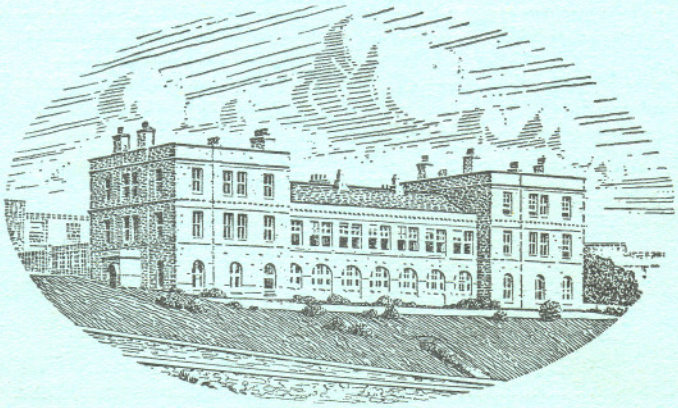
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



THE PLYMOUTH LABORATORY

VOLUME 33, No. 1

(issued February 1954)

CAMBRIDGE
AT THE UNIVERSITY PRESS
1954

Price Fifty-two Shillings net

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J. Mar. biol. Ass. U.K. (1954) 33, 1–19
Printed in Great Britain

THE EFFECTS OF WAVE-ACTION ON THE DISTRIBUTION AND NUMBERS OF THE COMMONER PLANTS AND ANIMALS LIVING ON THE PLYMOUTH BREAKWATER

By A. J. Southward and the late J. H. Orton, F.R.S.

(Text-figs. 1–7)

CONTENTS

Introduction

The breakwater in Plymouth Sound offers a simple case of two adjacent populations of intertidal organisms subjected to quite different degrees of exposure to wave-action. The other environmental factors, with a few exceptions, are approximately the same each side, and the effects of the wave-action should be clearly demonstrable.

Previous experience in the Isle of Man (Southward, 1953) has proved that evidence of variation in the numbers of the plants and animals is as important as variation in their zonation, and that the numbers may, in fact, show the greater changes in relation to differing degrees of exposure to wave-action. The work was planned on a quantitative basis, and the breakwater proved a fortunate choice, since it offers relatively uniform slopes throughout the greater part of the intertidal zone. The latter factor is almost a necessity for a quantitative study, but a quality lacking in most of the rocks exposed in the Plymouth area.

The investigation was suggested by Prof. Orton, and was planned and begun as a joint study with the aid of a grant from the E. T. Browne Fund of the Royal Society. Various factors intervened, not the least of which were delays due to inclement weather, and most of the field work was carried out by

A. J. S. alone, in September 1951. This account was prepared by A. J. S. and revised by Prof. Orton shortly before his death.

We are indebted to Mr F. S. Russell, F.R.S., and to various members of the staff of the Marine Biological Association for assistance, and to Dr R. G. Evans for helpful criticism of the typescript.

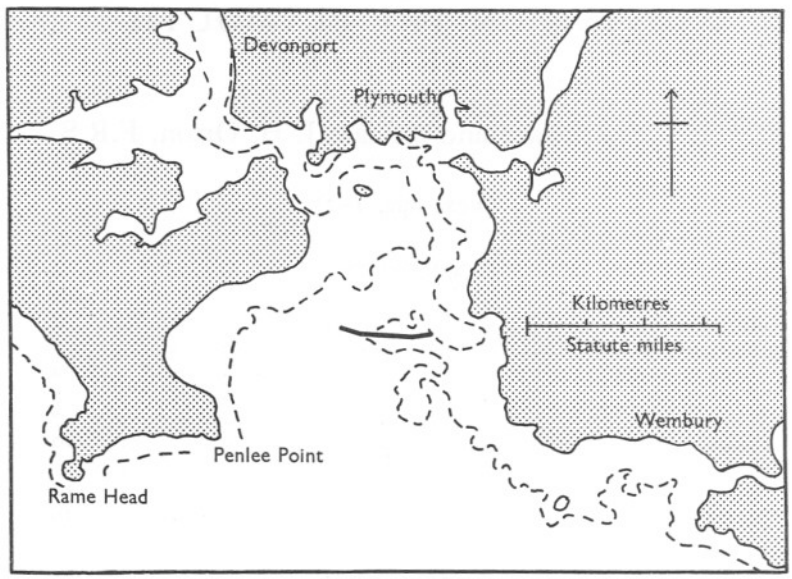

Fig. 1. Map of the Plymouth area showing the position of the breakwater. — —, approx. 5 fathom (9 m.) line.

The Environment

The general environment of the Plymouth area has been discussed by Orton (1920), Colman (1933) and Evans (1947*b*), and the main features of the breakwater have been described by Lysaght (1941). It is therefore only necessary to mention certain factors of importance to this study.

Substratum. The main constituent of the breakwater is a local limestone, and the northern side, down to low-water neap level, consists entirely of blocks of this material cemented together. From low-water neaps down, however, the north side consists of limestone and cement rubble of varying size: some of the larger masses may project as high as mid-tide level (Fig. 2).

On the southern side of the breakwater, the sharp ledges at each end (Fig. 2, sections *A* and *E*), the ledge at low-water neaps (sections *C* and *D*) and various other isolated parts (particularly on section *B*) are faced with granite. The granite frequently stands proud of the limestone by as much as 5 cm., indicating the extent to which the latter has eroded, and in places, especially towards the western end, this erosion has produced a somewhat irregular

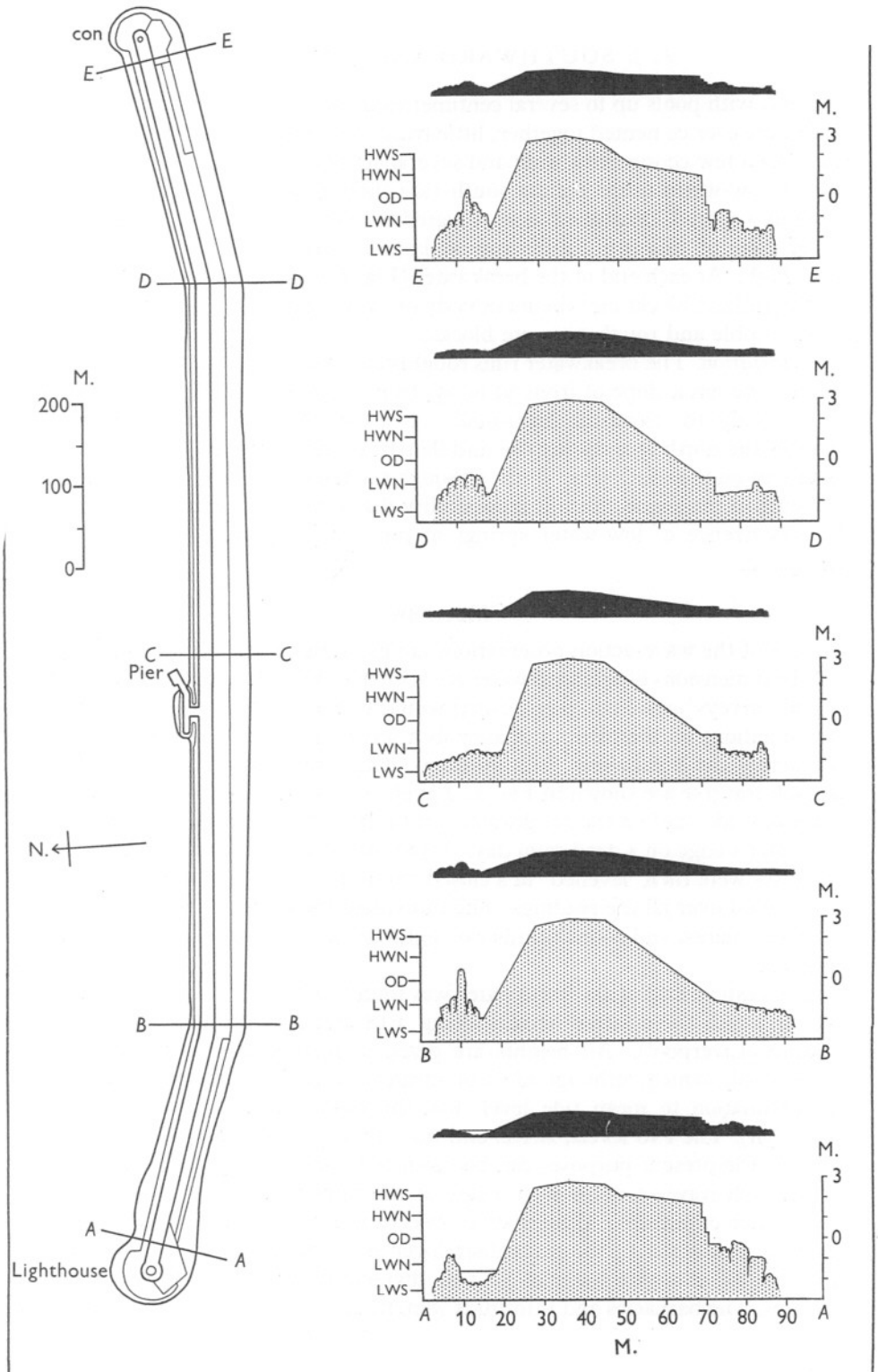

Fig. 2. Plan of the breakwater showing the traverses (*A–A* to *E–E*) and sections across them. The sections in black show the true profiles, while those stippled have their vertical scale exaggerated (five times the horizontal scale) to bring out the salient features.

surface, with pools up to several centimetres deep. If the blocks on the south side were ever cemented together, little trace of it remains, and there are deep crevices a few centimetres wide and several centimetres deep, between them. Below low-water neaps on the south side there is a gently sloping layer of roughly squared limestone blocks, covered with concrete in places, and at the outer edge of this is a double row of large concrete blocks, approximately 2 m. high. At each end of the breakwater (Fig. 2, sections *A* and *E*) the space between these blocks and the main body of the breakwater is filled with limestone rubble and rough concrete blocks.

Orientation. The breakwater runs roughly east–west (Fig. 1), and the north-facing side has a slope of from 30 to 35° from the horizontal, while the south face is only 10° from the horizontal. As a consequence, the sunlight that reaches the north face during the middle part of the day will be more oblique than that on the south face. Thus, although the north face cannot be regarded as being in the shade, there is a possibility that some of the adverse effects of the occurrence of low-water springs at midday (see Orton, 1920) may be mitigated.

Methods

Details of the wave-action observations are given in a separate section (p. 5).

The dimensions of the breakwater are such that the methods used previously ('grid surveys', see Southward, 1953) would be most impractical, and for the investigations on the plants and animals a series of five traverses was marked out across the breakwater. The positions of the traverses and cross-sections at each traverse are shown in Fig. 2. Levels were transferred from a recently surveyed tide-staff on the breakwater fort to the breakwater pier, by means of the water's edge on a dead calm day. Fixed marks at the highest points of the traverses were then 'levelled' in a closed circuit from the pier, the error being distributed over all the readings. The individual traverses were levelled from the fixed marks, and as the number of sightings was small, the error could be ignored.

The eastern end of the breakwater was found to be 0·3 m. higher than the western end, the relative heights being 3·18 and 2·88 m. above Ordnance Datum (Liverpool). All heights are given in metres above or below O.D. (Liverpool), which, although not true mean sea level, is at Plymouth a better approximation to mean tide level than the more recently established O.D. (Newlyn). The two levels, M.T.L. and O.D. (Liverpool) differ by only 0·07 m., and for the present purposes can be taken to be the same (see Table I).

On each traverse notes were made of the upper and lower limits of the commoner organisms. Quantitative observations were made at each 0·5 m. above or below M.T.L., the percentage cover of the plants being first estimated with the aid of a wire frame of 1 × 1 m. divided up into a hundred decimetre squares. The barnacles and littorinids were then counted on several decimetre

squares, or the whole metre square if sparse, and finally the limpets and top-shells were removed from the whole or half of the metre square for sorting. The length of the limpet shells was later measured to the nearest millimetre.

Owing to the broken nature of the region at L.W.N. on the north side the quantitative observations were not completed on certain traverses. Traverse *A* north side ended in a pool, and was not carried below M.L.W.N.

The animals and plants in the deep crevices between the blocks and in the pools were not included in the survey.

TABLE I. TIDE LEVELS FOR PLYMOUTH, BASED ON THE ADMIRALTY TIDE TABLES (1951)

Levels	Abbreviations used in text	Height relative to C.D. (Devonport)		Height relative to O.D. (Liverpool)		Height relative to O.D. (Newlyn)	
		Feet	Metres	Feet	Metres	Feet	Metres
Extreme high-water springs	E.H.W.S.	17·0	5·2	8·5	2·6	8·0	2·4
Mean high-water springs	M.H.W.S.	15·70	4·79	7·28	2·22	6·78	2·07
Mean high-water neaps	M.H.W.N.	12·25	3·72	3·83	1·15	3·33	1·00
Ordnance datum (Newlyn)	O.D.	8·92	2·72	0·50	0·15	0	0
Ordnance datum (Liverpool)	O.D.	8·42	2·57	0	0	−0·50	−0·15
Mean tide level	M.T.L.	8·20	2·50	−0·22	−0·07	−0·72	−0·22
Mean low-water neaps	M.L.W.N.	4·60	1·40	−3·82	−1·16	−4·32	−1·31
Mean low-water springs	M.L.W.S.	0·18	0·06	−8·24	−2·51	−8·74	−2·66
Chart datum	C.D.	0	0	−8·42	−2·57	−8·92	−2·72
Extreme low-water springs	E.L.W.S.	−1·6	−0·5	−10·0	−3·0	−10·5	−3·3

WAVE-ACTION

For the purpose of the present study, significant wave-action was defined as the occurrence of waves washing over the top of the breakwater at, or before, high water. With the tide at M.H.W.S. this requires a minimum height of wash of 0·6 m. above the true tide height at the western end of the breakwater, but at neap tides, of course, much greater wash is required. However, during rough weather the observations had to be made from the land (with the aid of field-glasses), as it was impossible to reach the breakwater, and no other criterion of wave-action was feasible over the distance involved (3·5 km.). During a period of 28 days' observations it was found that practically all winds with a velocity of 10 m.p.h. (16 km./hr.) or more, blowing from the sector W.–S.–S.E. were associated with waves breaking over the top of the breakwater. Some of the more extreme examples of the observations are shown in Table II: nearly all of these were recorded from the shelter of the laboratory.

