sand was then added and the dish stood in the light, shielded from the sun, for 16 days. In a similar manner water in which fresh Salthouse Lake (St. II) sand had been shaken was used to soak another small quantity of acid-cleaned Bullhill Bank sand. In the Salthouse Lake water silt was plentiful but no sand grains were present. A control sample of the acid-cleaned sand was soaked in filtered sea water in the same manner. The experiment also includes a control of the same acid-cleaned sand stored in distilled water.

Table VIII, recording the results, shows that the sand soaked in water in which Bullhill Bank sand had been shaken induced much better settlements than did any of the others. The sand from water in which Salthouse Lake sand had been shaken also showed an improvement over the control, but not so great. This improvement contrasts with the opposite effect observed when acid-cleaned or oolitic sands are buried amid the Salthouse Lake sand itself. The control sample soaked in clean filtered sea water seems to have been slightly more attractive than the sample stored in distilled water.

In order to make reasonably certain that the presence of a few small sand grains, which might pass through 100-mesh silk, would not significantly

affect the results obtained with sand samples enclosed in bolting-silk envelopes buried amid other sands, a test was made, in *Series C*, of acid-cleaned Bullhill Bank sand to which small quantities of fresh sands were added. The amounts added were appreciably larger than any which might be expected to pass through the meshes of the silk, and were 1 % or less of the whole sample. Although the conical vessel control was accidentally lost the results so far as they go confirm previous findings (Exps. 56A, B and 57A) that any effect is likely to be small.

As already mentioned (p. 215), Exp. 57 included a re-trial of the sands used in Exp. 56A.

Experiment 58 (Table IX)

An attempt was made to transfer the attractive factor in fresh Bullhill Bank sand, and the repellent factor in fresh Salthouse Lake (St. II) sand, to some artificial substance of as pure a composition as possible. I am indebted to Mr F. A. J. Armstrong for suggesting fused alumina, a reagent prepared by the Thermal Syndicate Ltd. The label on the bottle gave as the batch analysis; alumina 99·7%, carbon 0·0013%, alkalies 0·09%. The alumina is in the form of granular particles. By sieving through bolting silks it was possible to obtain a 'sand' similar in grade to that of the Bullhill Bank. The graded alumina (all of which was coarser than 86-mesh and most of it coarser than 60-mesh) was thoroughly washed in several changes of distilled water, to reduce yet further the soluble impurities and clean the large grains of adhering minute particles. The resulting 'sand' was clean and white. For *Series A* small samples were placed, in 100-mesh bolting-silk envelopes, amid fresh Bullhill Bank and Salthouse Lake sands, and there was the usual control in an envelope in sea water only. After 7 days the contents of the envelopes were removed and examined microscopically. No sand grains had penetrated the envelope which had been amid Bullhill Bank sand, but there were a very few quartz grains and some silt mixed with the alumina from the Salthouse Lake sand. Therefore all three samples were sieved separately, in filtered sea water, on 60-mesh silk about a third of each sample being lost through the meshes. This ensured the removal of all foreign sand grains and silt which had penetrated the 100-mesh silk. The sands were again examined to confirm this. No foreign grains among those of alumina could be seen, but various minute living organisms were observed on the grains, especially those which had been amid Salthouse Lake sand. The alumina grains which had been with the latter were tinged pale yellow.

The results indicate that an attractive factor from the Bullhill Bank sand had been transferred to the alumina, but they barely show any such transference of a repellent factor from the Salthouse Lake sand. They are not, however, completely negative in that respect, and taken in conjunction with earlier tests on similar lines (Exps. 53A, 55A and 57A) provide additional evidence, though slight, that such transference is possible.

In *Series B* some tests are made with activated charcoal. It is not at all clear how this reagent reacts with repellent sands to make them attractive. The grains of such sands are always darkened with adhering specks of the carbon which may do no more than stick to and cover up matter objectional to the larvae. The granular activated charcoal used in this experiment was the kind intended for gas absorption. The other kind was that used for decolorizing, the same batch having been used in all previous experiments with this substance. It is a very fine powder, and only a very small quantity of very small grains was obtained by washing away a large quantity of the still finer powder. There was sufficient for only a very small heap in the free-choice dish and not enough for a conical vessel as well. These very fine carbon particles attracted a large number of larvae, but the proportion to metamorphose was small, much smaller than amid the granular carbon or amid the same but still finer carbon particles mixed with the acid-cleaned sand. This suggests that while the activated charcoal is liked for its own sake the larvae require, among other factors, a substratum of reasonable solidity if metamorphosis is to proceed readily.

Exp. 58 also contained a re-trial of the sands used in Exp. 56B (see page 215).

Experiment 59 (Table X)

Some earlier tests, included in Exp. 55, had indicated that inoculation of an acid-cleaned sand with a few grains of a fresh sand, followed by incubation (in the dark at 22° C.) in a medium, increases slightly the attractiveness of the sand. The test for inoculation with fresh Bullhill Bank sand was repeated here, the concentration of medium which gave the best result in Exp. 55 being used. This was sea water enriched with 2·5 mg. per litre ammonium/nitrogen and 5 mg. per litre of glucose.

There was some difficulty in obtaining satisfactory larvae for this experiment. All the fertilizations intended for it failed and earlier fertilizations had to be used. The larvae put into the free-choice dish were 7 days old; those into the conical vessels were 6 days old and were from the fertilization which had supplied Exp. 58.

The result in the free-choice dish agrees with the earlier results that inoculation with fresh sand grains followed by incubation increases the attractiveness of the acid-cleaned sand. The incubated control which had not been inoculated was also made more attractive in comparison with some acid-cleaned sand which had been merely stored in distilled water until tested. Settlements in the conical vessels with younger larvae were all very similar and any slight distinctions are in line with the dish results.

Lack of suitable larvae prevented other tests, which had been planned, from being made.

Experiment 60 (Table XI)

In Exp. 51 (Wilson, 1953) an attempt to test the effect on settlement of different grades of fresh Bullhill Bank sand was only partially successful owing

to the unusually slow rate of development of the particular culture of larvae used. The experiment is repeated here with a larger number of grades. These were obtained by sieving fresh Bullhill Bank sand through bolting silks of the usual mesh-sizes used in these investigations (see Wilson, 1952, p. 59), the sieving being done in filtered sea water. The ungraded control sand was agitated on 100-mesh silk in sea water for a time equivalent to that needed for sieving the other grades. Only a very few of the smallest grains were lost. The heaps in the free-choice dish were all of the same size and were much smaller than usual. Hence the unusually small number of larvae settling in even the most attractive grades. Conical vessel tests were not used in this experiment.

The result supports earlier conclusions that grade size is not without influence on settlement. The grade in which the greatest number of larvae settled is that which until about 1950 comprised about 50% of the surface sand of the Bullhill Bank (see Wilson, 1952, p. 59 and Table II, p. 127). The settlement in the control ungraded natural sand was closely similar to that amid grains of 60–86-mesh sizes, the sizes which now form the greater bulk of the surface sand of the Bullhill Bank (Table I), and therefore of the control. Relatively large, or very small, grains attracted very few larvae or none at all.

Other Experiments

A number of other experiments were attempted towards the end of the breeding season late in July, but all were spoilt by the condition of the larvae. The fertilizations made in the latter half of July appeared to be excellent for the first 2 or 3 days. Afterwards the larvae went prematurely to the bottom and many stuck together in small and large clusters. This clumping has been shown to take place most readily when the water is in some way unsuitable for healthy development (Wilson, 1951). These fertilizations during the latter half of July were made in water from hydrographical station E 1 off Plymouth, after the Clyde water available earlier had all been used up. In spite of the condition of the larvae several experiments were set up. The results they gave were, as far as they went, in line with previous findings. Thus another attempt to transfer to oolitic sand the attractive factor from fresh Bullhill Bank sand seems to have been successful. Some tests based on filtering water in which fresh sands had been shaken gave promising, though imperfect, results. These are, however, sufficiently interesting to merit further attention at a later date.

Analysis of Sands for Nitrogen Content

Dr C. P. Spencer kindly undertook the analysis of surface samples of Bullhill Bank and Salthouse Lake (St. II) sands collected on 9 May 1952. The sands were first thoroughly washed in clean sea water, from the laboratory circulation, to remove silt. They were then sieved, in sea water, through 26-mesh bolting silk to remove large mineral particles and any larger living organisms

which might be present. The Salthouse Lake sand was further sieved through 40-mesh bolting silk, for it was found that a few small molluscs and worms had passed the larger mesh. Samples of the sand were then digested with the digestion mixture of Harvey (1951) and distilled with a Markham micro-stream distillation apparatus (Markham, 1942). The figures given below are the mean of triplicate estimations. The blank estimations were done on separate samples: Bullhill Bank sand, 490 μg. N/g. wet weight; Salthouse Lake (St. II) sand, 1760 μg. N/g. wet weight.

In view of the thorough washing, the nitrogen found must have been present on the sand grains in an insoluble form and is therefore probably organically bound. If this assumption is made, the nitrogen determination estimates organic matter, and so the Salthouse Lake sand, at least after washing, contained some three times as much organic matter as that from the Bullhill Bank.

The striking effect on settlement of treating sands with activated charcoal has frequently been demonstrated, repellent sands being made attractive by its use. I am much indebted to Mr F. A. J. Armstrong for making analyses in an attempt to find out whether the activated charcoal removed organic nitrogen compounds from the sand. The attempt was unsuccessful because the high nitrogen content of the activated charcoal masked any possible changes in the nitrogen content of the sand.

Discussion

In assessing the results of experiments such as these, and especially when comparing one experiment with another, it is essential to remember that they are based on the reactions of living organisms of which no two are ever quite alike. There is much accumulated evidence, not always set out in these papers, to show that different cultures of larvae vary in the readiness with which they will metamorphose under relatively unfavourable conditions, such as those of a clean glass vessel without sand. Some cultures remain free-swimming, with no tendency to metamorphose, longer than others of the same age, or younger, even when beside them on the same table under, practically identical conditions of temperature and lighting. Thus quite apart from variation in the numbers of larvae used from experiment to experiment, the results are to some extent affected by the characteristics of the particular batch of larvae used, as well as by their age when this is different. But in any one experiment very seldom has more than one fertilization of larvae been used, and all comparisons are made between larvae of the same age and with the same mixture of characteristics. Within each experiment results are thus strictly comparable. These remarks are necessitated by the long series of experiments which have now been published and which if compared without these points in mind may occasionally appear to be

contradictory. For instance, as pointed out above (p. 216), in Exp. 56 the particular larvae used showed a precocious tendency to metamorphose without sand of any sort. This may well explain why more of them settled in the acid-cleaned sand straight from storage in distilled water than was usual for larvae of their age. Though this peculiarity has to be remembered when comparing this particular experiment with any other, the comparisons between the different sands in the experiment are not in the least invalidated. On the whole, atypical results due to variable characteristics have been infrequent. Most results, indeed, have been consistently repeatable.

The results with mixtures may now be considered. Before the present series of experiments was started, a table was drawn up (using information gained in 1951) listing the mixtures it was proposed to test and forecasting the intensity of the settlements expected in them under both free-choice and conical vessel conditions. Twelve mixtures were proposed and, as already noted in the descriptions of the actual experiments, the results with them showed very little variation from those expected. Such small variations as occurred, chiefly in Exp. 53B and C, can be attributed to one component of each mixture not having been quite of the same neutral or repellent property as a similar sand previously assessed. Such variation between sands of different batches is only to be expected. The experiments, however, show that if the relative power to attract or repel of two sands be known then it is possible to forecast the intensity of settlement which will be given by 5-day-old larvae in a mixture of equal volumes. A mixture consisting of two attractive sands, or of an attractive with a neutral, remains attractive. A repellent sand with another repellent, or with a neutral, gives a repellent mixture. Neutral with neutral remains neutral. Attractive sands mixed with repellent sands produce results varying on either side of neutral in accordance with which factor, attractive or repellent, is dominant. Only when the opposing factors are equal will such a mixture be neutral.

That the attractive and repellent factors are distinct, and at least to some extent independent of the mineralogical characters of the sand grains which carry them, seems to be shown by several experiments in which they were transferred to sands consisting mainly of quartz grains (after drastic cleaning in hot concentrated sulphuric acid), to a mainly calcareous oolitic sand, and to a purely artificial sand of fused alumina. The attractive factor was transferred quite readily, though no sand so treated became nearly as strongly attractive as fresh Bullhill Bank sand itself. Before 1952 no experiment had shown transference of an attractive factor. Indeed the existence of such a factor was almost unsuspected before the results of the 1951 experiments had been considered. Evidently, the attractive factor is present only in fresh sand, particularly that from the Bullhill Bank, and that almost any treatment including sterilization, drying, subjection to hot concentrated sulphuric acid and red heat destroys it.

The repellent factor, on the other hand, is most readily demonstrated in sterilized or dried sand, particularly that from the Salthouse Lake (St. II), though hot concentrated sulphuric acid or red heat destroy it. Its transference, though imperfectly achieved both in earlier experiments and in 1952, seems to be no less real than the transference of the attractive factor. In 1952 its transference may have been complicated by the presence of the attractive factor, for both seem to be present on fresh Salthouse Lake (St. II) sand, which is normally almost neutral. Neutrality could result, as is shown by the mixture experiments, when the two factors are balanced against each other.

The manner in which transference takes place from fresh sands to acid-cleaned or other neutral sands is not clear. For the attractive factor, physical contact of the sand grains is evidently not necessary, since it was present in sea water in which fresh Bullhill Bank sand had been shaken (Exp. 57B). It seemed also to be present, though less concentrated, in water in which fresh Salthouse Lake (St. II) sand had been shaken (Exp. 57B). The latter experiment and certain inoculation experiments (Exps. 55, 56A and B) appear to show that the attractive factor is present on fresh Salthouse Lake sand and can be separated from the repellent factor which is also there. The repellent factor seems to be transferred less readily than the attractive factor, and then only to sands in close proximity if not in actual contact with it.

The experiments involving incubation of sands in media are difficult to interpret. In the first of these experiments (Exp. 52A), where fresh sands were put into a medium which produced a heavy growth of organisms, the sands became, even after thorough washing, less attractive than before. On the other hand, acid-cleaned sands inoculated with only a few grains of either of the fresh sands, and incubated in weaker media producing only a relatively small growth of organisms (Exps. 55 and 59), showed an increase in attractiveness after incubation. Even without enriching the sea water, and at room temperature both in the light and in the dark, the inoculation of acid-cleaned sand with a few grains of fresh sand of either sort, followed by a period during which a small growth could take place (Exp. 56A and B), resulted in a small increase in attractiveness. These experiments, taken by themselves, might indicate that the attractiveness or repellence of a sand depends not on two different factors but on the relative abundance of a single factor. It will be remembered that the organic nitrogen content of Salthouse Lake (St. II) sand was some three times that of Bullhill Bank sand (p. 221). None the less, other results are more easily interpreted if two factors are assumed, one of which, the attractive one, is readily destroyed by sterilization, drying and other simple treatments.

The natures of the attractive and repellent factors are still unknown, but on the evidence are probably organic or the products of organic activity. The results with sterilized sands suggest that the repellent factor may be non-living material; but the status of the attractive factor is even less evident.

Even when the nature of the factors is known the manner in which the larva perceives them, or is affected by them, has still to be sought. The experiments with activated charcoal may give a clue on how the attractive factor stimulates the larva to settle and metamorphose. This highly adsorbent substance appears to be attractive in itself (Exp. 58 B), although for maximum effect it must be present in particles of a size simulating a sand, or have sand grains mixed with it. The very property of adsorbing readily, by aiding the larva to get rid of excretions, may act favourably on the larva. The attractive factor might thus be something which removes excretory matter from the larva; though this is by no means the only possibility. It is well known that minute amounts of copper, or other substances, stimulate the larvae of certain species to metamorphose (see Wilson, 1952, pp. 51–2 for a brief discussion and references). The activated charcoal is not a pure substance and I am indebted to Mr F. A. J. Armstrong for showing that it contains an appreciable amount of copper. The decolorising charcoal, which has been used throughout these investigations, gave, on analysis, 320 parts per million of copper, while the granular charcoal used in Exp. 58 B gave 600 parts per million. From the natural sands themselves the amounts of copper extracted by heating to fuming with nitric and sulphuric acids were: Bullhill Bank, 6 parts per million; Salthouse Lake (St. II), 8 parts per million. The total copper contents, determined after opening the samples by hydrofluoric acid treatment, were 24 and 15 parts per million respectively. These figures are given without implying that they are of significance in relation to settlement in the natural sands; whether the larger quantities present in the charcoals influence metamorphosis cannot be decided on present evidence.

The experiments with activated charcoal again showed that if metamorphosis is to take place freely the particulate matter must be of a grade not too far removed from that of the natural sand itself. This is further brought out in Exp. 60 in which graded sizes of Bullhill Bank grains were tested under free-choice conditions. The grades which form the bulk of the surface sand of the Bullhill Bank attracted the largest settlements and there was a marked falling off in the numbers of larvae settling in coarser and finer grades. Sands can evidently be too fine or too coarse.

Much of the foregoing discussion is speculative, and further experiments will surely modify or refute some of the ideas put forward, but these ideas are of value in so far as they facilitate the planning of future work.

Summary

Previous conclusions that it should be possible to forecast the intensity of settlements which will be obtained in fifty-fifty mixture of two sands, provided the relative attractiveness or repellence of each component is known,

were fully confirmed. The results of these mixture experiments agree with the classification of sands as attractive, neutral or repellent.

It is shown that an attractive factor is capable of transference, in sea water, from fresh Bullhill Bank sand to acid-cleaned quartz sands, to calcareous oolitic sand and to an artificial sand of fused alumina. The attractive factor is present in water in which fresh Bullhill Bank sand had been shaken.

A similar transference of a repellent factor from fresh Salthouse Lake sand is not so clearly demonstrable. Water in which fresh Salthouse Lake sand has been shaken appears to contain the attractive factor. Thus both attractive and repellent factors may be present in Salthouse Lake sand.

Activated charcoal stimulates larvae to settle and metamorphose, but needs to be in granular form, or associated with neutral grains, to exert its full influence. The charcoal contains copper in minute proportion, but whether this has any significance for metamorphosis is undetermined.

The grade of a sand was again shown to be a settlement factor of some importance. The most attractive grain sizes appear to be those of which the bulk of the Bullhill Bank surface sand is composed.

Analysis shows that the surface sand of Salthouse Lake (St. II) contains some three times the organic nitrogen content of the surface sand of the Bullhill Bank.

TABLE I. GRADING OF SURFACE SANDS

Date	(6) >26 mesh	(5) 26–40 mesh	(4) 40–60 mesh	(3) 60–86 mesh	(2) 86–100 mesh	(1) 100–200 mesh	Silt	Mean category	Percentage floatability
From the middle of Bullhill Bank									
10. vi. 52	0·2	1·1	25·0	61·2	11·7	0·7	Slight	(3·15)	20–30
8. vii. 52	0·2	0·9	20·2	63·7	13·8	1·1	Slight	(3·07)	20–30
From the Salthouse Lake (St. II)									
10. vi. 52	0·3	1·1	16·0	33·9	32·0	16·7	Fair quantity	(2·54)	50–60

Categories (1) to (6) are percentage mesh sizes by weight, after washing away silt.

TABLE II. SCALE OF WORDS USED TO EXPRESS NUMBER IN TABLES III–XI

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

TABLE III. EXPERIMENT 52

(Begun 16. vi. 52 with larvae from a fertilization of 11. vi. 52.)
Results on 18. vi. 52.

Series A

	Bullhill Bank sand (10. vi. 52)			Salthouse Lake sand (10. vi. 52)	
	Rinsed in sea water	Incubated in medium for 6 days	Incubated in medium for 6 days after inoculation with Salthouse Lake sand (10. vi. 52)	Rinsed in sea water	Incubated in medium for 6 days
	Settlements in Dish				
Metd	Very many	None	None	Several	None
Meting		3 or 4	2 or 3		2
Unmet.	Few	Several	Several		Several
	Settlements in Conical Vessels				
Metd	Multitude,	Many	Many	Fair number,	Several
Meting	mainly metd.			mainly meting	
Unmet.	None	Several	Several	Several	

Series B

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude, mainly metd.	None	Many,	Multitude,
Meting		2	mainly metd.	almost all metd.
Unmet.	Few	1	Few	None

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude, mainly metd.	None	None	Very few
Meting		None	1	
Unmet.	Several	Several	Several	Few

TABLE IV. EXPERIMENT 53.

(Begun 17. vi. 52 with larvae from a fertilization of 12. vi. 52.)
Results on 19. vi. 52.

Series A

Acid-cleaned Bullhill Bank sand (9. v. 52)
In bolting-silk envelope in sea water for 5 days

	Stored in distilled water	In clean dish	Amid Bullhill Bank sand (10. vi. 52)	Amid Salthouse Lake sand (10. vi. 52)
		Settlements in Dish		
Metd	1	Few	Many, mainly metd.	None
Meting	1	Few	Many, mainly metd.	None
Unmet.	Few	Few	Few	Few
		Settlements in Conical Vessels		
Metd	Good number	Fair number	Multitude, mainly metd.	Few
Meting	Good number	Fair number	Multitude, mainly metd.	Few
Unmet.	Good number	Fair number	Few	Few

Series B

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) soaked 1 hr. in 5 % formalin in sea water	Mixture of the two sands	Mixture of the two sands
			Settlements in Dish	*Settlement in Conical Vessel*
Metd	Multitude, mainly metd.	None	*c.* 5	Many, mainly metd.
Meting	Multitude, mainly metd.	None	2	Many, mainly metd.
Unmet.	Few	None	1	None

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) washed (distilled water), boiled in distilled water 2–3 hr.	Mixture of the two sands	Mixture of the two sands
			Settlements in Dish	*Settlement in Conical Vessel*
Metd	Multitude	None	Fair number, mainly metd.	Multitude
Meting	Few	None	Fair number, mainly metd.	Very few
Unmet.	1 or 2	None	Several	None

Series D

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, treated activated charcoal 2–3 hr., normal sterilization	Mixture of the two sands	Mixture of the two sands
			Settlements in Dish	*Settlement in Conical Vessel*
Metd	Multitude	Fair number	Multitude	Multitude
Meting	Very few	Fair number	Few	Very few
Unmet.	Very few	Fair number	Few	None

TABLE V. EXPERIMENT 54

(Begun 25. vi. 52 with larvae from a fertilization of 20. vi. 52.)
Results on 27. vi. 52.

Series A

	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Salthouse Lake sand (10. vi. 52) boiled in distilled water 2–3 hr.	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	2 or 3	None	None	None
Unmet.	None	None	None	None

Series B

	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Salthouse Lake sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	1 or 2	None	None	None
Unmet.	Few	3 or 4	3 or 4	Very few

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Oolitic sand, normal sterilization	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude	1	Many	} Multitude
Meting	Several	None	Several	
Unmet.	None	2	Few	None

Series D

	Salthouse Lake sand (10. vi. 52) washed, dried, normal sterilization	Salthouse Lake sand (10. vi. 52) washed, dried, activated charcoal ¾ hr., normal sterilization	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	None	Fair number	None	Very few
Unmet.	1 or 2	Fair number	Few	Many

TABLE VI. EXPERIMENT 55

(Begun 30. vi. 52 with larvae from a fertilization of 25. vi. 52. Results on 2. vii. 52.)

Series A

Acid-cleaned Salthouse Lake sand (10. vi. 52)

	Stored in distilled water	In bolting-silk envelope in sea water for 6 days		
		Amid acid-cleaned Bullhill Bank sand (9. v. 52)	Amid Bullhill Bank sand (23. vi. 52)	Amid Salthouse Lake sand (23. vi. 52)
		Settlements in Dish		
Metd	1 or 2	1 or 2	25–30	None
Meting	1 or 2	1 or 2	Few	2
Unmet.	Few	Several	Several	Few
		Settlements in Conical Vessels		
Metd	1 or 2	3 or 4	Very many	5 or 6
Meting	Few	Few	Several	Few
Unmet	Good number	Good number	Several	Several

Series B

	Bullhill Bank sand (23. vi. 52) rinsed in sea water	Bullhill Bank sand (23. vi. 52) kept in distilled water for 3 days	Mixture of the two sands
		Settlements in Dish	
Metd	Multitude	Good number	Many
Meting	Few	Few	Few
Unmet.	None	None	None
		Settlements in Conical Vessels	
Metd	—	Multitude	Multitude
Meting	—	Few	Few
Unmet.	—	None	None

TABLE VII. EXPERIMENT 56

(Begun 1. vii. 52 with larvae from a fertilization of 25. vi. 52. Results on 3. vii. 52.)

Series A

Acid-cleaned Bullhill Bank sand (10. vi. 52)

	(1) Stored in distilled water	(2) In sea water in light for 10 days	(3) Sand no. 2 to which a few grains of Bullhill Bank sand (23. vi. 52) were added on 1. vii. 52	(4) On 21. vi. 52 a few grains of Bullhill Bank sand (10. vi. 52) added. In sea water in light for 10 days	(5) Sand no. 2 to which a few grains of Salthouse Lake sand (23. vi. 52) were added on 1. vii. 52	(6) On 21. vi. 52 a few grains of Salthouse Lake sand (10. vi. 52) added. In sea water in light for 10 days
			Settlements in Dish			
Metd	Few	Few	Several	Good number	Good number	Many
Meting	Several	Several	Few	Several	Several	Several
Unmet.	Several	Several	Few	Few	Few	Few
			Settlements in Conical Vessels			
Metd	Several	Many	Many	Very many	Many	Multitude
Meting	Very many	Many	Many	Many	Many	Many
Unmet.	Many	Several	Several	Very few	Few	Very few

Series B

Acid-cleaned Bullhill Bank sand (10. vi. 52), treated exactly as for Series *A* but nos. 2 (including 3 and 5), 4 and 6 kept in the dark for 10 days

			Settlements in Dish			
Metd	Few	Few	Several	Many	Several	Many
Meting	Several	Few	Several	Several	Several	Several
Unmet.	Several	Few	Several	Few	Few	Few
			Settlements in Conical Vessels			
Metd	*See A* 1	Many	Many	Very many	Many	Very many
Meting	*above*	Many	Many	Good number	Good number	Good number
Unmet.		Good number	Several	Few	Few	Very few

Series C

	Bullhill Bank sand (23. vi. 52) normal sterilization	Oolitic sand, normal sterilization	Mixture of the two sands
	Settlements in Dish		
Metd	None	2	None
Meting	Few	Few	Very few
Unmet.	Few	Few	Few
	Settlements in Conical Vessels		
Metd	Very many	Many	Many
Meting	Many	Many	Many
Unmet.	Very few	Good number	None

Series D

	Bullhill Bank sand (23. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, dried, normal sterilization	Mixture of the two sands
	Settlements in Dish		
Metd	Multitude	None	None
Meting	Good number	None	2 or 3
Unmet.	None	3 or 4	3 or 4
	Settlements in Conical Vessels		
Metd	—	Several	Many
Meting	—	Several	Many
Unmet.	—	Several	Many

TABLE VIII. EXPERIMENT 57

(Begun 14. vii. 52 with larvae from a fertilization of 9. vii. 52.)
Results on 16. vii. 52.