By applying the the results of the observations to wind records it is possible to produce an approximate exposure index. From Table III, columns 1 and 2, the values for 1950 and 1951 were 38 and 32% respectively. These figures are similar to those arrived at by Lysaght (1941), who, however, considered the sector S.W.–S.–S.E. to be critical (Table III, column 3).

TABLE II. SOME OF THE MORE EXTREME EXAMPLES OF WAVE-ACTION ON THE BREAKWATER

Date (1951)	Wind direction and velocity (m.p.h.) at 09.00 hr.	Maximum wind velocity (gusts in m.p.h.) during succeeding 24 hr.	West end			East end		
			Time of waves starting to wash over	Predicted height of tide at the time (metres relative to O.D.)	Calculated minimum height of wash (m.)	Time of waves starting to wash over	Calculated height of tide at the time (metres relative to O.D.)	Calculated minimum height of wash (m.)
26 Aug.	W.S.W. 16	42	09.30	0	2·8	No wash, even at high water		
27 Aug.	S.W. 18	35	11.00	0·4	2·3	No wash, even at high water		
28 Aug.	S.S.W. 23	48	11.45	0·3	2·4	13.00	1·3	1·7
29 Aug.	S.S.W. 20	44	12.00	−0·2	3·0	15.00	1·6	1·4
13 Sept.	S.S.W. 41	57	11.45	−1·3	4·1	12.30	−0·6	3·6
14 Sept.	S.S.W. 19	52	13.50	−0·2	3·0	No observation		

TABLE III. DISTRIBUTION OF WINDS AT PLYMOUTH

Month	Percentage days of wind blowing from the sector W.–S.–S.E. with a velocity of 10 m.p.h. or more		Percentage of winds (all velocities) blowing from the sector S.W.–S.–S.E., for the period 1893–1922 (from Lysaght, 1941, p. 43)
	1950	1951	
Jan.	32	29	33
Feb.	64	39	34
Mar.	45	42	32
April	30	27	30
May	16	16	35
June	47	40	38
July	35	10	36
Aug.	68	55	40
Sept.	43	43	30
Oct.	39	6	32
Nov.	20	40	26
Dec.	16	35	35
Year	38	32	33

All these exposure indices seem low compared with similarly derived indices for other places (e.g. 70% at Port St Mary, Isle of Man, see Southward, 1953), but there is no evidence that the breakwater is any less liable to wave-action. In spite of the wind and wave records there were only 3 days out of the 28 without any wave-action on the south side, and it would seem that refracted waves and swells play a much bigger part in the wave-action on the breakwater than at Port St Mary. There can be no precise comparison of the two places without long-period wave recordings.

In comparing the wave-action on the north and south faces of the breakwater we are on a firmer basis. It has already been pointed out that, during the period of wave-action observations, there were only 3 days when there was no appreciable wave-action on the south side; in the remaining 25 days the wave-action produced a wash of at least 0·3 m. above true tide height, if not more. On the north face, however, even during north-easterly winds reaching gale force at times, the wash was not seen to exceed 0·3 m., which is less than a tenth of the maximum wash observed on the south side.

It must be noted that, although the north face would appear to be almost completely sheltered from the mechanical effects of wave-action, it is wetted by wash when waves break over the top of the breakwater from the south side. This may accentuate the possible effects (noted on p. 4) of the obliquity of the sunlight received by the north face.

From the topography it might be expected that the eastern end of the breakwater would experience greater wave-action than the western end, which appears to be sheltered, to some extent, by Rame Head and Penlee Point (Fig. 1). However, this was not the case, and the observations show that there was considerably more wave-action on the western end than on the eastern end. The eastern end is only 0·3 m. higher than the western end, yet, on several occasions it was observed that the waves did not begin to wash over it until the tide had risen by from 0·5 to 1·6 m. above the level at which it stood when waves began washing over the western end (see Table II). On two occasions waves began washing over the western end more than 2 hr. before high water, but there was no wash at the eastern end even at high water.

The evidence thus indicates that the wave-action at the western end may be some 10–40% greater than at the eastern end of the breakwater. It is possible that the deeper water at the western end of the breakwater, which stands in 12–15 m. of water at low tide, may allow the closer approach of larger waves, with consequently greater wash on breaking. The eastern end stands in only 6–9 m. of water at low tide, and may be sheltered by the shoals to the south (Fig. 1).

Plants

At first impression there seemed to be a marked difference between the two sides of the breakwater, the south side appearing to be practically bare of algae, while the north face bore a comparatively dense population. In general, further investigation confirmed this view, but the population on the south side was greater than expected.

The species dealt with are described below: their nomenclature follows that of Newton (1931). The calcareous algae of the genera *Lithothamnion*, *Lithophyllum* and *Melobesia* were not identified and are referred to collectively as 'lithothamnia'.

Enteromorpha sp. Slight patches of *Enteromorpha* mixed with filamentous green algae occurred on the northern slope between M.H.W.N. and M.T.L. These patches were commonest towards the western end (traverses *A* and *B*) but did not exceed 20% cover. On the south side *Enteromorpha* was present all along the ledge just above M.L.W.N., but was commonest between traverses *C* and *D*; many of the limpets had a dense growth on their shells.

Laminaria saccharina and *Saccorhiza polyschides* (*bulbosa*) had similar distributions, at, and below, M.L.W.S. all along the northern side: on the south side they were restricted to the larger crevices between the rough blocks below L.W.N., occurring as high as 0·5 m. above M.L.W.S. This distribution confirms previous evidence (Evans, 1947*b*) that these species are generally restricted to places sheltered from wave-action. However, *Laminaria saccharina* seems to require a certain amount of water movement.

L. digitata. While some young plants of this species occurred as high as M.L.W.N. among the *Himanthalia* on the south side, the main upper limit, defined here as the zone of 50% or more cover, was a trifle higher on the north face, where it began at 0·4 m. above M.L.W.S. compared with M.L.W.S. on the south side. To some extent, this bears out a tendency, noted by Evans (1947*b*) and Southward (1953), for the main upper limit of *Laminaria* on steep or broken shores to be higher in sheltered places. However, on both sides of the breakwater there was practically 100% cover below M.L.W.S.

Fucus spiralis. As shown in Fig. 3, this species was well represented on the north side, where it occurred from M.H.W.S. down to M.T.L. The maximum cover was found at M.H.W.N. and just above, increasing from 20% on traverse *A* to over 50% on traverse *E*. It was poorly represented on the south side, only a few tufts being found on traverse *A*, and nowhere exceeded 6% cover. The zone varied quite widely on the south side but was always narrower than that on the north side. It is worth noting that on the north side of traverses *A*, *B* and *C*, the lowermost plants occurred side by side with the uppermost plants of *F. serratus*. This overlapping of the two species is not recorded by Colman (1933) or Evans (1947*b*), and has not been noticed in the Isle of Man, but may occur in Cardigan Bay (Evans, 1947*a*). It is possibly due to an unusually low lower limit of the *spiralis*, which itself may be due to the absence of *Ascophyllum*.

Fucus vesiculosus (Fig. 3). As might be expected from the known tolerance by this species of considerable wave-action (Kitching, 1935; Evans, 1947*b*; Southward, 1953), the differences between the populations on the north and south sides of the breakwater were much less than those of the other species of *Fucus*. In general, the cover was lower on the south side where it reached a maximum of 14% compared with up to 26% on the north side, but on traverse *A* the cover on either side did not exceed 2%. The upper and lower limits varied widely, the narrowest zone being found on the north side of traverse *A*, from 0·4 m. above M.T.L. to M.L.W.N. In other places, such as the

south side of traverse *B*, and the north side of traverse *E*, the species occurred up to M.H.W.N.

F. serratus (Fig. 3). This species is usually found higher up the shore in sheltered situations (Evans, 1947*b*; Southward, 1953). On the breakwater, the main upper limit on the north side was at approximately M.T.L., compared with about M.L.W.N. on the south side. With the exception of traverse *B*, the population was denser on the north side, where it exceeded 90% cover in places, compared with a maximum of 40% on the south side.

Ascophyllum nodosum was not found.

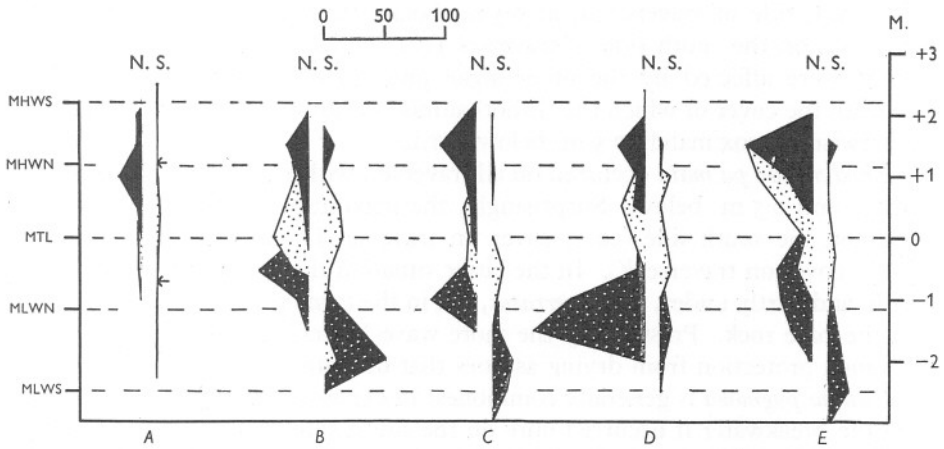

Fig. 3. The distribution of three species of *Fucus* on the breakwater. On the south side of traverse *A* the limits between the species are marked by arrows; on the north side of *A*, *F. serratus* could not be investigated and is not shown. On this, and subsequent diagrams, frequency polygons for the north and south sides of the traverses are indicated by the letters N. and S. respectively. ■, *F. spiralis*; ▤, *F. vesiculosus*; ▩, *F. serratus*. The scale denotes percentage cover.

Pelvetia canaliculata was not observed on any of the traverses other than the north side of *C*, where it occupied a narrow zone from 0·8 m. above M.H.W.N. to 0·3 m. above M.H.W.N., and reached 36% cover. Traverse *C* is very near the pier (see Fig. 2) and at high water is probably the most sheltered region of the whole breakwater. A few isolated tufts of *Pelvetia* were seen on the south side, between traverses *B* and *C*.

Himanthalia lorea formed a well-defined belt on both sides of the breakwater, from about 0·5 m. above M.L.W.N. down to almost M.L.W.S. On both sides it achieved 100% cover in places, but the region of maximum cover was higher on the north side, at approximately M.L.W.N., than on the south side, at 0·8 m. below this level. The species was least developed on the south side of traverse *A*, where it did not exceed 12% cover.

Porphyra umbilicalis. A few plants of this species, presumably the remnants of the settlement in the autumn of the previous year, were present all along the south side between M.H.W.S. and M.H.W.N., and at M.L.W.N. They were commonest just above M.H.W.N., where they reached 5% cover. A few tufts also occurred on the north side of traverse *A* at M.H.W.N.

'Lithothamnia'. The upper limit of the 'lithothamnia' and the associated *Corallina*, excluding pools and wet places, usually corresponds to the lower limit of the barnacles: on steep slopes or broken shores these limits are usually lower in the more wave-beaten places (Southward, 1953). This was the case on the breakwater, the lowest position of the limits being found on the south side of traverse *A*, at 0·5 m. below M.L.W.N., compared with at M.L.W.N. on the south side of traverses *C*, *D* and *E*. On the north side the limits were affected by the other algae present, especially *Fucus serratus*, beneath the cover of which the 'lithothamnia' occurred up to M.T.L., but was otherwise approximately 0·5 m. below M.T.L.

Rhodymenia palmata occurred on all traverses, with an upper limit between M.T.L. and 0·5 m. below. Surprisingly, the maximum densities were found both on the south side (20% cover on traverse *A*) and on the north side (20% cover on traverse *E*). In the latter situation the species was associated with, and partly under, *Fucus serratus*, but in the former it was largely present on the bare rock. Presumably the more wave-beaten situation (*A*) provides as much protection from drying as does that beneath *Fucus*.

Lichina pygmaea is generally commonest in exposed places (Evans, 1947*b*). On the breakwater it occurred only on the south side, but was quite sparse, not exceeding 4% cover. The zone, from E.H.W.S. to 0·3 m. above M.H.W.N., is narrow compared with other places in the Plymouth area where it may extend to M.T.L. (Colman, 1933; Evans, 1947*b*). The sparseness and restricted zone suggest that the breakwater is an unsuitable habitat for the species, possibly due to the smooth nature of the limestone blocks.

Animals

The nomenclature of the animals is based on that of the *Plymouth Marine Fauna* (Marine Biological Association, 1931), with the following exceptions: *Patella intermedia* Jeffreys 1865 = *P. depressa* Pennant 1777 (see Fischer-Piette, 1938, 1948); *P. aspera* Lamarck 1819 = *P. athletica* Bean 1844 (see Fischer-Piette, 1938, 1948); *Littorina saxatilis* Olivi 1792 = *L. rudis* Maton 1797 (see Colman, 1932).

Chthamalus stellatus. On all but the gentlest of slopes this species would appear to show the same relation to wave-action as does *Balanus balanoides*, i.e. an increase in exposure raises the upper limit, lowers the lower limit, and increases the population density (Hatton & Fischer-Piette, 1932; Moore, 1935; Southward, 1953). On the breakwater this was true of the southern face

as compared with the northern face, but the difference in number was much more noticeable than the differences in the upper and lower limits (Fig. 4). Thus, the upper limit on the north side was at approximately E.H.W.S. (0·3 m. above M.H.W.S.). On the south side the species did not occur any higher than the general upper surface of the breakwater (0·6 m. above E.H.W.S.), in spite of the numerous structures, such as shelters and blockhouses, which extended above this height. The lower limit on the south side varied from M.L.W.N. at the eastern end to 0·4 m. below this level at the western end. This is some 0·7–1·1 m. below the lower limit on the northern side, which was roughly 0·5 m. above M.L.W.N. (see Fig. 4).

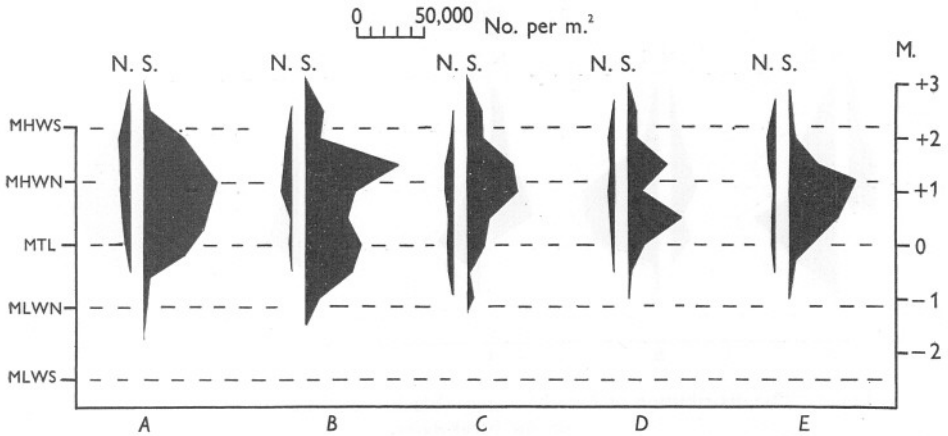

Fig. 4. The distribution of *Chthamalus stellatus* on the breakwater.

The counts showed much greater differences between the two sides of the breakwater. On each traverse, at the region of maximum abundance (about M.H.W.N.), the numbers of *Chthamalus* on the south side were over five times as great as those on the north side. The greatest number found on the north side was a mere 8000/m.², compared with a maximum of 68,000/m.² found on the south side. However, the latter figure refers to a granite substratum: on limestone the numbers did not exceed 40,000/m.², and were always lower than on the nearby granite. This difference in density of population between the two types of rock seems to be a fairly general phenomenon, and in this case may be related to the rougher surface of the granite.

It must be noted that on both sides of the breakwater the barnacle population was greater at the western end, although this was most marked on the south side (Fig. 4).

Balanus balanoides. At the time of the field work this barnacle had practically disappeared from the Plymouth area (Southward & Crisp, 1952). Isolated specimens were found on the south side of the breakwater, between M.T.L.

and M.L.W.N. on traverses *A*, *B* and *C*. The numbers did not exceed 2/m.^{2}, and the distribution was very erratic.

B. perforatus was found on both sides of the breakwater, between 0·8 m. above, and 0·8 m. below, M.L.W.N. The numbers were somewhat greater on the south side, with a maximum of 16/m.^{2}, compared with up to 6/m.^{2} on the north side.

B. crenatus occurred on the south side from 0·8 m. below M.L.W.N. to the lowest level investigated (E.L.W.S.), and was commonest towards the western end where it reached a density of up to 40/m.^{2} on traverse *A*. On the north side it was noticed only on the piles of the pier.

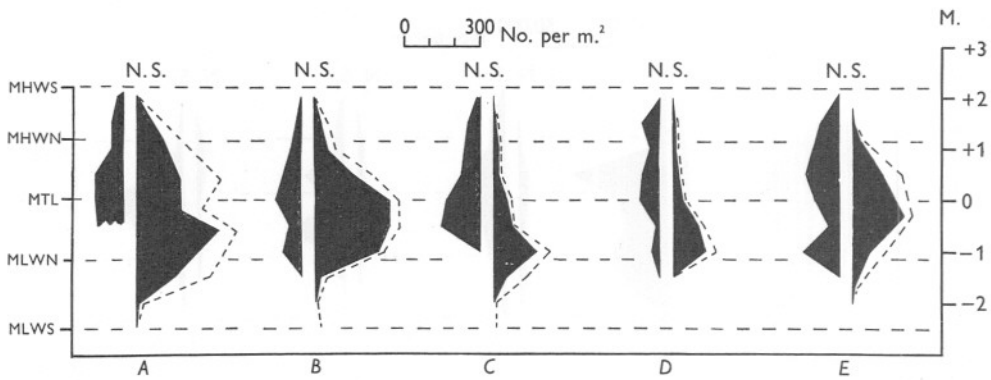

Fig. 5. The distribution of *Patella vulgata* (black) and total *Patella* (broken line) on the breakwater.

Patella vulgata (Fig. 5). The upper limit of *P. vulgata* was found to be practically the same on both sides of the breakwater, at 0·1–0·2 m. below M.H.W.S. According to previous evidence (Orton, 1929; Evans, 1947*b*; Southward, 1953) this species is not normally found above M.H.W.N., except on north-facing shaded surfaces, or on rocks receiving considerable wave-action. It would therefore appear that, on the breakwater, the effect of wave-action on the south side is more or less balanced by the effect of the greater shade from the sun on the north side. The moderately dense growth of *Fucus spiralis* on the north side up to M.H.W.S. may help to maintain the upper limit of *Patella* on that side by offering further protection from desiccation.

The lower limits differed by about 0·5 m.: on the north side the limit varied from 0·2 m. above, to 0·3 m. below, M.L.W.N., while on the south side it varied from 0·3 m. below M.L.W.N., to M.L.W.S. The occurrence of the lowest lower limit at the western end of the south side supports the view that this difference is due to the wave-action (cf. Southward, 1953), but it is possible that the higher lower limit on the north side is more a result of the layer of silt present on the rocks below L.W.N. rather than to the shelter from wave-action itself.

From Fig. 5 it will be seen that the largest numbers of *P. vulgata* occurred on the south side of each traverse, reaching a maximum towards the western end of the breakwater. In general, this agrees with findings in the Isle of Man, where, except on very steep slopes, the numbers tended to increase with increase in exposure to wave-action (Southward, 1953). However, the differences between the two sides of the breakwater were not very marked, especially on purely limestone substrata. For example, on traverses *C* and *D* the greatest numbers found on the north side were 134 and 76/m.^{2} respectively, compared with 156 and 130/m.^{2} on the south side. Above M.T.L., in fact, the north-side population was the greater. On the granite substrata of traverse *A*, however, the numbers reached 330/m.^{2} just below M.T.L., practically twice the population at the same level on the limestone rock, and nearly three times that at the same level on the north side.

The problem of population density in *Patella* is quite inseparable from that of size, since there is usually a broad inverse correlation between them (Jones, 1948; Southward, 1953). The smaller sizes present in wave-beaten situations (Fischer-Piette, 1941, 1948; Evans, 1947*b*) may therefore be due to the greater numbers present in such places (Southward, 1953). On the breakwater the limpets tended to be larger on the north side, i.e. there was a bigger proportion of the larger size-groups (40–70 mm. in length), and Fig. 6 shows a representative series of histograms for the M.T.L. samples. The size difference was most marked at, and above, M.T.L., the proportion of larger *Patella* on the south side increasing towards L.W.N. The absence of larger limpets was particularly noticeable on the densely inhabited granite substrata on the south side (Fig. 6*A*, *B*).

The restriction of *P. intermedia* and *P. aspera* to the more wave-beaten places (Fischer-Piette, 1948; Evans, 1947*a*, *b*) is clearly demonstrated by the limpet samples from the breakwater. On the north side, out of about 2000 specimens examined, only ten specimens of *P. intermedia* and six *P. aspera* were found, and these nowhere exceeded a density of 4/m.^{2}. On the south side these species were present on all traverses, and at certain levels constituted almost half of the total limpet population (see Fig. 5).

P. intermedia. The upper limit of this species was a little below that of *P. vulgata*, varying from 0·2 m. below M.H.W.S. at the western (more exposed) end, to about M.H.W.N. at the eastern end of the south side. The lower limit on the south side was at about M.L.W.N., except on traverse *A*, where the species occurred to 0·3 m. below this. The few specimens that occurred on the north side were found between M.H.W.N. and M.T.L. On the south side the greatest numbers were found around M.T.L., reaching a maximum of 160/m.^{2} on traverse *A*. The density was generally higher on the granite than on the limestone.

P. aspera. On the south side, the upper limit was higher at the western end, where it occurred at M.H.W.N. on traverses *A* and *B*, compared with M.T.L. on

traverse *D*. On traverses *A*, *B* and *C* the species was present down to M.L.W.S. and possibly below, but on *D* it did not occur more than 0·3 m. below M.L.W.N. Of the few specimens found on the north side, one occurred at M.T.L., the others at M.L.W.N. The numbers on the south side were generally greater on the limestone than on the granite, reaching a maximum of 80/m.^{2} on traverse *C*, just below M.L.W.N.

Gibbula cineraria was the least common of the three species of top-shells found on the breakwater. It occurred only once in the samples, at M.L.W.N. on the south side of traverse *B*, but it seems possible that it may have been present on the north side below the limit investigated (M.L.W.S.).

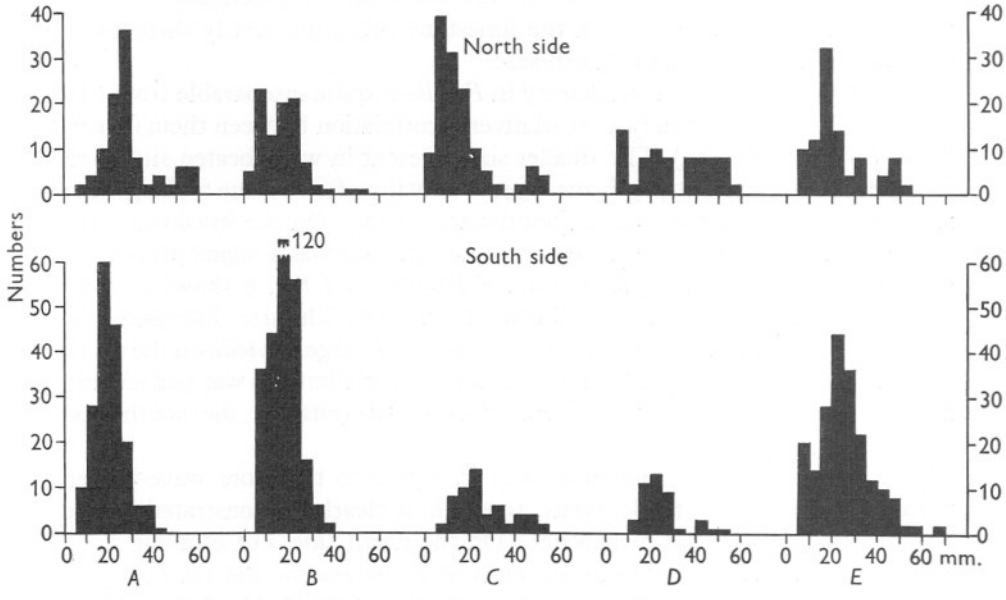

Fig. 6. Frequency distributions, in 5 mm. groups, of 1 m.^{2} samples of *Patella vulgata* taken at M.T.L. on each traverse on the breakwater.

G. umbilicalis was the commonest top-shell, but, except for one specimen in a pool at M.H.W.N. on the south side of traverse *D*, it was confined to the north side. It was absent from traverse *A*, sparse on *B*, but commoner on *D* and *E*, where it reached a density of 15/m.^{2}, and ranged from M.H.W.N. to M.L.W.N. In other places in the Plymouth area it has been found down to M.L.W.S. (Evans, 1947*b*).

Osilinus lineatus occurred only on the north side of traverse *E*, between M.H.W.S. and M.H.W.N.

Littorina neritoides. The distribution of this species on the breakwater has been described by Lysaght (1941). In the present work the observations were confined to the quantitative samples on the traverses, and the specimens which

occur in crevices and pools were ignored. Thus the numbers found in 1951 were generally less than those found by Lysaght.

When the crevice-living specimens were omitted it was found that the remaining specimens were associated with *Chthamalus*, occurring between the shells or inside dead ones. The distribution thus followed that of *Chthamalus* quite closely (Figs. 4, 7). However, there were a number of differences: the numbers were no higher on granite than on limestone, and the lower limit was approximately 0·5 m. higher than that of *Chthamalus*.