Series A

Oolitic sand from the Great Salt Lake

	(1) In bolting-silk envelope amid Oolitic sand in sea water for 16 days	(2) Sand no. 1 to which a few grains of Bullhill Bank sand from no. 3 were added on 14. vii. 52	(3) In bolting-silk envelope amid Bullhill Bank sand (23. vi. 52 and 8. vii. 52) in sea water for 16 days	(4) Sand no. 1 to which a few grains of Salthouse Lake sand from no. 5 were added on 14. vii. 52	(5) In bolting-silk envelope amid Salthouse Lake sand (23. vi. 52 and 8. vii. 52 in sea water for 16 days
			Settlements in Dish		
Metd	3	2	11	1	None
Meting	3	5	8	1	None
Unmet.	Several	Several	Several	Several	Several
			Settlements in Conical Vessels		
Metd	Good number	Good number	Good number	Good number	Few
Meting	Good number	Good number	Good number	Good number	Few
Unmet.	Several	Several	Several	Several	Good number

Series B

Acid-cleaned Bullhill Bank sand (10. vi. 52)

		Kept for 16 days, in the light in		
	Stored in distilled water	Filtered sea water	Sea water in which Bullhill Bank sand (23. vi. 52) had been shaken	Sea water in which Salthouse Lake sand (23. vi. 52) had been shaken
		Settlements in Dish		
Metd	None	Few	Many	Several
Meting	1 or 2	Few	Good number	Several
Unmet.	Several	Few	Many	Several
		Settlements in Conical Vessels		
Metd	Several	Good number	Very many	Many
Meting	Good number	Good number	Very many	Many
Unmet.	Many	Many	Many	Severa

Series C

Acid-cleaned Bullhill Bank sand (10. vi. 52)

	(1) Stored in distilled water	(2) Sand no. 1, to which on 14. vii. 52 1 %, or less, of Bullhill Bank sand (8. vii. 52) was added	(3) Sand no. 1 to which on 14. vii. 52 1 %, or less, of Salthouse Lake sand (8. vii. 52) was added
		Settlements in Dish	
Metd	None	None	None
Meting	None	None	None
Unmet.	Good number	Good number	Good number
		Settlements in Conical Vessels	
Metd	—	1 or 2	1 or 2
Meting	—	Few	Few
Unmet.	—	Good number	Several

TABLE IX. EXPERIMENT 58

(Begun 15. vii. 52 with larvae from a fertilization of 10. vii. 52.)
Results on 17. vii. 52.

Series A

Fused alumina

	Stored in distilled water	In bolting-silk envelope in sea water for 7 days: In clean dish	Amid Bullhill Bank sand (8. vii. 52)	Amid Salthouse Lake sand (8. vii. 52)
		Settlements in Dish		
Metd	2	1	26	1
Meting	2	4	32	None
Unmet..	Fair number	Fair number	Many	Fair number
		Settlements in Conical Vessels		
Metd	1 or 2	Several	Several	1 or 2
Meting	Several	Fair number	Many	Several
Unmet.	Many	Many	Very many	Several

Series B

	Acid-cleaned Bullhill Bank sand (10. vi. 52): Stored in distilled water	Mixed with activated charcoal powder	Small grains of activated charcoal	Granular activated charcoal
		Settlements in Dish		
Metd	None	Many	Several	Very many
Meting	None	Many	Several	Very many
Unmet.	None	Several	Very many	Good number
		Settlements in Conical Vessels		
Metd	Few	Multitude	—	Very many
Meting	Several	Multitude	—	Very many
Unmet.	Very many	Few	—	Very many

TABLE X. EXPERIMENT 59

(Begun 16. vii. 1952 with larvae from fertilizations of 9. vii. 1952 and 10. vii. 1952.)
Results on 18. vii. 1952.

Acid-cleaned Bullhill Bank sand (10. vi. 1952).

	Stored in distilled water	Incubated in medium for 5 days	Inoculated with Bullhill Bank sand (8. vii. 1952) and incubated in medium for 5 days
		Settlements in Dish	
Metd	1 or 2	Fair number	Many
Meting	3 or 4	Fair number	Many
Unmet.	Few	Good number	Fair number
		Settlements in Conical Vessels	
Metd	Few	Few	Few
Meting	Fair number	Good number	Many
Unmet.	Many	Many	Many

TABLE XI. EXPERIMENT 60

(Begun 19. vii. 1952 with larvae from a fertilization of 14. vii. 1952.)
Results on 21. vii. 1952.

Bullhill Bank sand (8. vii. 1952) graded by sieving through bolting silks in filtered sea water

	Sand shaken on 100-mesh silk in sea water	Sand grains > 26-mesh sizes	Sand grains 26–40-mesh sizes	Sand grains 40–60-mesh sizes	Sand grains 60–86-mesh sizes	Sand grains passing 86-mesh	Sand grains passing 100-mesh
			Settlements in Dish				
Metd	7	None	6	12	7	3	1
Meting	3	None	1	4	1	None	None
Unmet.	Few	Few	3	Few	Few	1 or 2	1 or 2

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THE BIOLOGY OF *ASTERIAS RUBENS* L.

V. A PORPHYRIN PIGMENT IN THE INTEGUMENT*

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(Text-figs. 1 and 2)

A porphyrin was extracted by MacMunn (1886), working at Plymouth, from the integument of *Asterias rubens* then known as *Uraster rubens*. He extracted whole starfishes with ethanol and ammonia and also with ethanol and sulphuric acid. After diluting his acid-ethanol solution with water he shook the extract with chloroform; the chloroform solution showed two absorption bands, at 607–593 and 566–548 mμ. On evaporation this extract yielded a brown amorphous residue which he found to be soluble in absolute ethanol giving a red solution with the same absorption spectrum. On adding ammonia four absorption bands appeared, at 632–622, 586–566, 548–529 and 516–490 mμ. He considered that this and similar pigments from the slug *Arion empiricorum*, and the coelenterates *Flabellum variabile* and *Fungia symmetrica* were identical with the haematoporphyrin of Hoppe-Seyler (1871) which was then the only porphyrin known. Experimental work has now shown that in other animals examined and found to contain porphyrin this pigment is never present in the form of haematoporphyrin. A reinvestigation has therefore been made of the occurrence of porphyrin in the starfish, *Asterias rubens*.

Certain details of the distribution in the Plymouth area of different colour forms of *A. rubens* have already been recorded (Vevers, 1949). In general, starfishes on the Rame-Eddystone grounds are dark brown or red-brown, but specimens coloured violet, violet-brown, red or even pale pink are not uncommon. In Plymouth Sound, on the other hand, a pure population of bright red starfishes was found in 1948; this population has now been greatly reduced in number but specimens can still be obtained.

In the present work four different colour types were used. The choice of these types was arbitrary, but they were nearly all colours which could be easily recognized in a sample of this species. The colour types used were dark brown, violet-brown and pale, all from the Rame-Eddystone grounds, and red (bright red) from the Plymouth Sound population. The violet-brown specimens were very close to the var. *violacea* of earlier writers.

* A shortened form of this paper was read by one of us (G.Y.K.) at the 2nd International Congress of Biochemistry in Paris 1952.

Chemical study of the pigment was carried out in two parts: (*a*) isolation and identification of the porphyrin, and its preparation in quantity; (*b*) determination of the porphyrin contents per gram of integument in starfishes of different colour groups.

Isolation and Identification of the Porphyrin

Isolation of Crude Free Porphyrin

The aboral integument of *Asterias* was stripped off with scissors, separated carefully from the adherent digestive caeca and both integument and caeca were shaken separately with ether:glacial acetic acid (mixture 5:1) for 2 hr. (Zeile & Rau, 1937). The resulting extracts were washed with distilled water until most of the acetic acid was removed, and extracted with 5% HCl (137 ml. conc. HCl/l.). The acid layer from the integument extract was purple and red-fluorescent in ultra-violet light, and greenish brown and non-fluorescent from the caeca extract.

The red-fluorescent acid extract from the integument was treated with fresh ether and saturated sodium acetate solution added to drive the porphyrin back into the ether. The ether extract was then washed free of salts with distilled water, dried roughly by filtering through ether-soaked paper, and evaporated to dryness.

A violet-red residue remained, which was used for subsequent experiments.

Hartridge Reversion Spectroscope

A little of the pigment dissolved in 'Analar' pyridine gave a spectrum showing the following bands:

I	II	III	IV
632	576	542	507 mμ

corresponding with that of *protoporphyrin.*

Chloroform solutions of the methyl ester of this pigment and of protoporphyrin dimethyl ester (prepared from haemoglobin) gave the following bands:

	I	II	III	IV
Asterias porphyrin methyl ester	630	575	540	507 mμ
Protoporphyrin dimethyl ester	631	575	541	507 mμ

Unicam Spectrophotometer

An absorption spectrum of a solution of the pure pigment in pyridine ('Analar') was recorded with a Unicam S.P. 500 Quartz Spectrophotometer. This spectrum is plotted in Fig. 1, and shows absorption maxima at:

624 568 535 500–506 406 mμ

Acid Test of Chu (1946)

A little of the porphyrin was dissolved in 'Analar' chloroform and 3 drops conc. HCl added. The solution turned *green* suggesting *protoporphyrin.* (Mesoporphyrin gives a purple colour.)

Paper Partition Chromatography

A small amount of the free porphyrin was dissolved in lutidine (a mixture of 2:4- and 2:5-dimethyl pyridines) and partition chromatograms were run on strips of Whatman no. 1 paper $3{\cdot}1 \times 75$ cm. at a temperature of 24° C. in an atmosphere of lutidine, water and ammonia vapour (Nicholas & Rimington, 1949) in a new apparatus for paper chromatography (Kennedy, 1953*b*). Pure protoporphyrin and coproporphyrin were used as markers, both in separate and in mixed spots. Papers were examined by ultra-violet light from an 'Osira' 125 W. black glass lamp after 15–16 hr. chromatography.

With protoporphyrin the *Asterias* porphyrin gave one spot only, and with coproporphyrin, two spots (Fig. 2).

A free *Asterias* porphyrin spotted on paper alone from lutidine solution gave one spot only, with an R_F value of 0·8, indicating the presence of a dicarboxylic porphyrin only, there being no other spots on the paper.

Column Chromatography

A sample of the porphyrin was esterified with methanol saturated with HCl gas at 0° C., and the dimethyl ester was dissolved in 'Analar' chloroform and applied to a column of grade III MgO (Nicholas, 1951) packed in chloroform. The chromatogram was developed by successive mixtures of chloroform: methanol in the proportions 100:0·5, 100:2 and 100:3 (Nicholas, 1951). Elution occurred only with the 100:3 chloroform: methanol mixture, indicating protoporphyrin and not mesoporphyrin or deuteroporphyrin.

Melting-point Determination

After chromatographing three times on grade III magnesium oxide the porphyrin dimethyl ester crystallized from chloroform and methanol in red prisms, melting at 225° C. A mixture of the porphyrin dimethyl ester with pure protoporphyrin dimethyl ester melted at 226° C.

Although Jope & O'Brien (1945) have criticized melting-point determinations as methods of identification of porphyrins, these results would indicate the identity of the *Asterias* porphyrin with protoporphyrin.

Formation of Lead Co-ordination Complexes

Lead co-ordination complexes were prepared from *Asterias* porphyrin, from pure protoporphyrin and from haematoporphyrin. All gave two-banded spectra:

	I	II
Pb—*Asterias* porphyrin	630	583
Pb—Protoporphyrin	630	584
Pb—Haematoporphyrin	620	578

This result indicates the identity of *Asterias* porphyrin with protoporphyrin and distinguishes it from haematoporphyrin.

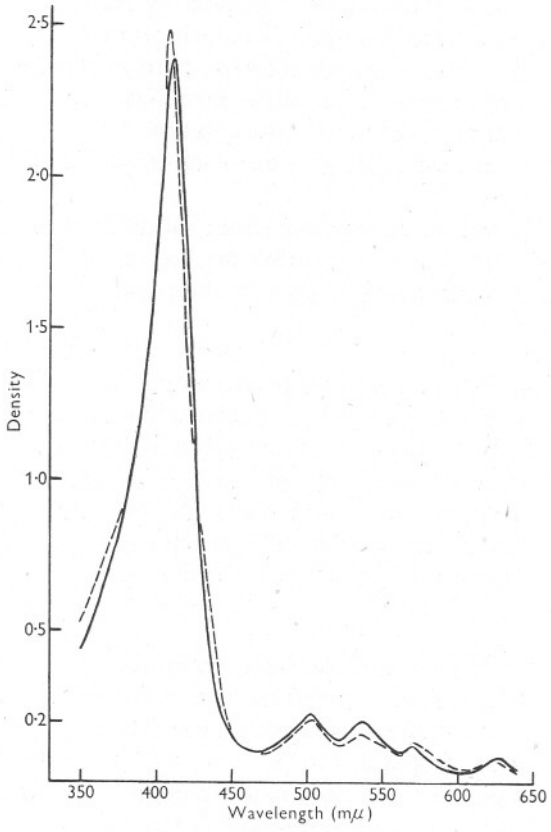


Fig. 1.

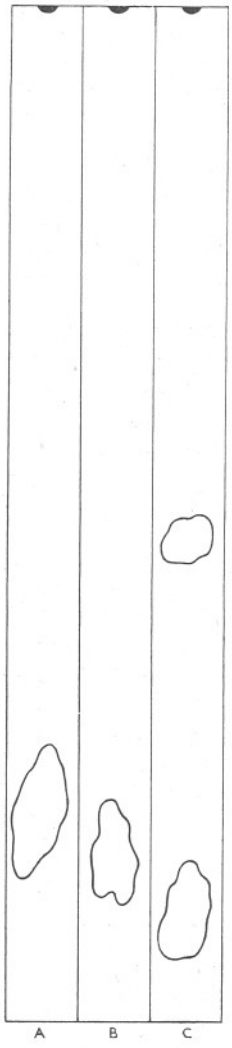


Fig. 2.

Fig. 1. Absorption spectra in pyridine of protoporphyrin (continuous line) and *Asterias* porphyrin (broken line).

Fig. 2. One-dimensional paper chromatograms run for 15–16 hr. at 24° C. in lutidine. The starting-points are marked as black semicircles: A, *Asterias* porphyrin alone; B, *Asterias* porphyrin and free protoporphyrin, mixed spot; C, *Asterias* porphyrin and coproporphyrin, mixed spot.