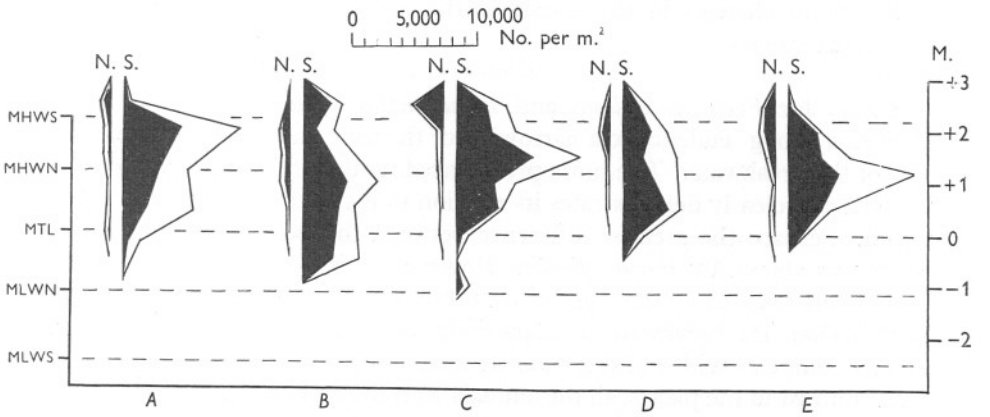

Fig. 7. The distribution of *Littorina neritoides* (■) and *L. saxatilis* (□) on the breakwater.

While *Littorina neritoides* was the dominant littorinid on both sides of the breakwater, the numbers present on the south side were at least three times as great as those on the north side; the maxima found were 1000/m.² at E.H.W.S. on the north side of traverse *C*, and 3000/m.² at M.H.W.N. on the south side of traverse *C*.

L. saxatilis was commonest on the south side of the breakwater, where it constituted half of the littorinid population between M.H.W.S. and M.H.W.N., and reached a density of 3000/m.² on traverse *E*. On the north side the numbers did not reach 500/m.², but the lower limit was some 0·5 m. lower than that of *L. neritoides* (Fig. 7).

It is interesting that *L. neritoides* should be the commoner littorinid on both sides of the breakwater since it is usually found in exposed places only (cf. Evans, 1947*b*). It is possible that there is some factor inimical to *L. saxatilis* on the north side.

Discussion and Conclusions

With the exception of *Lichina*, found only on the south face, and *Osilinus*, found only on the north face, all the species investigated were present on both sides of the breakwater. The factors influencing the distribution of *Lichina* are

somewhat obscure, but Evans (1947*b*) has shown some correlation with wave-action; the breakwater evidence, such as it is, would appear to confirm this. *Osilinus* and the other top-shells were scarce even on the north side of the breakwater. They have little power of attachment, and appear very intolerant of wave-action on steep faces or smooth slopes, where they are in great danger of being swept away. They reach their greatest abundance on flat, broken shores, where they may exist under moderately wave-beaten conditions.

The remaining differences between the two sides of the breakwater were restricted to changes in the extent of the zones, or in the abundance of individual species.

Zonation

Except for *Fucus vesiculosus* and *Himanthalia lorea*, most of the plants showed a strong tendency to a narrowing of the zone on the southern exposed side of the breakwater. The more pronounced nature of this narrowing at the western end clearly demonstrates its relation to increasing exposure to wave-action. Perhaps the greatest difference between the two sides of the breakwater was shown by *Fucus spiralis*. However, it is possible that the much lower limit reached by this species on the north side reflects the absence of *Ascophyllum* (the breakwater is apparently too steep for this species) which usually competes with *Fucus spiralis* in sheltered places.

In contrast to the plants, all the animals with the exception of the top-shells occupied a wider zone on the south side than on the north side. At the same time the zone was wider at the west end of the south face than at the east end. Obviously, increasing wave-action causes an extension of the upper and lower limits of these species, but the relationship does not appear to be a linear one. Thus, the wave-action on the south face has up to 10 times the amplitude of that on the north face, while along the south side, the amplitude is some 10–40% greater at the western end. Yet the zones may undergo a greater extension between the east and west ends of the south side than between the north and south sides of any traverse. For example, the zone of *Chthamalus* increases by 20% between the north and south sides of the breakwater, but increases by the same percentage between the east and west ends of the south side. Similarly, with the littorinids and *Patella vulgata*, the increase between the east and west ends of the south side is at least equal to, if not greater than, that between the north and south sides. While it is possible that other factors are involved in the case of *Patella*, we have observed elsewhere that the increase in the wave-action may be out of all proportion to the resulting change in levels. For example, in the Plymouth area, the normal upper limit of *Chthamalus*, excluding the upper parts of the estuaries, is approximately M.H.W.S., yet even in the most exposed places, where wave-action may have increased more than tenfold, it is rare for the species to extend more than a metre above this level.

A more direct relationship to increasing wave-action is shown by the upper

limit of *Patella aspera* (Southward, 1953) and possibly of *P. intermedia*, but this could not be demonstrated on the breakwater since too few specimens were found on the north side.

Abundance

The effect of wave-action on algae is comparatively well known (Stephenson, 1939; Evans, 1947*b*), and the breakwater evidence confirms previous investigations. Not only was the plant cover higher on the north side, but the maximum fucoid population occurred at its eastern end, the area with the least exposure to wave-action. In contrast, the lowest cover of all the species of plants investigated was found at the western end of the south side, the area of maximum wave-action.

The relationship of the numbers of the animals to wave-action is not quite so clear. The barnacles were most numerous towards the western end of the south face, but with *Balanus perforatus* and *B. crenatus*, which occur also in sheltered situations, this was probably an effect of the sparser plant cover. The numbers of *Chthamalus*, however, were almost directly related to the extent of the wave-action, and even the few specimens of *Balanus balanoides* present on the breakwater were commonest in the region of maximum wave-action. The present work offers no reason why the barnacles should increase in number with increasing wave-action, but from previous work it seems to be due to a greater initial settlement of larvae (Hatton & Fischer-Piette, 1932). It has been suggested that the increased water movement brings more food to the animals (Moore, 1935) and this may help to maintain the initial density (Southward, 1953).

The abundance of *Patella vulgata* is affected by the nature and amount of the plant cover (Fischer-Piette, 1948; Jones, 1948; Southward, 1953), which can provide both shelter and food, and large populations may exist both on comparatively bare but wave-beaten places and in comparatively sheltered but weed-grown places. In both habitats there appears to be a higher initial settlement of spat (Hatton, 1938; Southward, 1953), but the rate of growth is apparently greater in the algal-covered areas, hence the higher proportion of larger sizes in such places (Fischer-Piette, 1941, 1948).

The numbers of many intertidal organisms are affected by the nature of the substratum. Thus, on the breakwater, *Chthamalus stellatus*, *Patella vulgata* and *P. intermedia* all showed greater numbers on the rough granite than on the smoother limestone. It is possible that this is partly an effect on the settlement, since barnacles are well known to settle more densely on rough surfaces. It is also possible, with the limpets, that the rougher surface (of both the granite and the large barnacle population thereon) may restrict locomotion (Jones, 1948), thus reducing the feeding radius, and permitting the maintenance of a large population.

In conclusion, we can, on the evidence from the breakwater, divide up the

organisms dealt with into three groups according to their relations to wave-action. First, there are those species to which wave-action is unfavourable, and which show it by a narrowing of their zone (either a fall in the upper limit, or a rise in the lower limit, or both) and a reduction in their numbers. These are, in approximate order of decreasing sensitivity: *Osilinus lineatus*, *Gibbula umbilicalis*, *Pelvetia canaliculata*, *Fucus spiralis*, *Laminaria saccharina*, *Fucus serratus*, *Laminaria digitata* and *Fucus vesiculosus*. Then there are those species to which wave-action appears favourable, since under its influence their zone is widened and their numbers increased: these are: *Porphyra umbilicalis*, *Lichina pygmaea*, *Chthamalus stellatus*, *Patella intermedia*, *P. aspera*, *Littorina neritoides* and *L. saxatilis*. Finally, there are some species for which the breakwater evidence is inconclusive: *Himanthalia lorea*, *Rhodymenia palmata*, *Balanus perforatus*, *B. crenatus* and *Patella vulgata*.

Summary

This paper describes an investigation into the effects of wave-action on the distribution, zonation and numbers of the commoner intertidal plants and animals living on the Plymouth breakwater.

Comparison of observations of wave-action on the breakwater with local wind records produces an exposure index for the year of approximately 35% which, although similar to a previously calculated index, is much below similarly derived indices for places in the Isle of Man. It would appear that swells and refracted waves are more important at Plymouth.

The wave-action observations showed great differences between the two sides of the breakwater, the height reached by the wash on the south side being more than 10 times as great as that on the north side. At the same time, the wash on the south side was found to be some 10–40% greater at the western end than at the eastern end.

The distribution and abundance of the commoner plants and animals is described and discussed in relation to tide level and wave-action.

Most of the plants, with the exception of *Lichina*, showed their greatest abundance and widest zone on the north face, particularly at its eastern end, which is probably the most sheltered point on the breakwater. *Himanthalia lorea* and *Rhodymenia palmata*, however, showed little differences between the two sides of the breakwater.

The majority of the animals, most notably *Chthamalus stellatus* and *Patella intermedia*, were favoured by wave-action, the numbers being greater and the zone wider on the south side, and especially at its western end, the most wave-beaten part of the whole breakwater. The top-shells, however, were commonest on the north side. The evidence for *Patella vulgata* was inconclusive, but tended to confirm previous evidence that this species is abundant in both weed-grown and wave-beaten places, but that the zone is wider and the shell smaller in the latter situations.

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NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

I. THE PRESENCE OF *DIDEMNUM* (*LEPTOCLINIDES*) *FAERÖENSE* (BJERKAN) IN THE PLYMOUTH AREA

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The Plymouth Laboratory

(Text-figs. 1, 2)

Leptoclinides faeröensis Bjerkan (1905) is a little-known boreal species which has been found only in the Atlantic Ocean (Fig. 1). The most northerly record is from a little south of Spitzbergen, the most southerly from 37° 08′ N., off the North American coast. It occurs on the coast of Norway and in the Faeroe Islands, but it has not been reported nearer to Plymouth than these two localities. Most records are from deepish waters, though it occurs in the sublittoral zone along the Norwegian coast. It was in this zone, at Looe Island (50° 20′ 24″ N., 4° 26′ 53″ W.) near Plymouth, that we found a specimen of this species growing on a rock about 80 cm. below O.D.—just sufficiently low for it to remain covered by a few centimetres of water at the lowest tide of the year, the equinoctial spring tide.

The colony was 13 × 8 mm. across and 3 mm. thick. It was attached by the whole of its lower surface. The position of each zooid was marked by an area of sparse spicules showing darker against the white opacity of the spicule-packed common test. The spicules were concentrated in the upper layers of the colony. The deeper test was yellowish and of a firm consistency, almost free of spicules. The spicules were remarkably even in size, much more so than in most colonies of didemnids; they were 40–50 μ in diameter with a few long conical points; the formula was usually 1, 6, 9 or 3, 6, 9. They are illustrated by van Name (1945, p. 96, fig. 43). There was a single common cloacal cavity which was at the level of the abdomina of the zooids. The thoraces were completely embedded in the test while the abdomina had each a thin sheath of test-substance. Correspondingly, the atrial siphon of each zooid was prolonged backwards into a long funnel to reach the common cloacal cavity. This is the diagnostic generic character. The zooids were arranged vertically to the surface of the colony and each had six lobes to the branchial siphon. There were thirty-two oral tentacles of four orders of size arranged 1, 4, 3, 4, 2, 4, 3, 4, 1. The zooids varied from 1·1 to 2·1 mm. in length. The smallest had only three rows of stigmata, the majority four rows and the few largest had an incipient fifth row as shown in Fig. 2. The number of stigmata in each half-row was 9–12; in the fifth row, where present, this

number was only 3–5. There were usually three dorsal languets; a fourth was occasionally present if there was a fifth row of stigmata, though not always even then. The lateral thoracic organ was small, a little less than the height of a single row of stigmata, and lay between the third and fourth rows.

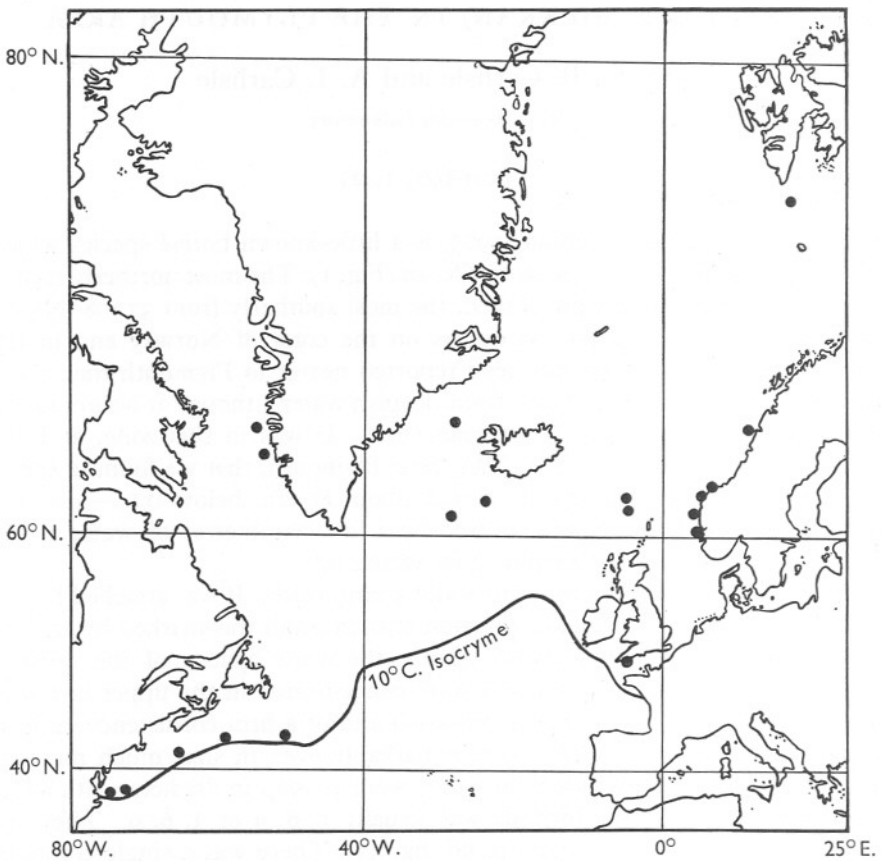

Fig. 1. The recorded distribution of *D.* (*Leptoclinides*) *faeröense*, showing agreement between the most southerly records of the species and the mid-winter sea temperature of 10° C.