Conversion to Mesoporphyrin

The *Asterias* porphyrin was reduced according to the method of Rimington (1938). The solid porphyrin was dissolved in 20 ml. of a mixture of glacial acetic acid (7·5 parts) and hydriodic acid (sp.gr. 1·94) (1 part) and boiled for 3 min. After cooling and adding peroxide-free ether, the excess iodine was removed with sodium acetate and sodium sulphite. The ether epiphase gave a spectrum of mesoporphyrin

I	II	III	IV
623	567	528	495 mμ

The ether layer was evaporated and the porphyrin esterified with methanol/HCl at room temperature and extracted with chloroform. This solution was diluted with ether and the porphyrin was taken into 5% (w/v) HCl. The porphyrin was then driven back into the ether with potassium acetate and the ether layer washed, dried by filtering through an ether-soaked paper, and evaporated. The residue was crystallized from chloroform-methanol, and melted at 212–214° C. A mixed melting-point with a specimen of mesoporphyrin IX di-methyl ester (m.p. 215–216° C.) gave 215° C.

This reaction enabled the original *Asterias* porphyrin to be characterized as being of similar constitution to blood pigments derived from aetioporphyrin III, and it could only be protoporphyrin IX, the vinyl groups having been hydrogenated to ethyl groups in mesoporphyrin.

These experiments confirm that *Asterias rubens* integument contains protoporphyrin IX and that the porphyrin is present in the free state.

Chlorophylls in Caeca

Digestive caeca were dissected out of living *Asterias* and ground with acid-washed glass powder in a mortar, and then extracted with 90% methanol. A deep green solution with a marked rosy-red fluorescence was obtained. This solution gave the spectrum of a chlorophyll mixture and was further identified by the formation of various degradation products of chlorophyll. There was no evidence of the presence of a porphyrin in the digestive caeca.

Preparation of *Asterias* Porphyrin

The aboral integument was dissected from a number of large red-brown living *Asterias*. This was scraped free of adhering caeca, washed in sea water, blotted on filter-paper and weighed. The weight of integument as starting material was 548·5 g.

The pooled integument was put through a tissue mincer, and mechanically shaken with 2500 ml. of ether-acetic acid mixture for 5 hr.

The ether-acetic acid mixture was made up as follows: peroxide-free ether ('Analar'), 5 parts; glacial acetic acid ('Analar'), 1 part; then petroleum ether 40–60° C. m.p. ('Analar'), 1 part, added to 9 parts of above mixture.

The petroleum ether helps to avoid emulsification which can be troublesome.

All the following operations were done in very subdued light to minimize the decomposition of the porphyrin.

After shaking, the mixture was allowed to stand overnight in the ice-chest. The following day the mixture was filtered, and the residue re-extracted with a further 1500 ml. of the ether-acetic acid solvent. This was repeated with 1000 ml. lots until the extract was no longer red-fluorescent to ultra-violet light. Even then, the residue was found to be strongly fluorescent due to adsorption of porphyrin on the calcareous spicules of the integument. The residue was therefore shaken with 25% by weight HCl and re-extracted (after neutralization with saturated sodium acetate until Congo-red paper gave a *grey* colour) with 500 ml. lots of ether until the ether-layer was no longer red-fluorescent to ultra-violet light. All the extracts were pooled.

The extracts were first washed with distilled water containing a little sodium acetate, and then with distilled water alone until most of the acetic acid had been removed. The porphyrin was then extracted from the ether solution with 100 ml. portions of 5% by weight HCl until the extracts were no longer fluorescent.

The acid extracts were then pooled in a separating funnel and the porphyrin driven back into 'Analar' ether with saturated sodium acetate. The ether layers were pooled, and washed, first with 2% NaCl, followed by distilled water until washings were no longer acid, and until they no longer gave a precipitate with silver nitrate. During washing a little 'Analar' ether was added from time to time to replace that removed by washing. (Ether dissolves in water to the extent of 7·5% at 20° C.)

All drops of water were shaken down and tapped off, the ether extract filtered through thick paper soaked with ether, into a distilling flask, and reduced to a small volume by distillation *in vacuo*.

The residue was poured into a 500 ml. beaker and evaporated to dryness on the water bath. The porphyrin remaining was rinsed with a little petroleum ether (b.p. 40–60° C.) when cool, and dissolved in the minimum amount of 'Analar' pyridine. This solution was chilled, and dry petroleum ether b.p. 60–80° C. added in the proportion of about 10 volumes with stirring. The porphyrin was precipitated and flocculated by placing the beaker on the hot-water bath until large flocks developed. The beaker was then well covered and, placed in the ice-chest overnight.

The supernatant liquid (if free from flocks) was pipetted off, and the remainder centrifuged. The residue was then washed with petroleum ether (b.p. 40–60° C.) and dried *in vacuo* at room temperature.

The total yield of crude porphyrin by this method was 38·4 mg.

Purification

The dry porphyrin was dissolved in absolute methanol saturated at 0° C. with dry HCl gas, and the mixture allowed to stand in the ice-chest for 48 hr. This esterified the porphyrin.

The solution in methanol/HCl was diluted with a little distilled ice water, chloroform was added with shaking, and the lower layer was tapped off. The whole process was repeated until the lower layer was no longer red-fluorescent. The pooled chloroform layers were washed carefully with 2% NaCl, followed by distilled water, the washings being monitored with ultra-violet light. Washing was continued until the washings were neutral to litmus.

The chloroform extract was filtered through a thick chloroform-soaked paper into a distilling flask, and the extract concentrated to small bulk, then transferred to a porcelain basin and taken to dryness on the water bath.

The dry residue was rinsed, when cool, with petroleum ether (b.p. 40–60° C.) and dissolved in a mixture of chloroform-petroleum ether 1:1, and passed down a column of Brockmann's alumina, packed in chloroform-petroleum ether 1:1 and monitored with ultra-violet light. (A new apparatus for fractional elution chromatography was used, Kennedy, 1953*a*.)

The main portion of the pigment passed through the column, preceded by a blue fluorescent band which was found to be due to fat. At the top of the column, a dark brown pigment remained which was eluted later with chloroform/acetic acid, and gave a greenish fluorescence and two-banded very indistinct spectrum (Eriksen, 1951).

The main bulk of the porphyrin passing through the column was collected and evaporated to dryness in the water bath. The pigment was then dissolved in pure dry chloroform and applied to a column of magnesium oxide grade III packed in chloroform. Development was done by successive mixtures of 0·5:100, 2:100 and 3:100 (v/v) methanol-chloroform (Nicholas, 1951). Only the 3:100 mixture caused elution, indicating the presence of protoporphyrin only.

It may be helpful to include here some details of the crystallization method from chloroform-methanol, a method which often leads to disappointment.

The eluted porphyrin was evaporated to dryness in a porcelain basin on the water bath, and redissolved, on the water bath, in a small volume of dry 'Analar' chloroform. This was allowed to evaporate *slowly* until the effect of 'tears of wine' was apparent on the sides of the basin: i.e. the solvent running back in streaks from a band about 5 mm. above the surface. At this point, 3 volumes—estimated roughly by eye—of *boiling* dry absolute methanol were added with stirring, and crystallization began at once. The basin was covered and set aside in the ice-chest for further crystallization overnight.

The crystals were removed by centrifugation, keeping the temperature as low as possible, and drained by standing the tube on end on a pad of filter-paper. They were then washed with dry petroleum ether (b.p. 40–60° C.).

The mother liquor was evaporated to dryness and the crystallization process repeated.

After two recrystallizations of the mother liquors, the crystals were dried *in vacuo* over P_2O_5 at room temperature. A yield of 33·6 mg. of fine dark red prisms was obtained, melting at 225° C.

It is essential that all solvents should be pure and dry. Methanol was dried by the Mg and I_2 method of Vogel (1948). Chloroform was dried over anhydrous calcium chloride for 72 hr. and then redistilled with a calcium chloride tube attached to the receiver. Petroleum ether, though 'Analar', was dried over calcium chloride and sodium chippings and redistilled.

Determination of Protoporphyrin in *Asterias* Integument

There are difficulties in the determination of protoporphyrin in the integument of *Asterias*, of which the following may be mentioned. (i) The presence of calcium spicules in the integument and consequently the presence of calcium salts during the extraction process is a nuisance when adjusting the pH with sodium acetate. (ii) Protoporphyrin in small concentrations is unstable to light, and the estimation process must be done rapidly in dull light. (iii) Since protoporphyrin has a high acid number, especially in the form of the dimethyl ester, the use of 8% HCl is required in the preparation of the final extracts, and this gives a fluorescence which is less than maximal according to Rimington (1943).

However, it was considered that even an approximate determination of the amount of porphyrin in the various colours of *Asterias* would make a desirable and interesting comparison, and a modified technique was devised which took all the difficulties into consideration and gave reproducible results, using the Rimington fluorimeter (Rimington, 1943).

The fluorimeter was calibrated using dilutions of a freshly prepared standard solution of protoporphyrin made up as described below. The lability of protoporphyrin necessitated fresh standard solutions for each determination.

Standard Solution of Protoporphyrin

1 mg. pure protoporphyrin IX was dissolved in 5 ml. 'Analar' glacial acetic acid and the volume was made up to 100 ml. with 8% HCl (219·2 ml. conc. HCl/l.). This solution contained 10 μg./ml. and was kept in a refrigerator in a black bottle. Dilutions for calibration of the Rimington fluorimeter (for these determinations) were made freshly as required from the solution, with 8% HCl in dim light, but the standard stock solution was not kept for longer than 2 days. Hence, although fluorescence in 8% HCl is not maximal, both standard and unknown solutions in estimations are exactly comparable.

Determinations on each colour group were done separately, each compared with a freshly made up standard of 2 μg./ml. protoporphyrin in 8% HCl.

Method of Determination

All steps were carried out in very subdued light.

(i) 2 g. aboral integument of each colour were weighed out separately, after drying roughly in a standard manner on Whatman No. 1 paper, into wide-mouthed stoppered bottles.

(ii) 100 ml. methanol saturated with HCl gas were added and the whole was stored at 0° C. for 36 hr. in the dark.

(iii) The extracts were filtered into separating funnels and the bottles, residues and papers washed with small volumes of 30 ml. methanol/HCl; the washings being added to the main extracts.

(iv) The extracts were diluted with 1 volume of distilled water and the carotenoids removed by shaking with petroleum ether (b.p. 40°–60° C.) until the *upper* layer was non-fluorescent to ultra-violet light. The upper layers were then discarded.

(v) Peroxide-free ether was then added to the lower layers, followed by saturated sodium acetate (red to congo red), and well shaken. This was repeated until the upper (ether) layers were non-fluorescent to ultra-violet light. The lower layers were discarded.

(vi) The ether extracts were pooled and washed with ice-cold distilled water repeatedly until the washings gave no cloudiness with silver nitrate. All drops were shaken down and tapped off.

(vii) The ether solutions were extracted repeatedly with 2 ml. lots of 8% (w/v) HCl until no further fluorescence was seen in the lower (acid) layer. The lower layers were collected in stoppered measuring cylinders, and the total volume noted. All drops in the funnels were shaken down, and the measuring cylinders were well shaken.

(viii) 5 ml. samples of the acid extract in Eggertz tubes were compared with a 2 μgm./ml. protoporphyrin standard in the Rimington fluorimeter. If the solutions were too strong they were diluted with 8% HCl and the dilution factor noted.

The means of five to ten readings were taken, and the porphyrin values read off from the calibration curve. The results are expressed as μgm. protoporphyrin/g. of integument.

Results

The starfishes were graded by colour as follows: violet-brown, VB; dark brown, DB; red, R; pale, P.

The results of the porphyrin estimations are set out in Table I. Each figure is the mean of three determinations on an extract derived from two or three selected starfishes of the appropriate colour.

TABLE I. PORPHYRIN IN *ASTERIAS*

Given in μgm./g.

	Grade of colour			
Determinations at	VB	DB	R	P
Plymouth	72·0	33·6	24·5	5·5
Sheffield	64·4	49·9	13·0	4·5
Sheffield	70·3	45·2	11·4	—
Sheffield	69·7	47·2	15·2	—

Tests on the Soft Parts of *Chlamys* and *Mytilus*

With a view to tracing the origin of the porphyrin in *Asterias rubens*, the soft parts of *Chlamys* and *Mytilus* were ground with washed sand and extracted with ether-acetic acid mixture. The extract was very faintly blue-fluorescent, but had no red-fluorescence at all. On standing exposed to light in the presence of hydrogen peroxide or quinhydrone no red-fluorescence developed, confirming the absence of a porphyrinogen.

Discussion

The results confirm the earlier evidence of MacMunn that a porphyrin is, in fact, present in the starfish, *Asterias rubens*, but extend his observations to the identification of the pigment as protoporphyrin, and not haematoporphyrin.

The determinations of protoporphyrin given in Table I show that in *A. rubens* integument the amounts per gram of this pigment are graded approximately according to the colour of the starfish. In general the darker starfishes have more protoporphyrin than the lighter ones. The main visible colour of the dark-brown and violet-brown starfishes is not, however, entirely due to the presence of this porphyrin, for these colours are known to be mainly carotenoid (including carotiproteins) (Vevers, 1952). The dark brown or black reticulate pattern has been shown to be a melanin pigment (Vevers, in preparation). At the same time there appears to be some parallelism between the porphyrin content and the colour of the carotenoid pigmentation. The measurements are relative and not absolute; and although there is much variation in pigmentation in *A. rubens*, the range of figures in Table I gives an excellent indication of the order of the porphyrin content in these varying shades of starfish.

The chromatographic results indicate that the protoporphyrin is present in the starfish body in the free state and not as an ester, since it takes up the clear position of protoporphyrin on the paper and does not travel all the way with the mobile phase as do the esters.

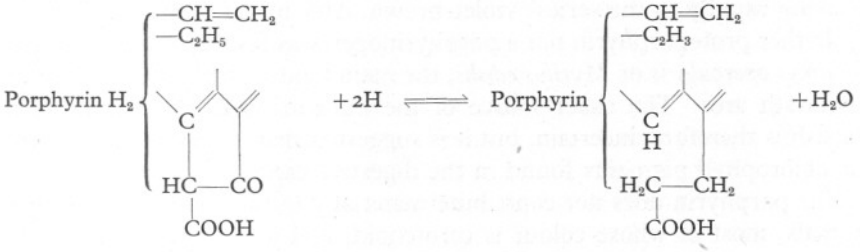
The problem of the origin of the free protoporphyrin in *Asterias* is intriguing. It is entirely absent from the soft parts of the lamellibranchs *Chlamys opercularis* and *Mytilus edulis*, which form the principal food of *Asterias rubens* in the Plymouth area. Uroporphyrin and coproporphyrin are widespread in mollusc shells, but do not occur in the soft parts that alone are eaten by the starfish.