The abdomen was little, if at all, larger than the thorax. The neck was sharply constricted. The oesophagus was straight and opened into a globular or squarish stomach. The post-stomach was horizontal and formed the bottom of the gut loop. The mid-intestine was short and sharply delimited from the post-stomach and from the rectum by grooves. The rectum was curved into an abrupt S; the final bend lay to the left of the stomach. The ovary lay entirely within the intestinal loop, the testis to the left side of the loop extending rather behind the mid-intestine. It was undivided. The sperm duct made four

or five turns around it before running to the atrium. The atrial siphon was very long and possessed a strong sphincter muscle.

The colony was collected on 18 March 1953, and was not then breeding. The larva is unknown.

The species does not appear to have any synonyms, other than misspellings of the trivial name, and is clearly distinguishable from any other species. For references to the literature see van Name (1945).

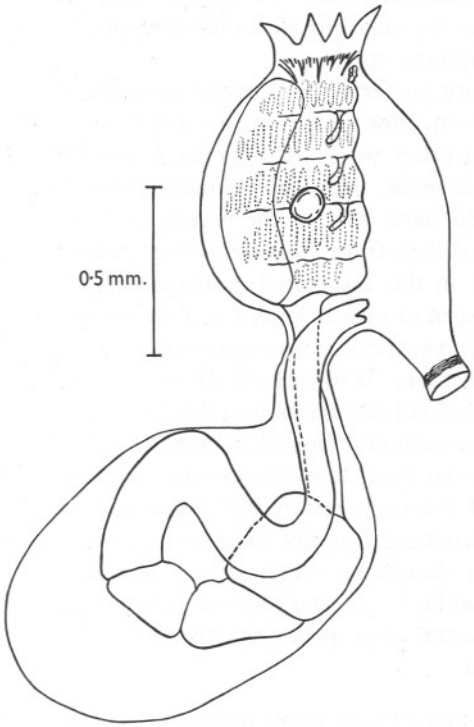

Fig. 2. Drawing from the left side of a large zooid of *D. faeröense*, omitting the reproductive system. Note especially the incipient fifth row of stigmata only present in the largest zooids.

Six species of the genus *Leptoclinides* are known, the north Atlantic *L. faeröensis*, the south Atlantic *L. brasiliensis*, known only from Michaelsen's (1923) description of his type specimen, *L. glauerti* Michaelsen (1930), likewise known only from the type specimen, *L. dubius* (Sluiter), *L. ocellatus* (Sluiter), and *L. madara* Tokioka. The last three of these species are not significantly different, except in the condition of the atrial system, from the subgenus *Polysyncraton* of *Didemnum*. Sluiter's species were indeed described under this genus, but they were separated from it by Michaelsen (1930) and Tokioka (1953). *Leptoclinides madara*, according to its author,

may be merely a colour variant of *L. ocellatus*. *L. brasiliensis* is rather similar to *L. faeröensis*, but has an unlobed branchial siphon, and the common cloacal cavity is completely below the level of the zooids so that the atrial siphon is even more prolonged. Michaelsen suggests that the common cloacal cavity may open on the lower side of the colony, but this cannot happen in *L. faeröensis* because the whole lower surface is attached to the substratum. In *L. glauerti* the atrial siphons open direct on to the lower surface of the colony and the common cloacal cavity system is abolished altogether, or represented at most by small local depressions on the lower side into which individual atrial siphons open.

If, then, the more posterior level of the common cloacal system is worthy of generic distinction, how much more should be the complete abolition of the system. If, in other words, the genus *Leptoclinides* has any validity as separate from *Didemnum*, then *Leptoclinides glauerti* should be in a further separate genus. But how valid is the genus *Leptoclinides*? *L. faeröensis*, the type of the genus, differs from *Didemnum* only in the position of the common cloacal cavity and in the associated prolongation of the atrial siphon. The same condition is seen to a lesser extent in *Trididemnum tenerum* and *T. niveum* (see Carlisle, 1953), yet there is no suggestion that the condition is worthy of generic distinction there. Within both *Didemnum* and *Trididemnum* there is great specific and individual variation in the degree of development, the extent and the level of the common cloacal system, and it is for this reason that the genus *Leptoclinum* has been abandoned—the character on which it is based is too variable. Now it is on precisely this character that the genus *Leptoclinides* is based. The distinction does not hold so the genus collapses. *L. faeröensis* and *L. brasiliensis* should be received into the genus *Didemnum*, retaining subgeneric rank, while *L. glauerti* requires a new genus for its reception. The name *Sinecloaca* seems appropriate as expressing the lack of common cloacal cavity in this form.

Sinecloaca n.gen.: an ascidian genus presenting typical didemnid characters possessing usually four rows of stigmata; sperm duct coiled around testis; differing from *Didemnum* Savigny in the reduction, apparently secondary, of the common cloacal cavity system to a few local depressions on the lower surface of the colony into which individual atrial siphons open direct to the sea, and in the corresponding elongation of the atrial siphons to open in this manner on the side of the colony opposite to the branchial siphons.

Type species: *Sinecloaca glauerti* (Michaelsen) (= *Leptoclinides glauerti* Michaelsen, 1930).

The remaining species of the abandoned genus *Leptoclinides* Bjerkan become *Didemnum* (*Leptoclinides*) *faeröense* (Bjerkan) and *D.* (*Leptoclinides*) *brasiliense* (Michaelsen), while *L. dubius*, *L. ocellatus*, and *L. madara* go back to *Polysyncraton* where they belong—*Didemnum* (*Polysyncraton*) *dubius* (Sluiter), *D.* (*P.*) *ocellatus* (Sluiter), and *D.* (*P.*) *madara* (Tokioka) or *D.* (*P.*) *ocellatus* (Sluiter) forma *madara* (Tokioka).

SUMMARY

Didemnum (*Leptoclinides*) *faeröense* Bjerkan is present in Plymouth waters. The adult is briefly described. The genus *Leptoclinides* Bjerkan is not valid and two of its six species should be included in *Didemnum* as a separate subgenus, a third species, *L. glauerti*, must remain separate in a genus of its own for which the name *Sinecloaca* n.gen. is proposed, while the remaining three species are returned to the subgenus *Polysyncraton*.

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J. Mar. biol. Ass. U.K. (1954) 33, 27–32
Printed in Great Britain

NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

II. THE NUMBER OF ROWS OF STIGMATA IN *DIDEMNUM GELATINOSUM* MILNE EDWARDS AND IN *DIDEMNUM MACULOSUM* (MILNE EDWARDS)

By D. B. Carlisle
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Milne Edwards (1841) established the genus *Leptoclinum* for those Didemnidae which possessed a largely expanded common cloacal system. His type of the genus was *L. maculosum* which is now placed in the genus *Didemnum* Savigny (1816). Other species were *L. asperum* and *L. durum* which are now regarded as mere forms of *Didemnum maculosum* (e.g. Harant & Vernières, 1933); *Leptoclinum fulgens* which is closely related (see Harant & Vernières), if not a mere colour form of *Didemnum maculosum*; *Leptoclinum listerianum* which has been transferred to the genus *Diplosoma* Macdonald; and *Leptoclinum gelatinosum*. This last has often been considered synonymous with *Diplosoma listerianum*, but my reasons for disagreeing with this assumption are elsewhere expressed (Carlisle, 1953). Milne Edwards did not of course describe the course of the sperm duct by which *Diplosoma* is distinguished from *Didemnum*. In the absence of a description of this diagnostic character we are reduced to a consideration of numerous details of the anatomy of *Leptoclinum gelatinosum* in order to decide where to place it in the classificatory scheme. When such a point-by-point comparison of shape and curvature of the gut, position of anus, form of atrial and buccal apertures, nature of test, etc., is made, it appears that the one point in which the drawings and descriptions given by Milne Edwards differ between *L. gelatinosum* and *Didemnum gelatinosum* Milne Edwards (1841) is the presence of a common cloacal system in the former and its absence in the latter. But the presence or absence of a common cloacal system is no longer regarded as a generic distinction (see, for example, van Name, 1945). Berrill (1950), on the basis of one specimen, and presumably with Milne Edwards's authority, states that *D. gelatinosum* has no common cloacal system. I am unable to agree with this statement. Large colonies of *D. gelatinosum* collected and identified by myself, and other large colonies collected and determined by Miss P. Kott (and in the type collection of this laboratory), frequently contain a large common cloacal system stuffed with faecal pellets, whereas small colonies are without such cavities. It seems to me then, that we have no clear grounds for separating *Leptoclinum gelatinosum* Milne Edwards from *Didemnum gelatinosum* Milne Edwards (1841) and, so far as it

is possible to recognize the species from his descriptions, these are most probably synonymous, particularly as his drawings and descriptions of *D. gelatinosum* are based on a small specimen with only a small number of zooids, while his description and drawings of *Leptoclinum gelatinosum* are evidently based on a much larger colony. The identification of the one with the other can of course never be certain. Certainly, however, the description of *L. gelatinosum* is unlike that of any other species of didemnid found in the English Channel.

One thing stands out as strange in Milne Edwards's drawings and descriptions—he states that in *L. gelatinosum*, 'Le sac branchial est garni de cinq rangées de fentes stigmatiformes' (1841, p. 300), and he illustrates it so. Similarly, he states of *Didemnum gelatinosum*, 'Le thorax est gros et n'offre que cinq rangées transversales de stigmates branchiaux' (p. 296). He figures it with sometimes three, sometimes four, and sometimes five rows of stigmata (his plate 7, figs. 5*a–e*), so that his 'n'offre que cinq rangées' must mean 'has no more than five rows,' rather than 'has only five rows'. How is all this to be squared with the usual statement that the Didemnidae possess either three or four rows of stigmata—e.g. van Name's statement (1945, p. 78) 'There are (apparently always) either three or four rows of stigmata'—or with the diagnostic difference usually offered between *Didemnum* and *Tridemnum* that the former has four rows and the latter only three rows of stigmata? Clearly, since the species *Didemnum gelatinosum* is ascribed to Milne Edwards this matter requires a fresh investigation. If the species that we now call *D. gelatinosum* Milne Edwards always possesses four rows of stigmata then it cannot truly go by this name in view of Milne Edwards's deliberate statements. At the same time it would be as well to examine other species ascribed to Milne Edwards to determine the situation there.

Two species of *Didemnum* are to be found in the Plymouth area in abundance, *D. gelatinosum* and *D. maculosum* auctt. (?Milne Edwards). An examination of a large number of colonies of these two species has shown that the majority of zooids possess four rows of stigmata apiece. Before the breeding season commences, however, when the colonies are growing actively by vegetative means, the zooids around the edges of the colonies are much smaller. These zooids have only three rows of stigmata each, and usually one or two less than usual in each row. During the period of sexual reproduction the number of these smaller zooids is progressively less, and successive tracing of the outline of colonies at Salcombe (Devon) at monthly intervals has shown that during this season growth in area of the colonies is almost at a standstill. At any season of the year in colonies with more than about forty zooids one can usually find one or a few thoraces with five rows of stigmata in both species. Sometimes, when a colony, from the abundance of the faecal pellets and from the general appearance, seems particularly well fed, quite a high proportion of the zooids possess five rows of stigmata, even as high a proportion as 15%

when the animals are not in the breeding season. Towards the end of the breeding season, when the larvae are fully developed, the thoraces of the zooids are resorbed and only abdomina, larvae, and faecal pellets are to be seen in the colonies.

The zooids around the edges of the colony are always smaller than those nearer the centre. The difference of size is more pronounced in the thorax (which, in a zooid from the centre of the colony, may be $2\frac{1}{2}$ times as large [linear measure] as in one from the periphery) than in the abdomen (which is rarely more than $1\frac{1}{2}$ times as big). The difference in size of the abdomina is reflected in that of all the organs. In particular, the spermduct makes fewer turns around the testis in the smaller zooids, and the mature ovum is smaller. Correspondingly, the larva developed from a smaller ovum, derived from a smaller peripheral zooid, is smaller than one produced from a larger central zooid, and such a smaller larva is furnished with only three rows of stigmata instead of the usual number of four which is found in the larvae in the centre of the colony. Larvae from the centre of the colony of *D. gelatinosum* have five pairs of anterior ectodermal ampullae, while the smaller peripheral ones have only four pairs; in *D. maculosum*, which has a smaller larva, the central larvae have four pairs of ampullae, or rarely five, while those from the periphery have only three (see also Kott, 1952). Further differences are to be seen in the anatomy of the zooids from the centre and from the periphery of the colony. The course of the gut is less circuitous in the smaller peripheral zooids. Thus in *D. maculosum* the rectum in the larger central zooids makes two sharp bends to form a shallow S (see Milne Edwards, 1841; Millar, 1949); in the smaller zooids from the periphery the course of the rectum is almost straight. This may perhaps be correlated with the larger amount of food that the larger thoraces may supply to the central zooids, which requires a longer gut for its digestion. The mid-intestine is relatively rather larger in the smaller zooids, though absolutely slightly smaller.