The experiments with the soft parts of *Chlamys* and *Mytilus* have also shown that there is no extractable substance present comparable with a porphyrinogen, which could be converted simply to a porphyrin by oxidation in the starfish. The formation of protoporphyrin in *Asterias* must therefore be more metabolic.

There appear to be three possible sources of the free protoporphyrin: (i) haem enzymes and cytochromes, (ii) chlorophyll of the digestive caeca, (iii) complete synthesis *de novo* from simple substances in the diet.

Haem enzymes and cytochromes are present in the starfish in very small (physiological) amounts, and the amount of protoporphyrin in the integument is vastly greater. No transformation of the porphyrin *c* of cytochrome *c* (haematoporphyrin) to protoporphyrin has yet been demonstrated conclusively.

Chlorophyll is present in the digestive caeca in very large amounts, and could be converted into protoporphyrin by a dismutation, two hydrogen atoms being lost from the nucleus and two from the ethyl side chain. The addition of six hydrogen atoms would bring about opening of the isocyclic ring in chlorophyll and the formation of the second propionic acid side chain of protoporphyrin.



(*After Lemberg & Legge*, 1949.)

The third idea involves complete synthesis of the porphyrin nucleus from glycine, acetate, or α-ketoglutarate as shown by Muir & Neuberger (1950) and Shemin & Rittenberg (1946).

On the whole, the formation from chlorophyll is the most likely source of protoporphyrin in this organism, and will be the subject of future study.

The porphyrin in the integument clearly does not contribute to the colour pattern of the animal, as has been mentioned already. The pigment may therefore be either an excretory by-product formed from chlorophyll, or be concerned in integumentary photoreception, since porphyrins are known to render tissues more sensitive to light.

In the starfish the pigment apparently is only accumulated in the hard leathery integument, as repeated tests have shown it to be entirely absent from the gut, gonads and digestive caeca. Direct ultra-violet examination has also shown that there is no porphyrin pigment in the calcareous spicules embedded in the starfish integument. This is interesting since porphyrin is so frequently associated with calcium compounds, e.g. protoporphyrin in the hen's egg-shell, the porphyrins of mollusc shells (Nicholas & Comfort, 1949), and uroporphyrin in the bones of the squirrel *Sciurus niger*.

SUMMARY

A porphyrin pigment found in *Asterias rubens* has been re-examined and found to be protoporphyrin, and not haematoporphyrin as originally reported by MacMunn. The pigment was identified by spectroscopic and spectrophotometric observations as well as by chemical tests and the use of paper partition and column chromatography. The protoporphyrin is absent from the gut, gonads and digestive caeca, and is present only in the leathery integument.

The porphyrin occurs in the integument as free protoporphyrin; a yield of 33·6 mg. of red crystalline protoporphyrin dimethyl ester was obtained from a sample of 548·5 g. of *Asterias* integument.

The relative amounts of protoporphyrin in different coloured specimens of *A. rubens* were determined by a fluorimetric method. With four well-defined colour types the amount of porphyrin per gram of integument was found to decrease rapidly in the series: violet-brown, dark brown, red and pale.

Neither protoporphyrin nor a porphyrinogen was found in the soft parts of *Chlamys opercularis* or *Mytilus edulis*, the main food animals of *Asterias* in the Plymouth area. The exact source of the integumentary porphyrin in this starfish is therefore uncertain, but it is suggested that it may be derived from the chlorophyll pigments found in the digestive caeca.

The porphyrin does not contribute materially to the colour pattern of the animals, most of whose colour is carotenoid, and it is most likely that it is a by-product of excretion formed from chlorophyll.

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THE REGENERATION OF THE SIPHONS OF *CIONA INTESTINALIS* L.

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(Text-figs. 1–16)

The urochordates exhibit remarkable powers of asexual reproduction and regeneration, and have provided a fertile field for experiment along these lines. Much of this work has been performed upon ascidians such as *Diazona violacea* Savigny, *Archiascidia neapolitana* Julin, *Archidistoma aggregatum* Garstang and members of the Clavelinidae, etc., since they are relatively easy to obtain and their methods of budding are such as to lend themselves to experimental techniques. Species such as *Ciona intestinalis* (L.) and *Ascidiella aspersa* (Müller), etc., that do not reproduce asexually have, however, been little studied. A few experiments upon *Ciona intestinalis* have indicated that regenerative powers indeed exist, for they have shown that regrowth of the tunic, mantle, siphons, neuroglandular complex and much of the pharynx is possible. With the exception, however, of the regeneration of the tunic and neuroglandular complex, no detailed histological study of the processes involved has been undertaken. The present work attempts to remedy that deficiency with respect to the siphons and anterior end of the pharynx.

Historical

Hirschler (1914), using young *C. intestinalis*, discovered that the posterior region of the body could regenerate all the missing anterior organs (provided that a small portion of the pharynx were present). The anterior region could not, however, regenerate posterior organs. He noted that brown pigmented cells (the cells with orange pigment of Pérès, 1943) tended to accumulate at cut surfaces and attributed to them a phagocytic function. The numbers of regenerating 'ocelli' often varied considerably from those normal to the siphons.

Kammerer (1923) again demonstrated the ability of the siphons to regenerate, and added that the new growths were larger and longer than normal. He even stated that the offspring of the operated animals inherited the ability to grow longer siphons. This was disproved by Fox (1924) who showed that the length of the regenerating siphons was dependent upon nutrition and non-heritable.

Pérès (1948*a*) removed the tunic of *Ciona* and gave a very detailed account of the histology and chemistry of the growth of the new test. The epidermis

and three types of cell, said by him to be mesenchymatous, are involved: (i) cells with granules and acidophil 'bâtonnets' at first carrying carbohydrates, later to be replaced by proteins; (ii) cells with a single, large, refringent granule of a protein nature; and (iii) cells with a small acidophil vacuole containing a carbohydrate of high molecular weight, possibly glycogen. The epidermis secretes the ground substance of the tunic, formed as a result of the polymerization of carbohydrates. This is speedily invaded by the three types of cells just described and the chemical changes necessary for the full development of the tunic are then completed.

The regeneration of the neuroglandular complex of *C. intestinalis* has been said by Pérès (1943) to be the work of 'neoblasts'. The nature of these cells will be discussed later.

Much of the work on regeneration and asexual reproduction in ascidians has been performed on *Clavelina lepadiformis* (Müller), by Huxley (1926), Brien (1930) and Berrill & Cohen (1936). Brien has shown that, while the main sources of newly differentiating tissues and organs in the bud chambers are mesenchymatous and blood cells which he calls lymphoblasts, in the artificial regeneration of the thorax in adults the epicardium is largely responsible. Berrill & Cohen, who isolated pieces of stolon artificially, state that the cells forming the blastogenic mass are derived from a mesenchymatous septum which has disintegrated. They appear not to recognize the participation of the blood cells, noted by Brien.

The work of Brien (1927) on *Aplidium zostericola* Giard. indicates that the majority of the new tissues, cardiopericardiac vesicle, pharynx, peribranchial cavities, epicardium and oesophagus, of the blastozooid are constituted from the fragment of epicardium in the bud. This appears also to be true of *Diazona violacea* Savigny, and *Archidistoma aggregatum* Garstang (see Berrill, 1948 *a*, *b*), and of *Archiascidia neapolitana* Julin (see Brien, 1933).

Selys Longchamps (1916) has described the method of pallial budding in *Heterocarpa glomerata* Hartmeyer, and *Stolonica socialis* Hartmeyer, and Drach (1948) that of the Botryllidae. In the stolidobranchs it is apparently ectoderm and mesenchyme which are responsible for organogenesis and tissue formation.

Berrill (1951), in a review of regeneration and budding in tunicates, states that in ascidians 'while the external tunic of cellulose is readily regenerated by exposed or renewed epidermis... regeneration of other tissues and structures may be accomplished by a variety of internal tissues depending upon the level of the zooid from which regeneration takes place.... Siphons reform from epidermis and atrial epithelium.... At post abdominal levels all structures other than epidermis are reconstituted from epicardial tissue alone when it is present, or from septal mesenchyme.'

Finally, Berrill (1950) is of the opinion that the epicardium is probably mesodermal and not endodermal as has hitherto been thought, but does not pursue the subject.

METHODS

The *Ciona intestinalis* upon which operations for siphon removal were performed were adults varying in length from 1·9 to 7 cm., averaging 3·7 cm.

The specimens were first anaesthetized in menthol in sea water for 1 hr. to avoid siphon retraction and then put into fresh sea water from Plymouth Sound. The water was changed daily and kept at a temperature varying between 15 and 16° C. The siphons were removed in such a way that the cut edges were flush with the body surface (Fig. 1). In several specimens this involved the removal of a small part of the anterior end of the pharynx.

Thirty-nine specimens were used for observations upon the living animal during the period of regeneration. In nine of these, both siphons were removed, in sixteen the inhalant, and in fourteen the exhalant siphons alone. In these experiments the animals were kept alive for a minimum of 6 days to cover the period of growth of siphons normal in all respects other than length. Two animals in which both mouth and atriopore were removed were kept alive for a fortnight for study of the further development of the newly regenerated organs. In five of the above operations the neuroglandular complex was also removed. In three further experiments, in which both siphons were removed the neuroglandular complex was damaged but not excised.

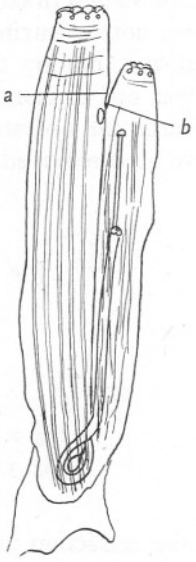

Fig. 1. *Ciona intestinalis*, showing level of cut for removal of inhalant siphon (*a*) and exhalant siphon (*b*).

Ninety-nine animals were operated upon for the removal of both siphons and then preserved for observations upon the histological changes involved in regeneration. The animals were killed at regular intervals, hourly up to 6 hr., two-hourly from 6 to 24 hr., and thereafter at four-hourly periods up to 96 hr., three being killed at each stage. The anterior thirds of the animals were fixed, either in Bouin-Dubosq or Benoit. The sectioned material was cut at 4 or 6μ and stained in Unna's pappenheim, or toluidine blue and eosin after Bouin-Dubosq, or with iron haematoxylin counterstained with light green after Benoit. The Feulgen test was carried out in many cases.

In addition, sixteen animals from which both siphons had been removed were used for experiments in *intra-vitam* staining. The material was immersed either in neutral red or janus green B. The concentration for each was 1 ml. of a 0·1 % solution of the stain in 9 ml. of sea water. Neutral red was used as an indicator of the presence of intracellular neutral red vacuoles, regarded by Baker (1945, 1949) as a component of the Golgi apparatus, janus green B as

an indication of the distribution of mitochondria. Six injured and five uninjured animals were immersed in neutral red, and two injured and two uninjured in janus green B for a period of 24 hr. in each case.

Observations on the Living Material

For half an hour to 1 hr. after operation the effect of the anaesthetic is still evident; the reduced siphons, if the two apertures remaining after operation can be so described, still gape. From 1 to $2\frac{1}{2}$ hr. later, however, the 'siphons' close, not by intrinsic muscular movements, since the sphincters have been removed, but by differential movements of the anterior end of the body. When one siphon alone has been removed, the anterior end bends over towards the operated side, thus effectively closing the aperture; when both have been removed, differential contractions of the mantle musculature result

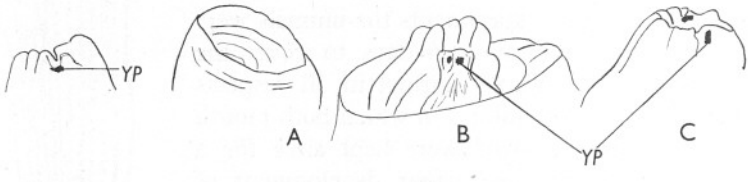

Fig. 2. Fig. 3.

Fig. 2. Mouth, 1 day after operation. *YP*, yellow pigment.

Fig. 3. Two days after operation. A, mouth (specimen IV); B, mouth (specimen I); C, atriophore (specimen II).

in the retraction of the siphon region into the anterior end of pharynx and atrium. However, in 3 hr. time the apertures are again open. Momentary stimulation with a dissecting needle fails to produce any closure of the actual apertures, and only prolonged stimulation provokes a repetition of the bending or retraction movements described above.

During the first day (Fig. 2) certain reactions to the operation are observed. The cut edges bend inwards, and in 60% of the specimens are the site of the irregular deposition of yellow pigment, a phenomenon noted by Hirschler (1914).

In the course of the second day (Fig. 3) very slight growth of the siphons occurs, sufficient to restore a regular, but not characteristically lobed shape. The new growth is extremely delicate and there is no visible evidence of regeneration of a new tunic by the new tissue. In a few animals the yellow pigmentation becomes more intense, but in most it was noticeably paler, and from one had entirely disappeared.

The changes initiated during the second day continue into the third. As a result of continued but differential growth, the siphons acquire the characteristic lobes. Further, during this period, secretion of tunicin by the new

tissues begins. The siphons now open and close normally, an indication of the presence of sphincter muscles. By the end of the fourth day the 'ocelli' have differentiated (Fig. 4).

Thus, in 4 days, siphons complete in all respects other than length are regenerated. There were only two exceptions. In one specimen the pigment spots appeared on the third day, and in another they were not visible until the fifth. The removal of only one instead of both siphons does not expedite the repair process for the stages and speed of regeneration are identical in each.

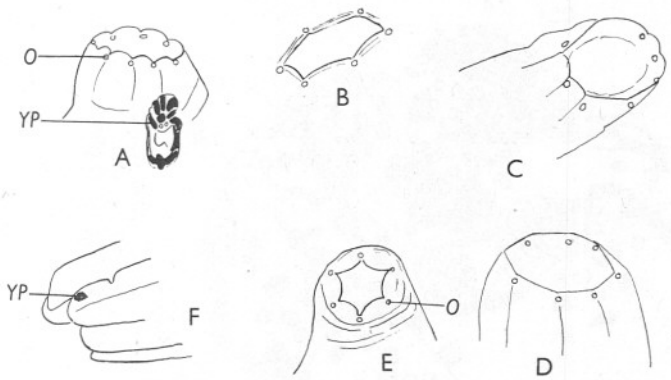

Fig. 4. Four days after operation. A, mouth (specimen I); B, atriophore (specimen II); C, mouth (specimen III); D, mouth (specimen IV); E, exhalant siphon: neuroglandular complex injured but left *in situ*; F, exhalant siphon: neuroglandular complex removed. *O*, ocellus (pigment spot); *YP*, yellow pigment.

However, a profound effect is produced by the removal of the neuro-glandular complex together with the siphons (Fig. 5). Regeneration of the siphons is much slower, not being completed until 13 or 14 days have elapsed. If the complex is damaged by maceration with a needle, but is not excised, the regeneration rate is normal.

The process of regeneration subsequent to the fourth day involves a thickening of the new tunic and continued growth in length of the siphons, the normal size being regained in 2–3 weeks after operation.

Neutral red staining was used to indicate the presence of intracellular 'neutral red vacuoles' regarded by Hirsch (1939) as the Golgi pre-substance, and by Baker (1945, 1949) as part of the Golgi apparatus. This cell organelle is generally regarded as being connected with secretory activity. It was therefore hoped that this technique would give evidence of increased secretory activity by the cells of the injured region. Observations over a period of 2 days in fact indicated that the injured region took up neutral red to a greater extent than the undamaged tissues. Microscopic examination after 24 hr. showed, however, that cells other than those with yellow pigment granules, muscle and test cells, betrayed a much greater affinity for the stain that had been expected.

Both cytoplasm and nucleus of haemoblasts, of cells with many refringent granules or with a single granule, of lymphocytes, and of amoebocytes took up the stain. The granules where present coloured more intensely than the cytoplasm. In phagocytes and in senescent cells in the tunic, the contents of the vacuoles took up the stain intensely; the cytoplasm and nucleus were coloured to a much smaller degree. As the senescent cells moved towards the edge of the tunic the intensity of the neutral red within the growing vacuoles steadily increased.

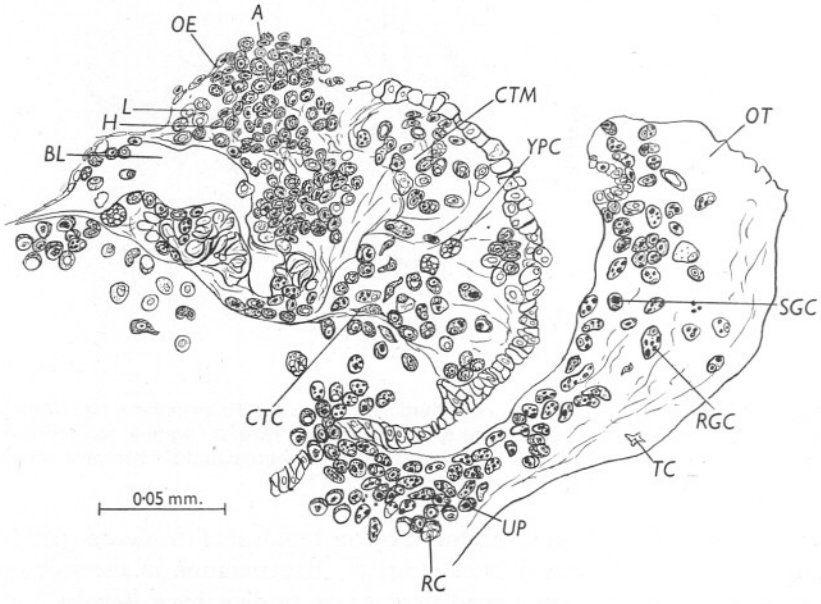

Fig. 5. Six hours after operation—atriopore. *A*, amoebocyte; *BL*, blood lacuna; *CTC*, connective tissue cell; *CTM*, connective tissue matrix; *H*, haemoblast; *L*, lymphocyte; *OE*, original epidermis; *OT*, original tunic; *RC*, reticulate cell; *RGC*, refringent granule cell; *SGC*, cell with single granule; *TC*, tunic cell; *UP*, univacuolar phagocyte; *YPC*, yellow pigment cell.

Janus green B was used in an attempt to stain mitochondria. The reactions observed were interesting, since, with minor exceptions, those regions within the cells which showed an affinity for neutral red also took up janus green B. So widespread was the staining within the cells that it was impossible, in general, to distinguish the mitochondria, which according to Pérès (1943) are never large.

Observations on Preserved Material

Examination of sectioned material shows that during the first hour after siphon removal no visible changes in the affected tissues occur.

During the first 6 hr. after operation two reactions to the injury by the affected tissues can be distinguished. The first may be described as comprising

direct traumatic responses to the operation; the second as comprising processes that represent the earliest stages in the regeneration of the new siphons.

The earliest direct traumatic response is the approximation and apposition of the cut epidermal edges of the mantle which bend inwards and meet (Fig. 6). At first they may overlap, but later they appear to fuse. The epidermal cells in this region alter in shape from characteristic small flattened cells to a cubical, and in places almost columnar, epithelium. In many specimens, where the two epidermal layers do not meet, the gap is plugged by a clump of orange pigment cells, the largest of the blood cells. In other regions the gap is plugged by muscle cells. The differing degrees of intensity of the yellow pigmentation round the cut siphon edges is thus explained. The effect of these processes is to protect the subepidermal tissue from exposure to the water.

The orange pigment cells appear not to have a very long life, since even at this stage a few can be seen degenerating, a process which can occur in two ways. The first is characterized by a change in colour of the pigmentation from yellow or orange to green, and finally by a complete disintegration of the cell and release of the granules into the neighbouring subepidermal regions. Very probably the freed granules are then removed by two types of blood cell, univacuolar phagocytes and amoebocytes. The second method is that described by Pérès (1943). The orange pigment cells again change in colour to green and become reticulate cells (Fig. 5) with an achromatic nucleus. In the hyaline compartments between the refringent bars, one or two small granules can be seen, but in time even these disappear.

Another direct result of the operation is the damage or destruction of cells at the site of the injury. The removal of these cells is performed by amoebocytes and univacuolar phagocytes (Fig. 5). These appear in large numbers from two sources: (i) by emigration from the blood stream, and (ii) by differentiation on the spot, from haemoblasts and lymphocytes. At this stage the number of haemoblasts, although increasing, is not yet large, and, while differentiation of the two types of phagocytic cells is to be observed, the number produced by this means can only be relatively small.

The mobilization of the cells that take part in the regeneration of the siphons begins during this period. These cells are the haemoblasts, the basic blood cells which originate on the epicardium. Examination of sections shows that the initial increase in numbers of the haemoblasts is only, in very small measure, due to the divisions of haemoblasts already present in the injured area (Figs. 5, 6). The number of dividing cells is always small, although it increases slightly during the period of regeneration. Examination of both injured and uninjured animals, vitally stained with neutral red, which facilitates the recognition of haemoblasts in living material, shows that the cavities of the tentacles form reservoirs of haemoblasts. From the bases of the tentacles of injured animals chains of these cells extend towards the cut edges

of the siphons. There is no evidence of a similar migration in uninjured animals. Further examinations of portions of the epicardium from both injured and uninjured animals give pertinent results. While the epicardium of uninjured animals shows a large number of haemoblasts, that of injured animals shows a vastly increased population. So dense is it over considerable areas that the cells are actually touching each other.

Thus the operation would seem to stimulate the liberation of haemoblasts from the tentacles, and to increase the rate of proliferation of these cells by the epicardium. They then enter by way of the blood stream into the zone of regeneration.

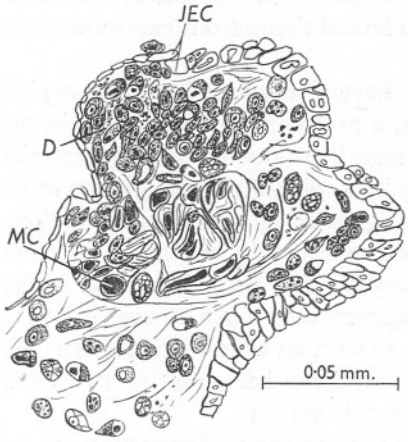

Fig. 6.

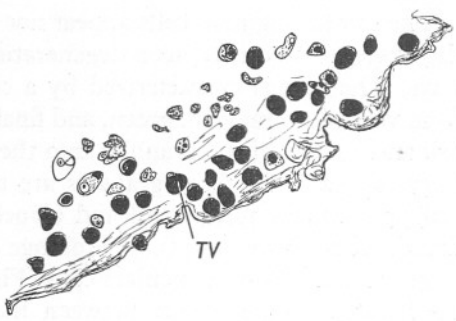

Fig. 7.

Fig. 6. Six hours after operation—mouth. The scale refers to this figure. *D*, debris; *JEC*, epidermal junction; *MC*, muscle cell showing extent of thymonucleic acid in cell.

Fig. 7. Edge of old test showing senescent cells. *TV*, cell with thymonucleic acid vacuole.

The first signs of differentiation from haemoblasts of the three types of cells that participate in the regeneration of the tunic can be distinguished 6 hr. after operation (Fig. 6). In addition, the number of these cells in a fully developed condition in the region has also increased. Some of these have already passed into the tunic adjacent to the old mantle (Fig. 6).

Finally, certain cells are eliminated from the animal by a process analogous to desquamation. Senescent cells, having migrated into the tunic, move towards the free edge and undergo degenerative changes *en route*. No matter what their origin, they all acquire a relatively enormous vacuole, and at the same time cytoplasm and nucleus decrease in volume. Ultimately only a crescentic area of cytoplasm containing a very small achromatic nucleus is to be distinguished (Fig. 7). The vacuole stains deeply with toluidine blue, the methyl green of Unna's Pappenheim, and is positive to Feulgen. These

reactions suggest that as an accompaniment to the deterioration of the cells there is a nuclear breakdown which results in a liberation of thymonucleic acid into the vacuole. In other words, there appears to be a modified form of chromatolysis (cf. Wigglesworth, 1942). At the free edge the cells seem to drift together (Fig. 7) and as they pass out of the tunic become surrounded by a thin bag formed by the test. This finally breaks away from the animal altogether.

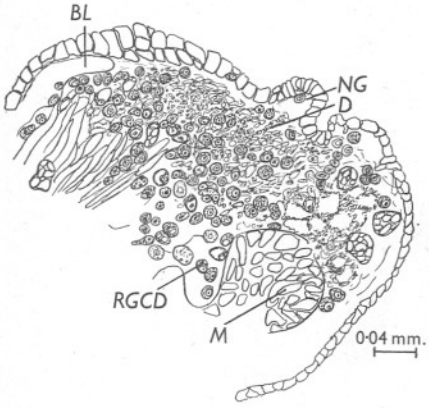

Fig. 8.

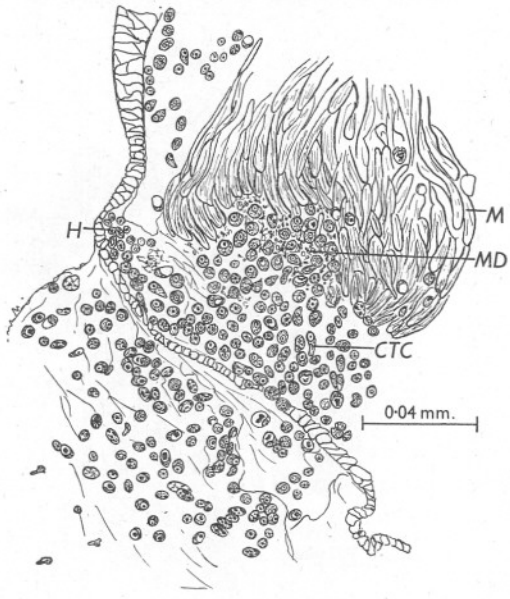

Fig. 9.

Fig. 8. Twelve hours after operation—mouth. *BL*, blood lacuna; *D*, debris; *M*, muscle; *NG*, new growth; *RGCD*, differentiating refringent granule cell.

Fig. 9. Sixteen hours after operation—mouth. *CTC*, connective tissue cell; *H*, haemoblast; *M*, muscle; *MD*, muscle debris.

Twelve hours after the operation (Fig. 8) the two epidermal layers have completely fused all round the reduced 'siphons'. In the subepidermal connective tissue there is a slight decline in the number of orange pigment cells, univacuolar phagocytes, amoebocytes and reticulate cells and an increase in the free pigment granules and in the numbers of small and medium-sized cells with refringent granules. The processes described as occurring 6 hr. after the operation continue.

By 16 hr. (Fig. 9) the numbers of those cells concerned with the regeneration of the missing siphons has markedly increased. The subepidermal lacunae are packed with haemoblasts and with cell-types differentiating from them. Univacuolar phagocytes, amoebocytes and yellow pigment cells remain, but they are now few.

In certain regions (Fig. 9) there appears to have been a mass migration of cells with one or several refringent granules into the tunic. In one specimen in which, as a result of the operation, the old test was torn from the epidermis posterior to the actual cut, new test growth was well advanced. The epidermal cells had already laid down the ground substance of the tunic, and cells with one or many granules were in the process of adding to the ground substance exactly as described by Pérès (1948*a*).