Evidently Milne Edwards was correct in his statement that *D. gelatinosum*, 'n'offre que cinq rangées transversales de stigmates branchiaux' (1841, p. 296), and that in *Leptoclinum maculosum* the structure of the zooids 'est essentiellement la même' (p. 298). His descriptions and figures suffer simply from the general fault of those of his period—he always worked from the largest and best developed, never from the typical or average specimen. This is true not merely of his descriptions and figures of didemnids but also of other ascidian species. More recently the emphasis has been on examining average specimens; in determining the species of a colony of a didemnid it is usual practice to cut off and dissect a 'typical portion', neither too thick nor too thin, nor too near the edge. Such a portion would contain almost entirely zooids with the usual number of rows of stigmata for the colony—four in *Didemnum*. It is notoriously difficult to count the number of rows of

stigmata, especially in dead and preserved specimens, and indeed neither Michaelsen (1923) nor Hartmeyer (1924) was able to determine the number of rows in the species which the former named *D. helgolandicum*. Accordingly, it is usual to examine a number of zooids and discount as impossible to observe properly any which seem to show an unusual number of rows of gill-slits. Thus any zooids in a *Didemnum* colony with other than four rows of gill-slits would be unlikely to be taken for examination and if examined would most probably be discounted. It is only when one examines living zooids of *Didemnum* species that the number of rows of gill-slits is seen, with practice, clearly enough for one to be unable to mislead oneself with a belief that unusual zooids are actually ones which are difficult to observe correctly and not really unusual at all. It comes as rather a shock to see five rows of gill-slits in a living zooid of *D. maculosum*, with each slit clearly outlined by a ring of beating cilia, and to realize that preserved ones, previously discarded as poorly preserved and misleading, really did show five rows after all. It may be noted that Milne Edwards (1841, p. 219), unlike most of his successors, examined his specimens alive.

The account given by Salfi (1933, 1950) of budding in the Didemnidae gives some explanation of the way in which the number of rows of stigmata may increase with the age of the zooid. He describes budding in these forms as proceeding by the formation of half-buds. The oesophageal region of a zooid buds off separate thorax and abdomen, either together or at different times. Either of these half-buds can complete itself by the formation of the other half, or two halves formed simultaneously and near together may combine. But more thoracic buds are formed than abdominal buds and not all of these go to form fresh zooids. Some of them are used by the parent zooid to replace its own thorax which is worn out. The old thorax is resorbed and the newly budded one takes its place, or rather the other way round, for the new one begins to function before the old thorax is resorbed. This may apparently happen several times in the life of a single zooid. This I can confirm from my own observations. At a time when the colony of *D. maculosum* is growing most actively by vegetative means, most new zooids are formed from abdominal buds which then form their own thoraces. Such buds are small, chiefly around the edge of the colony and most have only three rows of stigmata. As they grow larger a new thoracic bud is formed, by each such zooid, which takes over the function of the old thorax which is now too small and worn out. This new thorax has the typical four rows of stigmata. This may happen several times as the zooid grows larger until when it is very large indeed a new thorax may be large enough to have five rows. The limiting factor for the number of rows of stigmata seems to be the size of the thorax at the time when it begins to take over its functions. Once it is functioning there seems to be no further development of fresh rows of stigmata, although there may occasionally be an increase in the number in one row. When the

breeding season starts most of the energy of the colony goes to the production of embryos. New budding and growth of the colony stops and vegetative growth seems confined to necessary repair work: zooids may replace their thoraces but not form entirely new buds. This may account for the decrease in number of zooids with three rows of stigmata during the breeding season. Small zooids naturally produce small eggs and the larger, older zooids produce larger eggs. Again the size of the thorax of the larva seems to determine how many rows of stigmata it shall have—as many rows develop as there is room for. Small larvae have only three rows, larger ones four. It is probable that in the past larvae with only three rows of stigmata from *D. maculosum* have been interpreted as not fully developed, but this is not so, for free-swimming, small larvae may possess only three rows up to metamorphosis, beyond which I have not observed them further. Such differences in the structure of larger and smaller larvae may be compared with similar differences which I have observed in such oviparous species as *Phallusia mammillata* (Cuvier), between larvae obtained by artificial fertilization of eggs from the oviduct and of eggs from the ovary, which are always slightly smaller and produce larvae with a simplified sensory system and other simplifications and reductions in their anatomy.

The situation with respect to the number of gill-slits is similar in *Didemnum* (*Leptoclinides*) *faeröense* (Bjerkan) as described in part I of these notes (Carlisle & Carlisle, 1954). In *Diplosoma* spp., however, I have never seen other than four rows of stigmata in any zooid.

Summary

The number of rows of stigmata in three species of *Didemnum* varies from zooid to zooid within the colony. Peripheral, newly formed, small zooids have only three rows, most zooids have four rows, while a few very large ones have five rows. This is probably a result of the replacement of the thorax by partial budding during the life of the zooid, accompanied by a steady increase in size. The number of rows of stigmata seems to be always four in *Diplosoma* species. The importance of examining living specimens is stressed.

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J. Mar. biol. Ass. U.K. (1954) 33, 33–48
Printed in Great Britain

THE ANNUAL GROWTH AND REPRODUCTIVE CYCLE OF THE ASCIDIAN *DENDRODOA GROSSULARIA* (VAN BENEDEN)

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(Text-figs. 1–11)

This paper is the second of a series dealing with the annual cycle of growth and reproduction in British ascidians; a previous paper (Millar, 1952) dealt with the species *Diplosoma listerianum* (Milne Edwards), *Ciona intestinalis* (Linnaeus), *Ascidiella aspersa* (Müller), and *Botryllus schlosseri* (Pallas).

The present investigation is concerned with the growth, the course of sexual reproduction and establishment of new generations, and the histological changes in the gonad throughout the year, in the ascidian *Dendrodoa grossularia* (family Styelidae).

Samples were examined from two widely separated localities: Fambridge in the River Crouch, Essex; and Farland Point, Isle of Cumbrae, in the Firth of Clyde. The specimens from Essex were dredged from a depth of about 2 m. (below L.W.O.S.T.), and were attached to the empty shells of the European oyster, *Ostrea edulis* L. and to the shells of *Crepidula fornicata* L. The specimens from the Firth of Clyde were attached to stones on sheltered parts of the shore. The animals here extended over a zone of the shore from about Chart Datum + 2·5 ft. (0·76 m.) to about Chart Datum + 7·5 ft. (2·29 m.). Only in very sheltered positions, such as under large boulders, did *Dendrodoa* occur in abundance. The two habitats were thus very different in nature, one being sublittoral on the south-east coast of Britain and the other being littoral on the north-west coast. The value of comparisons is limited by the different nature of the conditions to which the sublittoral and the littoral populations were exposed.

The observations on growth in the Clyde extended over most of 1951 and 1952, and nineteen samples were taken. The ten samples in 1952 were used for examination of breeding condition, and of histological state of the gonad. Eleven samples from Essex covered the period December 1951–December 1952, and supplied data on growth, breeding condition, and histological state of the gonad.

I wish to thank Mr M. N. Mistakidis and Mr G. Duncan Waugh, of the Ministry of Agriculture and Fisheries, for collecting and sending the samples from Essex, and for providing temperature records for that area.

Growth

About 200 animals in each sample were measured, to the nearest 0·1 mm., the antero-posterior length of the body being taken as a measure of the size. Care was taken to measure the length of the body itself, excluding the basal expansion of test which is very variable in extent. The length of the body is not an entirely satisfactory measure of the size since the ratio of length to height varies to some extent with the habitat, but length was found to be the best practical measure available.

The Essex population

Histograms of the distribution by size of *D. grossularia* in the samples from Essex during 1952 are shown in Fig. 1. The samples of 15 December 1951 and 15 January 1952 represent the winter state of the population. There are two clearly separable components: (i) individuals up to about 1·5 mm. long, and (ii) the remaining individuals from about 1·5 mm. to about 8·5 mm. in length.

There is no clear indication of spring growth until after 25 March 1952, although it may have started somewhat earlier than this. Considerable growth occurred in the animals of the 0–1·5 mm. group, between late March and early May, and continued throughout June and July so that they had attained a length of 4·0–5·5 mm. by 22 July 1952. No significant change is seen in the form of the histograms, for sizes over 4·0 mm., in the period up to 22 July 1952. This is due to the growth of small animals up into the size groups over 4·0 mm. and, we may suppose, little growth in the large animals together with death of the oldest ones.

During May and to a less extent in April and June the spring settlement of 1952 took place. This period of settlement is confirmed by the presence of larvae in breeding individuals (see pp. 38–40). Little or no settlement took place in August.

The autumn settlement occurred mainly in September, October and November, and these animals attained a length of up to 1·5 mm. before growth stopped for the winter. The histogram of 5 December 1952 again shows the winter structure of the population, with two components: (i) individuals under 1·5 mm. representing the autumn 1952 settlement, and (ii) individuals from about 1·5 mm. up to the maximum size, representing the spring 1952 settlement together with an unknown proportion of the 1951 settlements.

During a considerable part of the year, from the time of establishment of the autumn generation through the winter and on into the spring there are present at least three groups of individuals of separate origin. Thus in the autumn of the year n these groups represent (i) the autumn settlement of the year n, (ii) the spring settlement of the year n, and (iii) the autumn settlement of the year $n-1$, together with some unknown proportion of the settlements of spring $n-1$, and of the year $n-2$.

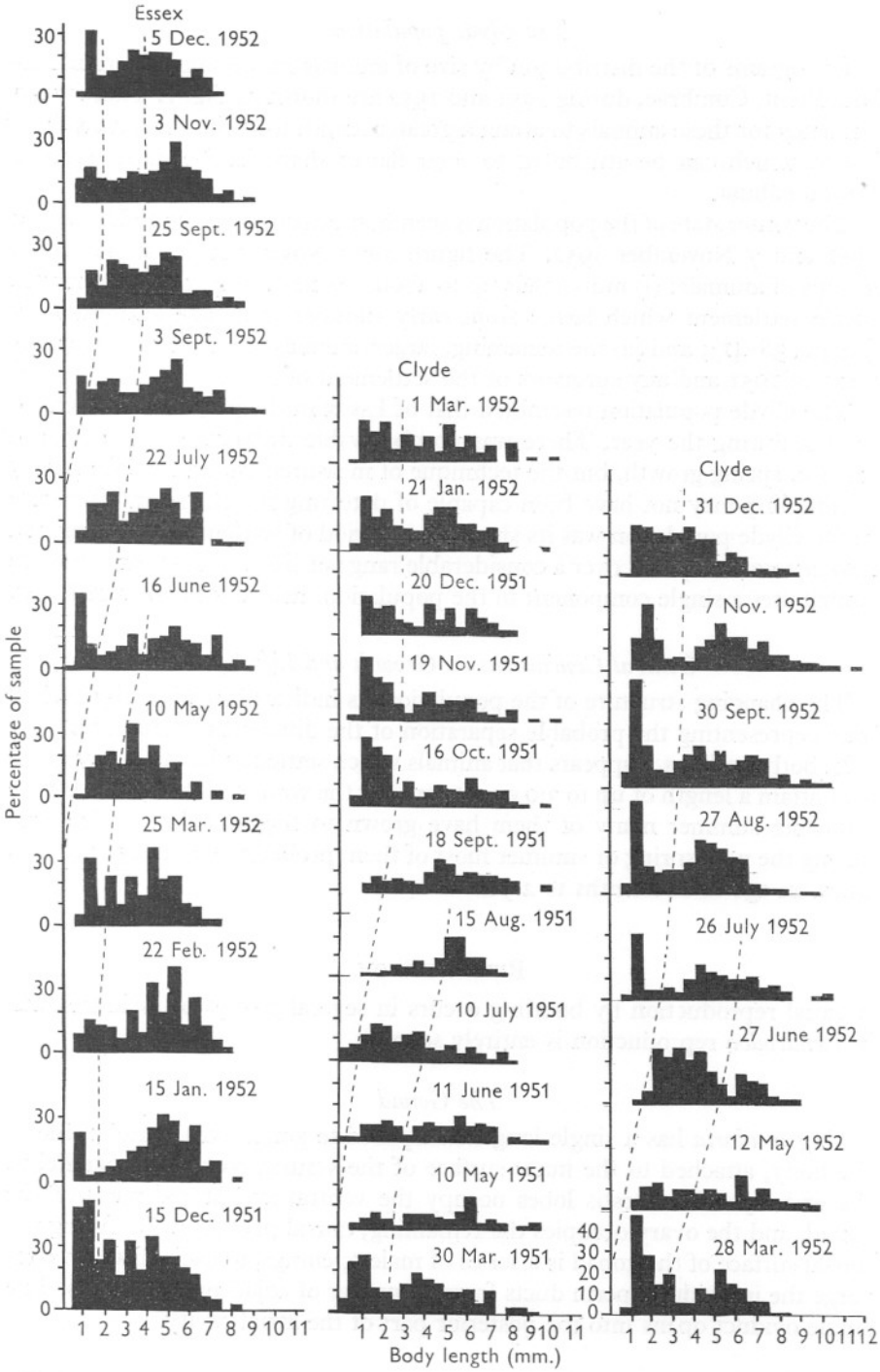

Fig. 1. Size-frequency histograms of *D. grossularia* samples from Essex and the Clyde.