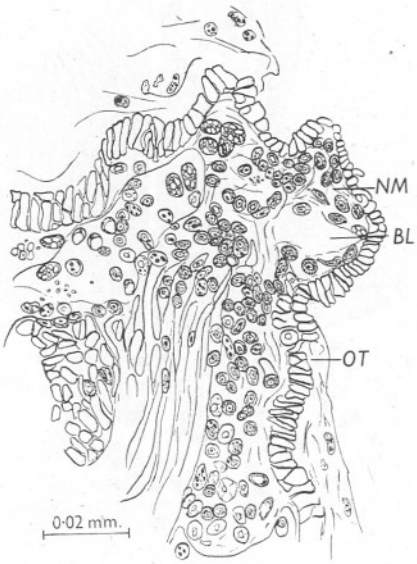

Fig. 10.

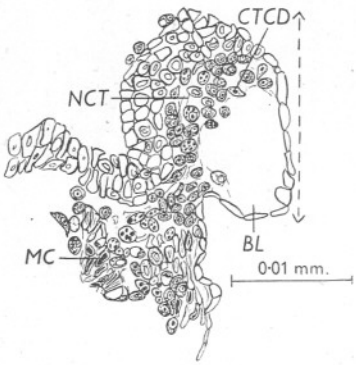

Fig. 11.

Fig. 10. Twenty-four hours—mouth. *BL*, blood lacuna; *NM*, new connective tissue matrix; *OT*, original tunic.

Fig. 11. Thirty-six hours—mouth. *BL*, blood lacuna; *CTCD*, differentiating connective tissue cell; *MC*, muscle cell; *NCT*, new connective tissue matrix; - - - , new growth.

In 24 hr. (Fig. 10) the haemoblast population is at its maximum density and the first evidence of actual regeneration of the mantle is observed. Between 16 and 24 hr. after the operation, the epidermal cells have begun to divide and a slight outpushing of the epidermis all round the cut edges of the siphons is apparent (Fig. 10). The bulge becomes filled with blood plasma carrying haemoblasts. Elsewhere in the region differentiation of haemoblasts and lymphocytes continues, in particular into cells with many granules or with a single granule, some of which migrate into the tunic, while others remain in the subepidermal region to perform other functions.

By 36 hr. (Fig. 11) the epidermal outpushing has increased in length and, in the subepidermal space, all stages in the differentiation of the haemoblasts into connective tissue cells can be distinguished (Fig. 11). The many cells

which have fully differentiated have begun to secrete the connective tissue matrix (Fig. 11) characteristic of the subepidermal regions of the normal mantle.

It is to be remarked that very rarely in the regenerating regions do the different types of blood cell differentiate from lymphocytes. Differentiation almost invariably appears to involve haemoblasts alone. Pérès has stated (1943) that the various blood elements, in young animals differentiate from

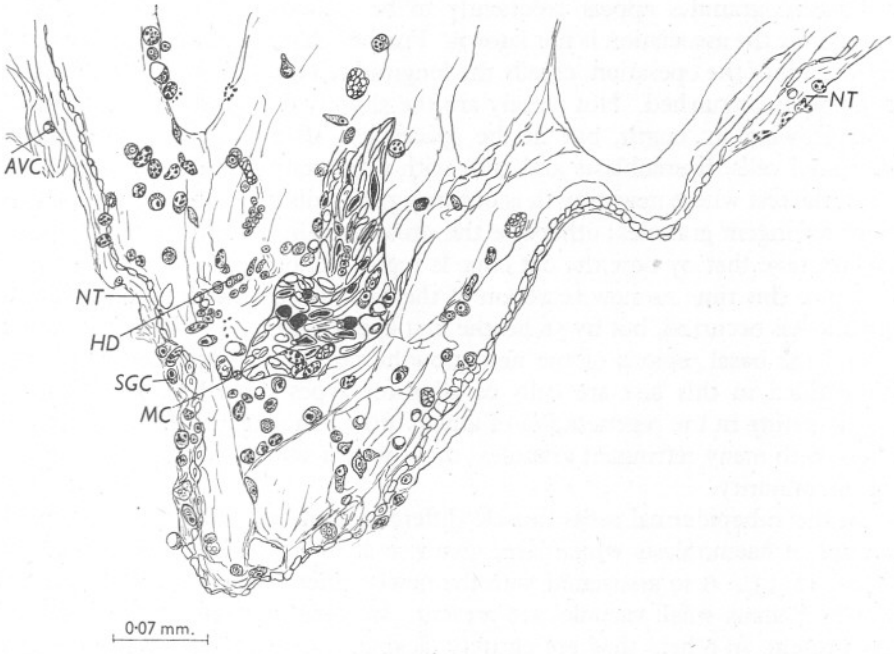

Fig. 12. Fifty-six hours—mouth. *AVC*, acidophil vacuole cell; *HD*, dividing haemoblast; *MC*, muscle cell; *NT*, new tunic; *SGC*, cell with single granule.

haemoblasts, in older animals from lymphocytes. It would therefore appear that in old specimens of *Ciona* regeneration is associated with the type of cell differentiation characteristic of young animals.

In one specimen killed 36 hr. after operation, the inhalant siphon had been cut sufficiently far posteriorly to involve the anterior pharynx. Here regeneration of new gill bars was in process. It was of interest to note that the endoderm and ectoderm, separated by connective tissue, which lined the anterior end of the pharynx, were identical in appearance. The ectoderm and endoderm had joined round the cut edge, and could not be distinguished from each other. Laterally and internally, slight bulges were visible at intervals, the forerunner of new gill bars and papillae.

By 48 hr. the ectodermal outpushings at the apices of the regenerating siphons have become even longer. In the posterior region of the regenerated zone a clumping of haemoblasts in the recently secreted connective tissue matrix has occurred. Differentiation of these cells along three lines has already been initiated. One group has begun slightly to elongate to form new muscle cells, another to differentiate into cells with refringent granules, and a third, much smaller than the others, consists of haemoblasts in the process of differentiation into cells with a single refringent granule. Cells with several refringent granules appear frequently to be associated with muscles. The reason for the association is not known. Further changes in muscles damaged as a result of the operation, chiefly the longitudinal muscles of the mantle, can now be distinguished. Not merely are the slightly damaged cells now intact and growing in length, but in the spaces left after the removal of badly damaged cells, haemoblasts and cells with refringent granules are found.

In the test where new growth is occurring the cells present are mostly those with refringent granules; otherwise the situation is little different from that at 24 hr., save that by now the cut edge is regular in outline.

Up to this time no new secretion of the tunic by the newly differentiating mantle has occurred, but by 56 hr. the first appearance of a very delicate tunic round the basal regions of the new growths can be distinguished (Fig. 12). Embedded in this test are cells of all three types described by Pérès, as participating in the regeneration of a new tunic. In general, the majority are those with many refringent granules; those with a small acidophil vacuole are in the minority.

In the subepidermal areas muscle differentiation continues and additional groups of haemoblasts which later give rise to muscle cells can be observed (Figs. 12–13). Also associated with the newly differentiated muscles are cells in which many small vacuoles are present. In some only one or two granules are present, in others they are entirely lacking. These are probably stages in the degeneration of the refringent granule cells.

In certain regions of the newly regenerating siphons there appears to have been no appreciable growth in length between 48 and 56 hr.; in others growth has continued. The third day sees the appearance of lobes characteristic of the siphons; and the differential growth just mentioned is undoubtedly associated with the development of these lobes. This type of growth continues through the third day.

By 60 hr. the picture of the regenerative processes is very little different from that observed at 56 hr. In the new tunic, which by now has here and there fused with the old, the number of cells with single acidophil vacuoles has increased, and some of the cells with many refringent granules have entered into the second phase of activity (Pérès, 1948*a*). The original granules are much paler and, in some cells, difficult to distinguish, while densely staining spheroidal masses, probably proteins, are appearing.

From 76 hr. until the end of the fourth day (Figs. 14–16) the regeneration of the siphons continues by repetition of the processes already described. For the first time near the outer edge of the tunic secreted by the newly regenerated mantle, a few cells with large vacuoles whose contents stain with toluidine blue, methyl green, and are positive to Feulgen, are seen.

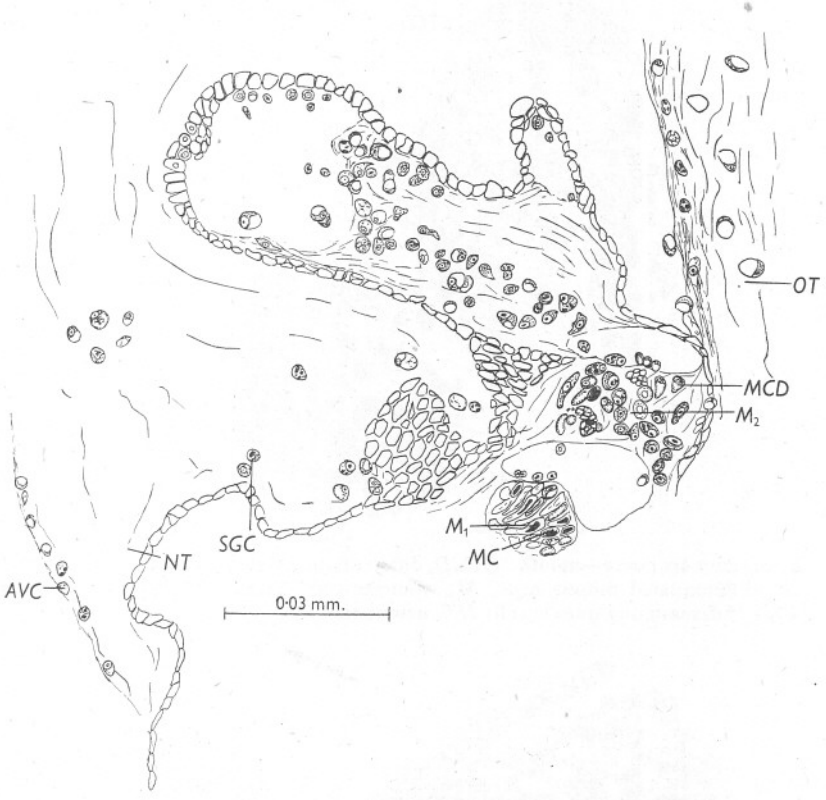

Fig. 13. Sixty hours—atriopore. *AVC*, acidophil vacuole cell; M_1, almost completely differentiated muscle cells; M_2, younger cells differentiating into muscle cells; *MC*, muscle cell; *MCD*, differentiating muscle cell; *NT*, new tunic; *OT*, original tunic; *SGC*, single granule cell.

The peculiar nature of the muscle cells should now be mentioned. Sections through individual fibres of fully developed muscles show that thymonucleic acid is evenly distributed throughout the cell, positive results having been given by both the Feulgen test and Unna's pappenheim. During the development of a fibre from a haemoblast the thymonucleic acid in the cytoplasm steadily increases in amount. With its appearance the small nucleus present during the earlier stages of growth ceases to be microscopically recognizable. The fully differentiated muscle cell is therefore apparently non-nucleated.

The description of the regeneration processes just given is characteristic of the majority of animals examined. There are minor variations in the time of tunic secretion by the newly formed mantle. It may be slightly earlier than has been stated.

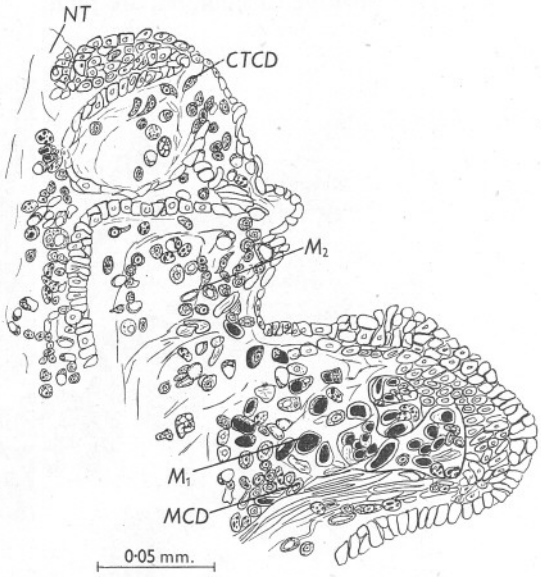

Fig. 14. Seventy-six hours—mouth. *CTCD*, differentiating connective tissue cell; M_1, almost fully differentiated muscle cells; M_2, younger cells differentiating into muscle cells; *MCD*, differentiating muscle cell; *NT*, new tunic.

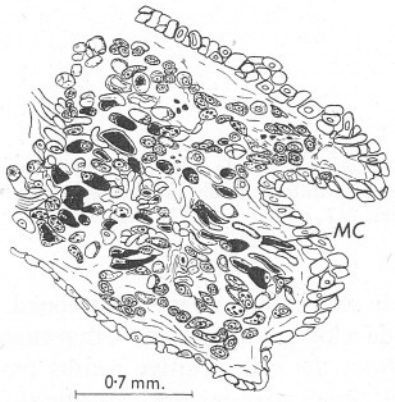

Fig. 15.

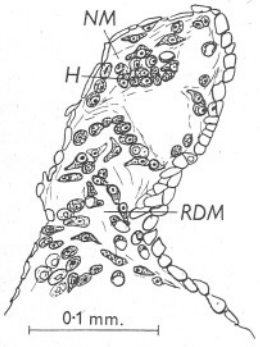

Fig. 16.

Fig. 15. Eighty hours—mouth—further growth. *MC*, muscle cell.

Fig. 16. Eighty hours—atriopore—continued growth. *H*, haemoblast; *NM*, new connective tissue matrix; *RDM*, recently differentiated matrix.

Discussion

The regenerative processes of specimens of *Ciona intestinalis* from which the siphons have been removed involve the haemoblasts, ectoderm, and a small region of the pharyngeal endoderm. The operation stimulates division and migration of the haemoblasts to such an extent that in a few hours a large number will have appeared at the site of the injury. Once there, they may differentiate directly into connective tissue cells, muscle cells and into various types of blood cell, or, much less frequently, differentiation of the blood cells may be preceded by a division of the haemoblast into two lymphocytes. The ectoderm and endoderm of certain regions of the pharynx are microscopically indistinguishable, and in some animals from which the most anterior region of the pharynx has been removed it is impossible to tell whether ectoderm or endoderm is proliferating to give rise to the new endoderm. The participation of haemoblasts in the formation of the new epithelia is in doubt. Certainly in some sections haemoblasts are seen wedged among the new ectoderm and endoderm cells. They may, however, be merely in transit through the epithelium.

There have been many accounts of asexual reproduction and regeneration among ascidians, but almost all of them suffer from inadequate accounts of the histological processes involved. Nevertheless, an attempt will be made to review the processes, to relate the phenomena of siphon regeneration in *Ciona* to those of ascidian regeneration in general, and to formulate certain principles which may go some way towards explaining the outstanding powers of asexual reproduction and regeneration in ascidians.

Asexual reproduction and regeneration in the phlebobranchs and aplousobranchs (in Harant's classification, 1933) either involve internal organogenesis from the epicardium, a pharyngeal outgrowth, or from a mesenchymatous septum, or from the blood cells, as in *Ciona*. As will be seen later both organogenesis from the epicardium and from the septum can occur in one and the same animal.

Before these methods can be discussed, more detailed descriptions of the processes involved must be given.

Asexual reproduction in *Diazona violacea* involves the formation of buds by transverse constrictions of the post-abdomen (Berrill, 1948*b*). Each bud contains ectoderm, muscles, two sections of the alimentary canal and part of the epicardium. From the epicardium are proliferated cells from which the pharynx, peribranchial sacs, cerebral ganglion and the anterior part of the post-pharyngeal alimentary canal of the new individual develop. The pericardium also has its origin in this structure. The two sections of the parent alimentary canal unite posteriorly and the loop so formed increases in length. This account lacks any detailed histology.

However, Brien's (1933) work on *Archiascidia neapolitana* (Julin) may

clarify the picture. When the thorax has been naturally or artificially lost, the ventral wall of the epicardium thickens and proliferates cells, described by Brien as embryonic in appearance, and it is from these cells that the two pharyngeal lobules develop that later coalesce to give the definitive pharynx. Significantly Brien describes the cells proliferated by the epicardium as 'embryonic', and very probably these are haemoblasts. In 1933 Pérès had not described the various types of blood cell to be found in ascidians and this term was therefore unknown to Brien. As noted above, Berrill (1948*b*) describes the epicardium in *Diazona* as dividing to give rise to cells from which the branchial sac, atrium, etc., develop. These cells may well be similar to those described as embryonic in *Archiascidia* and thus also be haemoblasts.

Asexual reproduction in *Clavelina phlegrea* (Salfi) is in its major aspects (Brien, 1930) an almost exact replica of that of *Archiascidia neapolitana*, but in another species, *Clavelina lepadiformis*, and in the Perophoridae, an apparently different process is involved. Here each of the bud chambers that develop upon a stolon contains a 'blastogenic mass', consisting in *C. lepadiformis* of a mass of blood cells, which Brien (1930) calls lymphoblasts, and of mesenchyme cells. These cells are the products of the disintegration of a mesenchymatous septum to which the lymphoblasts had become attached. In this mass an 'endoblastic vesicle' appears, on the future dorsal side of which there develops a neurogenital 'massif'. This vesicle is the forerunner of the pharynx peribranchial cavities, epicardium and the remainder of the alimentary canal. The neural and genital cells dissociate from each other. Further development consists of the differentiation of organs whose 'anlagen' have now appeared.

It therefore seems that, according to the current view, two methods of asexual reproduction and regeneration can occur in the one genus *Clavelina*. Further, it has been found experimentally that the two processes can occur in the same species *C. lepadiformis*. Asexual reproduction is normally at the expense of a mesenchymatous septum. If, however, as a result of transverse cuts, a bud chamber is formed in the oesophageal region, the new organs develop from the epicardium (Brien, 1933; Berrill & Cohen, 1936). The epicardium, a pharyngeal outgrowth, is generally said to be an endodermal organ. Thus, clearly, according to current ideas, two very different methods of asexual reproduction can occur, not merely in the same genus, but even in the same species. This conclusion seems to be very much open to doubt.

As already stated, the epicardium is generally regarded as an endodermal structure. Berrill (1951) has, however, suggested that it may possibly be of mesodermal origin. There are now good reasons for the acceptance of this idea. Enterocoely is common among protochordates and echinoderms, possible ancestors or close relations to the ancestors of the urochordates. To suggest, therefore, an enterocoelic origin for the epicardial tubes seems not unreasonable. This would make the cavity of an epicardial tube a portion of the coelom. In its continued growth, in *Ciona* the epicardium comes to invest the abdominal

organs; the potential cavity in this double-layered structure would thus form a perivisceral coelom, whose absence in ascidians has seemed anomalous. Further, the epicardium has been stated by Pérès (1947*c*) to be the centre of haemoblast proliferation, particularly in larval life. He also notes that in the Polyclinidae it undergoes haemoblastogenesis in the adult condition. In cephalochordates and in most vertebrates, according to Brachet (1944), blood cells are of mesodermal origin.

If the mesodermal nature of the epicardium be conceded, then epicardial and septal budding are but different manifestations of a single process. This hypothesis, however, does not account for the phenomena of siphon regeneration in *Ciona* and an attempt will now be made to show that this apparent defect can be overcome.

Pérès (1947*c*) pointed out the epicardial origin of haemoblasts, and Brien (1933) noted the proliferation of embryonic cells from the epicardium during asexual reproduction in *Archiascidia neapolitana*. He also described the mesenchymatous septum in *Clavelina lepadiformis* as consisting, very largely, of lymphoblasts (haemoblasts). It might also be suggested that the already differentiated mesenchymatous cells would be unlikely to play an important role in organogenesis. Thus, in both species the same cells, the haemoblasts, are almost certainly extensively involved in asexual reproduction, and in both they have the same origin, the epicardium. Berrill's (1948*b*) account of asexual reproduction in *Diazona violacea* offers no serious objection to this view. The epicardial origin of cells involved in organogenesis is noted. It is true that he states that blood cells play no part in the process, but this statement refers to the nutritive trophocytes.

It therefore seems reasonable to suggest that, in the phlebobranchs and aplousobranchs, asexual reproduction occurs at the expense of haemoblasts, cells proliferated in an undifferentiated condition, from the epicardium. The position of *Ciona*, a phlebobranch, in so far as regenerative processes are concerned, is now clear. In this animal, the cells responsible for the regeneration of all tissues other than the epithelia, of the siphons, are haemoblasts originating in the epicardium.

As a point of interest, according to Pérès (1947*b*), the cerebral ganglion of *Ciona* differentiates from neoblasts. These cells appear to be very like haemoblasts. The cerebral ganglia of *Diazona* and *Clavelina*, if the views outlined above be accepted, also differentiate at the expense of haemoblasts.

Finally, a third method of asexual reproduction in ascidians is seen in the pallial budding of the stolidobranchs. Here mesenchyme and ectoderm alone are involved in organogenesis; the mesenchyme is that tissue lying between the two layers of ectoderm of the outer atrial wall. The vesicle that gives rise to the future pharynx, nerve complex and atrial cavity is lined, at its first appearance, by ectoderm. A neural tube and two peribranchial cavities evaginate and become isolated from the parent vesicle, after which process

differentiation to form the definitive organs takes place. The future genital cells are carried from the parent gonads to the bud by the blood stream (*Botryllus*), or differentiate from mesenchyme cells, *in situ* (*Heterocarpa glomerata*). It must be emphasized that detailed histological investigations of the processes involved have never been attempted, and further work along these lines may considerably modify our present information.

I would like to express my most grateful thanks to the Director of the Marine Biological Laboratory at Plymouth, Mr F. S. Russell, F.R.S., for having afforded me the facilities of the Laboratory where all the observations upon the living material were made and much of the work upon the preserved material was carried out. I would also like to express my gratitude to Mr C. C. Hentschel of Chelsea Polytechnic and to Prof. P. B. Medawar, F.R.S., of University College, London, for their most helpful suggestions and criticisms of the manuscript. Finally I wish to acknowledge the most valuable assistance of the University of London Grants Committee, from whom I have on loan a Dixon Fund Microscope without which the work could not have been completed.

Summary

Regeneration of the siphons of normal specimens of *Ciona intestinalis* has been found to be completed, in every particular other than that of length, in 4 days.

Removal of the neuroglandular complex retards the regeneration rate.

With the exception of the epidermis and of the basic tunic, the new structures differentiate from haemoblasts.

A tentative synthesis has been made of the information available upon asexual reproduction and regeneration in the Ascidiaceae.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

An Experimental Study of the Scattering of Light by Natural Waters

By W. R. G. Atkins and H. H. Poole

Proc. Roy. Soc. Lond., B, Vol. 140, 1952, pp. 321–38

A blue-sensitive multiplier phototube was used to measure light scattered from a parallel beam in distilled, tap and sea water, the first-named serving as a check upon errors from extraneous sources of light. Forward and back scatter are closely the same for distilled water, but with natural waters by far the greater part of the effect occurs through angles less than 25°. A minimum is found for a deviation of about 110°, back scattering increasing somewhat for greater angles. The relative importance of forward scatter increases with turbidity, and in sea water about three-quarters of the effect is due to matter removable by filtration through a collodion filter of average pore diameter 1 μ or by sedimentation; further passage through 0·6 and 0·2 μ filters produces little change. Scattering is greater in blue light. Plymouth tap water scatters more than surface coastal water and the latter more than surface water 20 miles out, station E 1. Surface water scatters more than deeper—the water column being remarkably homogeneous even when a well-marked thermocline had existed for weeks, but a small increase was detectable at the top of the cold water. E 1 surface water increased in scattering between August and January, and decreased till May. Deep water showed little change. Extinction due to scattering between 20 and 155° amounted to less than one-sixth of that found for a similar sample with a Pulfrich photometer, so probably much scattering occurs below 20°. This explains why Pulfrich extinctions are so much greater than vertical extinction coefficients found in the sea.

The preponderance of forward scattering within the range 20–155° and the effects of filtration suggest that such scattering is due chiefly to refraction through transparent mineral particles, large compared with the wave-length of light. The refractive index of organic matter is too near that of water to produce refraction through angles as large as 20°. Such matter may, however, be responsible for some of the scattering through smaller angles, which apparently accounts for most of the turbidity found with the Pulfrich photometer. W.R.G.A.

D-Amino-acid Oxidase in the Molluscan Liver

By H. Blaschko and Joyce Hawkins

Biochem. Journ., Vol. 52, 1952, pp. 306–12

The livers of two cephalopods, *Octopus vulgaris* and *Eusepia officinalis*, contain the enzyme D-amino-acid oxidase. Since this is the first time that the enzyme has been described in invertebrates, some of its properties have been studied and compared with those of the corresponding mammalian enzyme. The pattern of substrate specificity of the cephalopod enzyme is similar to the mammalian enzyme, but there are a few differences; for instance, the octopus enzyme oxidized D-glutamic acid more rapidly. Both homogenates and acetone-dried powders were active.

The enzyme was also found in *Mytilus edulis* and in *Helix*, but not in *Anodonta*. H.B.

Observations on Amine Oxidase in Cephalopods

By H. Blaschko and Joyce Hawkins

Journ. Physiol., Vol. 118, 1952, pp. 88–93

This paper contains a report on the occurrence of the enzyme amine oxidase in the tissues of *Octopus vulgaris* and *Eusepia officinalis*.

In *Octopus*, very high amine oxidase activity was found in liver extracts; in order of decreasing activity, the enzyme was present in the posterior salivary glands, the anterior salivary glands and in brain tissue. The high amine oxidase activity of the tissues of *Eusepia* already described in earlier papers has been confirmed. Differences in the pattern of substrate specificity between the enzymes from the two species are also reported.

The significance of these findings in relation to the presence of amines in the tissues of cephalopods is discussed.

Amine oxidase activity was not detected in *Mytilus edulis* and *Helix*. H.B.

The Mechanical Analysis of the Responses from the End-Organs of the Horizontal Semicircular Canal in the Isolated Elasmobranch Labyrinth

By J. J. Groen, O. Lowenstein and A. J. H. Vendrick

Journ. Physiol., Vol. 117, 1952, 329–46

The mechanical properties of the cupula-endolymph system were subjected to experimental tests by means of the oscillographic method of recording from the nerve supplying the horizontal semicircular canal of the isolated labyrinth of the ray (*Raja clavata*).

Tests on torsion-swing and turn-table furnished conclusive evidence that the cupula-endolymph system behaves like a true pendulum, and a differential equation describing the behaviour of this system is suggested.

During the tests, sense organs deviating from the expected mode of behaviour were encountered, but a simple physical model (the characteristic curve of an electronic valve) renders possible a unified description of all behaviour types of semicircular canal organs. O.L.

Muscle Activity and Drug Action in the Body-wall of the Sabellid Worm *Branchiomma vesiculosum* (Montagu)

By J. A. Colin Nicol

Physiol. Comp. et Oecol., Vol. 2, 1952, pp. 339–45

The effects of certain autonomic drugs on strips of body-wall of *Branchiomma vesiculosum* (Sabellidae) are described. In preparations lacking central nervous system, acetylcholine and nicotine cause contractions of the longitudinal musculature. The threshold concentration lies about 1/100,000, and nicotine has the stronger effect. Eserine and DFP potentiate the contraction due to acetylcholine. Atropine, pilocarpine, and adrenaline are without effect. Repetitive electrical stimulation of body-wall (with and without nerve cord) shows no augmentation of response after eserinization. D-tubocurarine does not black neuromuscular transmission in *Branchiomma*. The contrast with the earthworm preparation in which augmentation or facilitation occurs is brought out. Evidence for antagonistic systems, adrenergic and cholinergic, in annelids is reviewed, and the differences in the blocking action of curare within this group are taken to indicate considerable diversity in receptor mechanisms concerned with neuromuscular transmission.

J.A.C.N.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888. The cost of the building and its equipment was £12,000 and, since that date, a new library and further laboratory accommodation have been added at an expenditure of over £25,000.

The Association is maintained by subscriptions and donations from private members, scientific societies and public bodies, and from universities and other educational institutions; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. 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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Founders		100	0	0
Governors		500	0	0

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

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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