The Clyde population

Histograms of the distribution by size of animals in the samples from Farland Point, Cumbrae, during 1951 and 1952 are shown in Fig. 1. There was a tendency for these animals to attain a greater length than the specimens from Essex, which can be attributed to their flatter shape in the more exposed littoral habitat.

The winter state of the population is seen in the histograms for 20 December 1951 and 7 November 1952. The figure for 7 November 1952 shows two groups of animals: (i) individuals up to about 3·5 mm. long, representing the single settlement which lasted from early summer until late autumn 1952 (see pp. 38–41); and (ii) the remaining, larger, animals representing the settlement of 1951 and any survivors of the settlement of 1950.

The Clyde population resembled that of Essex in the period and extent of growth during the year. There was no observable difference in the time of onset of spring growth, but the technique of measurement and the frequency of sampling may not have been capable of detecting it. The main difference in the Clyde population was its single long period of settlement resulting in a new generation spread over a considerable range of sizes. Each year therefore contributes a single component to the population, instead of two as in Essex.

General Conclusions on Growth and Life Span

The changing structure of the populations is indicated in Fig. 1 by broken lines representing the probable separation of the different components.

In both localities it appears that animals which settled as larvae in any given year attain a length of up to 3·0 or 3·5 mm. by the winter of that year. By the following summer many of them have grown to their maximum size, and during the next spring or summer most of them probably die. They may thus attain an age of 18 months to 2 years.

Reproduction

Asexual reproduction by budding occurs in several groups of ascidians, but in *Dendrodoa* reproduction is entirely sexual.

The Gonad

D. grossularia has a single long hermaphrodite gonad on the right side of the body, attached to the inner surface of the ventral body wall, parallel to the endostyle. The testis lobes occupy the ventral and lateral parts of the gonad, and the ovary occupies the remaining, dorsal part (Fig. 6). Along the dorsal surface of the gonad is a series of male openings, to each of which converge the individual sperm ducts from a number of adjacent testis lobes. The single oviduct opens into the posterior part of the atrial cavity.

Brood Filaments and the Retention of Developing Eggs

When the eggs are extruded from the oviduct they are not carried away by the exhalent stream of water out through the atrial siphon. Instead they are retained within the atrial cavity by a group of specially developed filaments which do not seem to have been described and which may be called the brood filaments. These filaments, which are numerous and closely spaced, stretch across the atrial cavity from the outer surface of the posterior part of the branchial sac to the inner surface of the body wall opposite (Fig. 2). They are

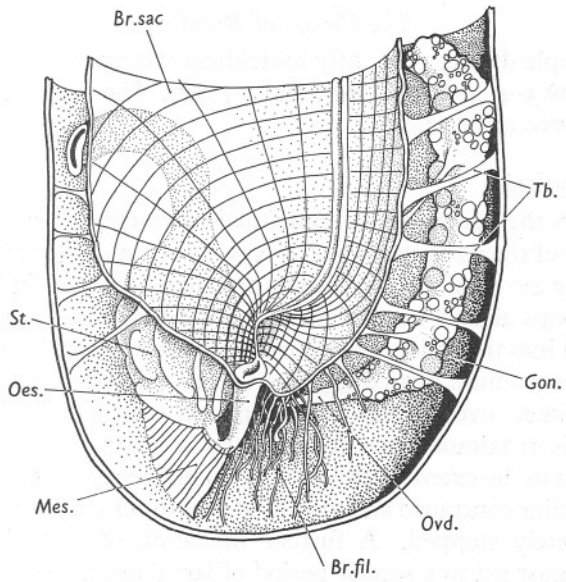

Fig. 2. Ventral half of the posterior part of the body of *D. grossularia*. *Br. sac*, branchial sac; *Br.fil.*, brood filaments; *Gon.*, gonad; *Mes.*, mesentery; *Oes.*, oesophagus; *Ovd.*, oviduct; *St.*, stomach; *Tb.*, trabeculae.

probably homologous with the trabeculae which pass from the branchial sac to the body wall in other parts of the body, and which maintain the branchial sac in position. Since the opening of the oviduct lies immediately ventral to this mass of brood filaments, the eggs as they are shed become entangled amongst the filaments. Here they are fertilized and remain during subsequent development to the larval stage. The larvae, however, when fully developed tend to become free of the brood filaments and to spread into the adjacent part of the atrial cavity, on the right. Eggs and larvae are prevented from passing into the left part of the atrial cavity by a vertical mesentery uniting the body wall to the oesophagus, stomach and posterior end of the branchial sac. The liberation of the larvae from the entangling brood filaments may be due both to muscular movements of the larvae and to the pressure of increasing numbers of eggs shed from the oviduct. When thus freed from the

brood filaments the larvae can be carried by the exhalent current to the exterior. The mechanism ensures that it is the most advanced larvae, that is those developed from eggs first shed from the ovary, that are freed from the brood filaments to pass to the exterior, while the younger developing eggs are still retained.

The brood filaments are often found to be attached only at one end, usually to the outer wall of the branchial sac, a condition resulting from their being broken by the pressure of large numbers of eggs.

The Course of Breeding

In each sample during 1952, fifty individuals were dissected, ten in each of the size groups 3–4, 4–5, 5–6, 6–7, and 7+mm. The eggs and larvae in the atrial cavity were counted.

The Essex Population

Fig. 3 shows the percentage of individuals with eggs or larvae in the atrial cavity, in each of the size groups 3–4, 4–5, 5–6, 6–7 and 7+mm., during 1952. Animals under 4·0 mm. in length took practically no part in breeding. The remaining groups all had a rather similar pattern of breeding activity. Eggs were first shed into the atrial cavity during March when, although 30% of the specimens over 7·0 mm. carried eggs, no larvae were yet present. On 10 May 1952 all specimens over 6 mm., and many between 4·0 and 6·0 mm., were breeding. This maximum was followed by a sharp decline, many larvae having escaped to the exterior while few or no further eggs were shed from the ovary. The decline continued and produced a minimum in July, when breeding almost completely stopped. A further liberation of eggs from the ovary starting in August led to a second period of larval development from August to November. This was followed by a decline to the winter non-breeding state of the population.

The Clyde Population

Fig. 4 shows the course of breeding at Farland Point, Cumbrae, during 1952. As in Essex, individuals under 4·0 mm. in size took very little part in breeding, and amongst the Clyde population only a small percentage of the 4–5 mm. group bred. Animals from 5·0 to 7·0 mm. started to breed in April or early May, had one maximum of breeding activity in June, a second in September or October, and then returned to the winter non-breeding state. In the 7+mm. group, however, there was no reduction in the percentage of animals breeding in July and August, such as was observed in smaller animals.

Intensity of Breeding

The mean number of eggs and larvae per breeding individual may be taken as a measure of the intensity of breeding, and Figs. 3 and 4 also show the

variation in these numbers during 1952. The rise and fall in breeding intensity coincided with the fluctuations in the percentage of animals breeding, closely in Essex, and approximately in the Clyde.

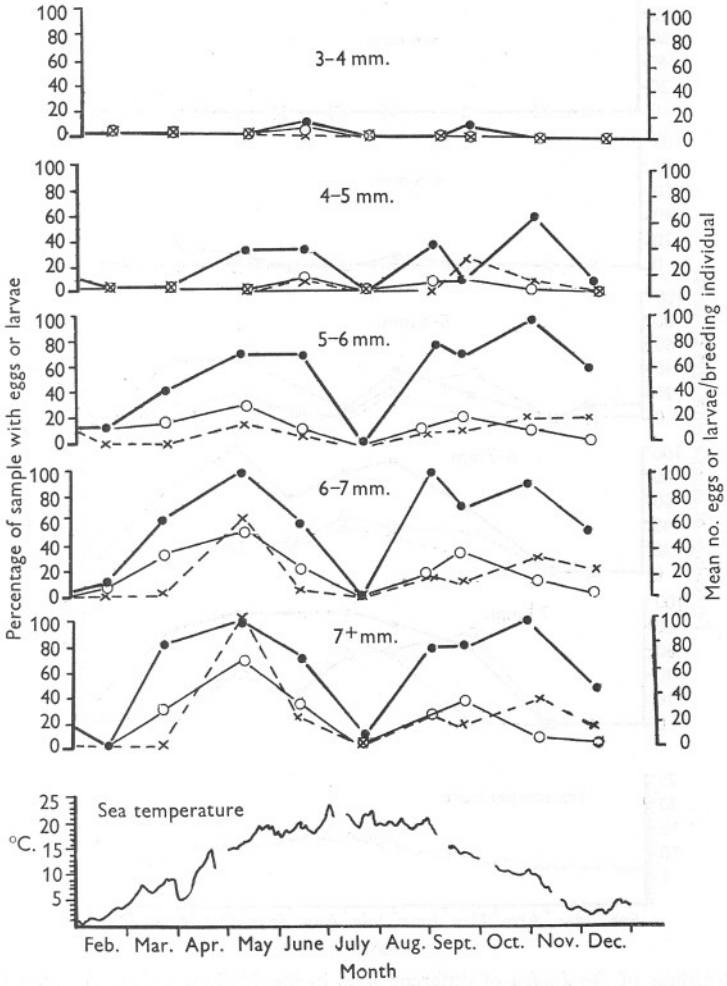

Fig. 3. Breeding of *Dendrodoa* of different sizes in the Essex samples. ●—●, percentage with eggs or larvae; ○—○, mean number of eggs per breeding individual; ×—×, mean number of larvae per breeding individual.

The Essex Population

In all animals over about 5·0 mm. long the intensity of breeding in May exceeded that in autumn. The relation between the length of the body and the number of eggs and larvae found in the atrial cavity is shown in Fig. 5. In

general, breeding started at a body length of between 4·0 and 5·0 mm. and increased in intensity with increasing body length. The greatest number of eggs plus larvae found in any individual was 266.

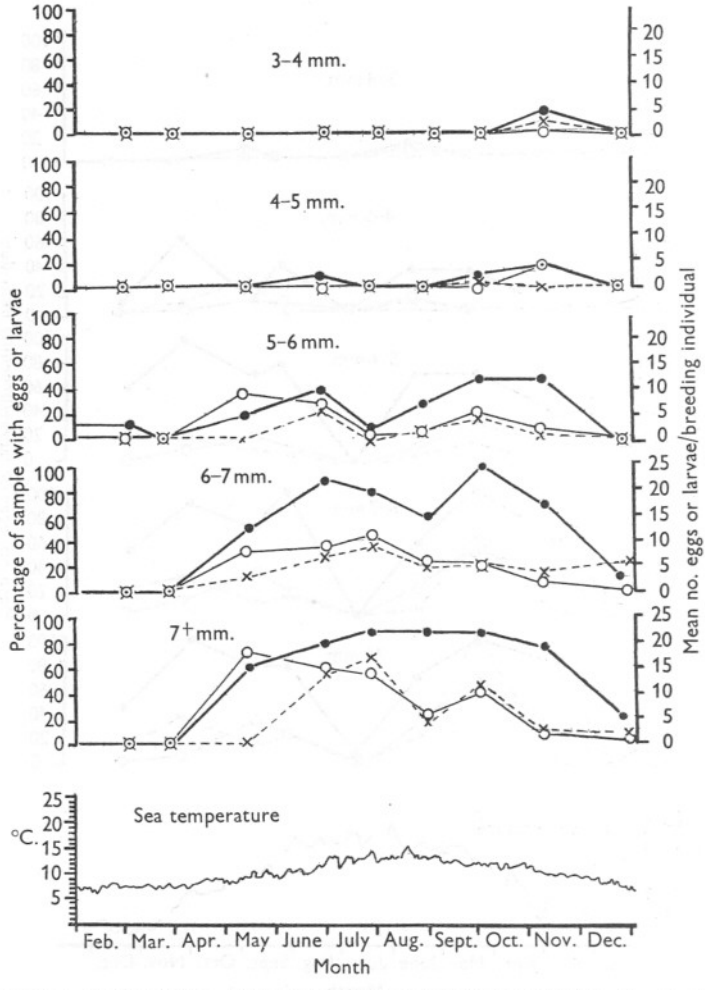

Fig. 4. Breeding of *Dendrodoa* of different sizes in the Clyde samples. ●—●, percentage with eggs or larvae; ○—○, mean number of eggs per breeding individual; ×—×, mean number of larvae per breeding individual.

The Clyde Population

The first maximum in breeding intensity, in July, was greater than the second maximum, in September or October. It has already been noted that there was a slight depression in breeding activity in summer, observed both in the percentage of breeding individuals and in the breeding intensity, in

animals of 5·0–7·0 mm. In animals over 7·0 mm. the percentage of breeding individuals remained high throughout the summer, but the intensity of breeding dropped in August, to rise again in September.

Fig. 5 shows the relation between the length of the body and the number of eggs and larvae in the atrial cavity. Very few eggs were produced by animals under 6·0 mm., and in larger animals the number of eggs increased with increasing body length.

The intensity of breeding was much less in the Clyde population than in Essex. The greatest value of the mean number of eggs plus larvae per breeding individual at the height of the reproductive season was only thirty-two in the Clyde, but 172 in Essex, in specimens over 7·0 mm. The greatest number of eggs plus larvae found in any specimen in the Clyde population studied was fifty-five, compared with 266 in Essex. These figures indicate that breeding was about five times more intense in Essex than in the Clyde.

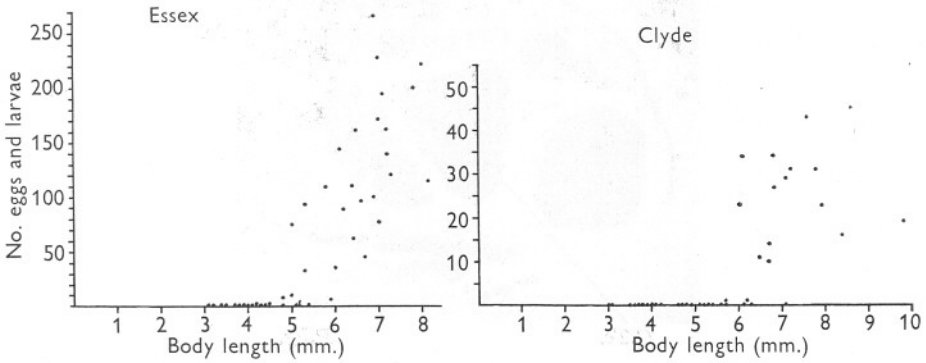

Fig. 5. Relation between length of body and the number of eggs and larvae in the atrial cavity, of specimens from Essex (10 May 1952) and the Clyde (26 July 1952).

Histological Condition of the Gonad throughout the Year

In each of the ten samples taken during 1952 the gonads of ten specimens from each of the size groups 5–6, 6–7 and 7+mm. were fixed in Bouin, sectioned at 12 μ, stained in Ehrlich's haematoxylin and eosin, and examined. Little variation was found in the condition of gonads from the different size groups and the following account therefore applies in general to all mature animals.

The Essex Population

In the overwintering condition the testis lobes consisted of a solid central mass of cells with 'resting' nuclei, and a narrow peripheral layer of phagocytes with dark-staining contents (Fig. 6). There were no spermatozoa or spermatids.

The ovarian part of the gonad had germ cells ranging from a small size up to oocytes 60–120 μ in diameter. None of these oocytes had yet acquired yolk.

There were also present the remains of yolk-laden eggs which had been retained in the ovary at the end of the previous breeding season, and which had been attacked by phagocytes.

The changes which transformed the gonad from this quiescent state to the height of its breeding activity, and the subsequent changes leading again to the overwintering condition, can be best represented graphically (Figs. 7 and 8). During the late winter and early spring the nuclei of the undifferentiated testicular cells started to divide, and by March all specimens had at least some fully developed sperm within the now hollow testis lobes. Meanwhile the peripheral phagocytes had almost completely disappeared from the testis.

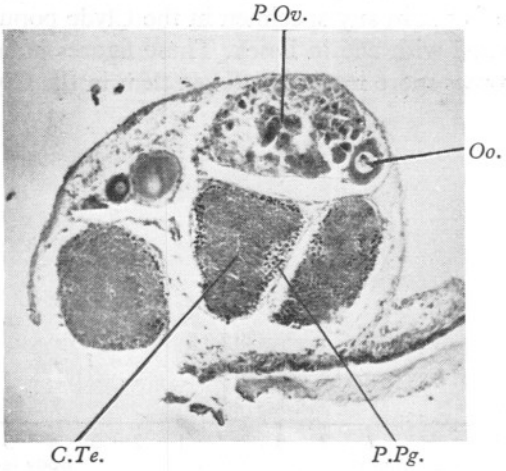

Fig. 6. Photomicrograph of transverse section through gonad in the overwintering condition (Essex, 15 January 1952). 12 μ. Ehrlich's haematoxylin and eosin. *C.Te.*, central part of testis lobe; *Oo.*, oocyte; *P.Ov.*, phagocytosed ovum; *P.Pg.*, peripheral phagocytes of testis.

During the period January–March the ovarian part of the gonad had also undergone changes. The remains of the previous year's unshed eggs disappeared by the completion of the process of phagocytosis which had been slowed down or halted in winter. Oocytes increased in size and yolk started to appear in the protoplasm, so that by the end of March all gonads had yolk-laden oocytes. In early May the gonad reached its fullest development (Fig. 9).

When spawning was intense, sperm began to disappear from the testis lobes, and at the same time phagocytes started to invade the testis. The number of eggs with yolk fell sharply as a result of ovulation, and residual eggs, together with almost ripe oocytes, were attacked by phagocytes (Fig. 9). Resorption of residual germ cells was rapid in July; phagocytes retired to the periphery of the testis lobes and disappeared, and the remains of unshed eggs were also quickly removed.

During August and September the gonads were again brought to breeding condition by a series of changes similar to those that occurred in late winter and early spring. After the autumn spawning the gonads were returned to their overwintering condition by the same processes that followed the earlier spawning.

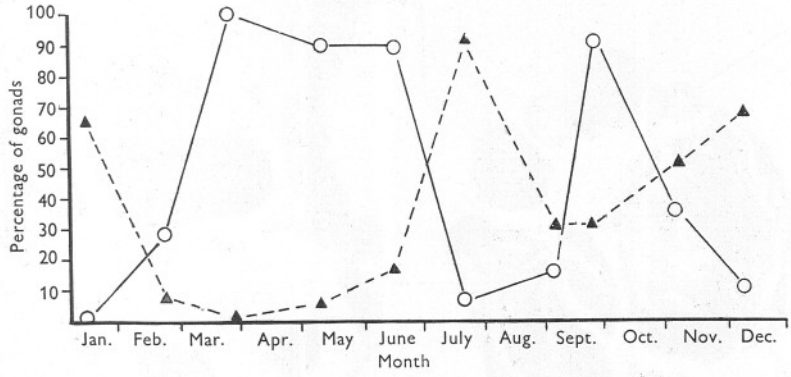

Fig. 7. Histological condition of ovary throughout the year in the Essex samples. ○—○, percentage of gonads with yolk-laden eggs; ▲—▲, percentage showing phagocytosis of eggs.

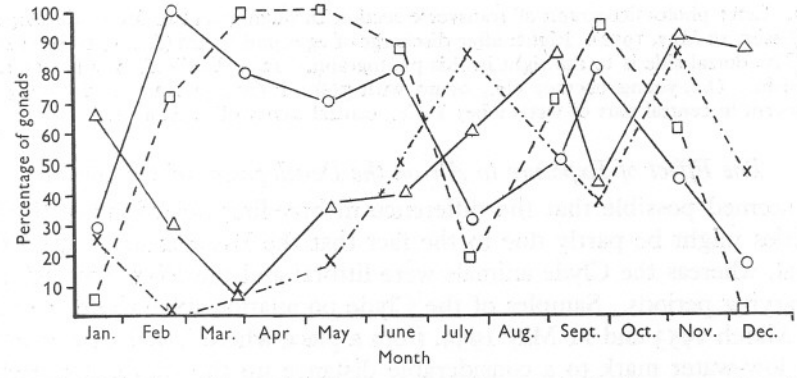

Fig. 8. Histological condition of testis throughout the year in the Essex samples. ○—○, percentage of gonads with dividing male nuclei; □ - - - □, percentage with sperm; △—△, percentage with phagocytes in peripheral part of testis; × — ×, percentage with phagocytes in central part of testis.

The Clyde Population

Figs. 10 and 11 show the cycle of changes in the gonads of the specimens from Farland Point, Cumbrae. The overwintering condition and the condition at the height of the breeding season were the same in the Clyde as in Essex. But in the Clyde there was relatively little suppression of breeding at any time

in the summer, and correspondingly little phagocytosis and reversion of the gonad towards a non-breeding state, either in the testicular or ovarian part. The extent of these processes can be seen in the trend of the graphs in August.

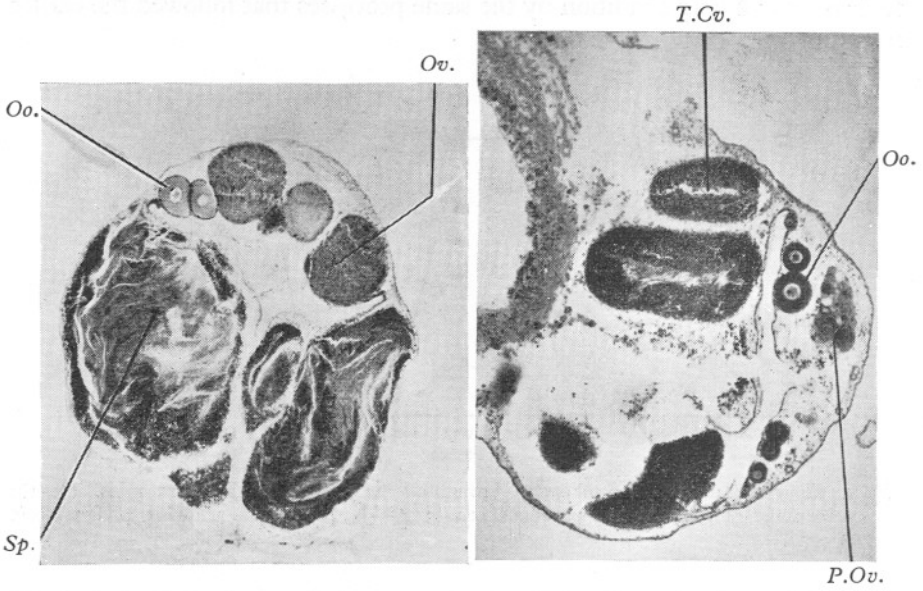

Fig. 9. Left: photomicrograph of transverse section through gonad at fullest development (Essex, 10 May, 1952). Right: after discharge of eggs and sperm (Essex, 16 June 1952). (The dorsal side is to the right in this photograph.) 12μ. Ehrlich's haematoxylin and eosin. *Oo.*, young oocyte; *Ov.*, ovum with yolk; *P.Ov.*, phagocytosed ovum; *Sp.*, sperm in central part of testis lobe; *T.Cv.*, central cavity of testis lobe.

The Effect of Exposure to Air on the Development of the Gonad

It seemed possible that the difference in breeding behaviour in the two localities might be partly due to the fact that the Essex animals were sublittoral, whereas the Clyde animals were littoral and therefore exposed daily for varying periods. Samples of the Clyde population were therefore taken on 6 March 1953 and 11 May 1953, from a place where *Dendrodoa* extended from low-water mark to a considerable distance up the shore. On each of these dates one sample was taken from the level of Chart Datum +2·2 ft. and one from the level of Chart Datum +7·1 ft. The lower position has a mean exposure of 3·0 hr. per day and the upper position 13·7 hr. per day. The gonads were sectioned and examined as in the main series of samples, and the results are shown in Table I.

The number of gonads examined (about twenty in each of the four samples) was rather small, but the results leave little doubt that a difference in level of 4·9 ft., on the Clyde shore in question, had only a slight effect on the rate of gonad development in spring. It therefore appears likely that the exposure

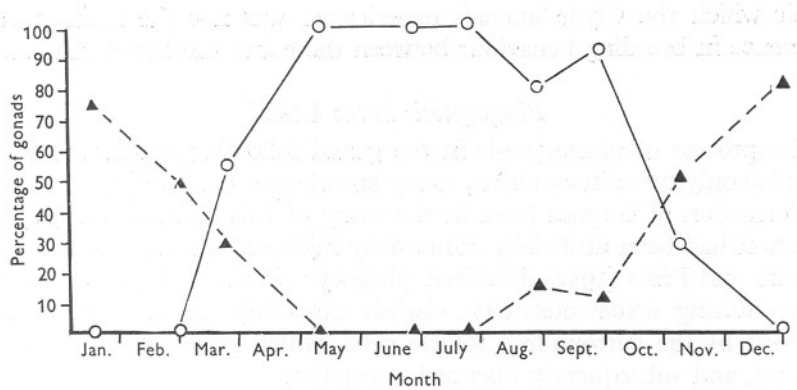

Fig. 10. Histological condition of ovary throughout the year in the Clyde samples. ○—○, percentage of gonads with yolk-laden eggs; ▲—▲, percentage showing phagocytosis of eggs.

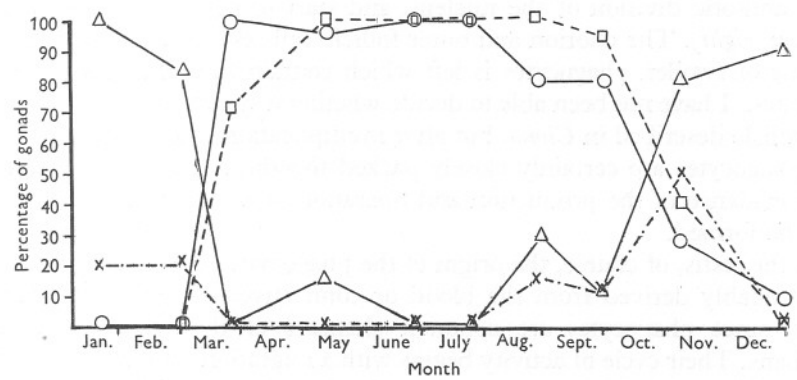

Fig. 11. Histological condition of testis throughout the year in the Clyde samples. ○—○, percentage of gonads with dividing male nuclei; □ - - - □, percentage with sperm; △—△, percentage with phagocytes in peripheral part of testis; × – · – ×, percentage with phagocytes in central part of testis.

TABLE I. EFFECT OF LEVEL ON THE SHORE ON GONAD DEVELOPMENT

		Percentage of gonads with						
Date	Level on shore (ft. above Chart Datum)	Male nuclei dividing	Sperm	Phago-cytes in central testis	Phago-cytes in peri-pheral testis	Yolk in eggs	Phago-cytosis of eggs	Diameter of largest oocytes (μ)
6 March 1953	2·2	100	58	10	2	30	15	135
	7·1	91	50	7	21	22	30	126
11 May 1953	2·2	100	100	0	0	100	0	279
	7·1	100	100	0	0	96	0	287

to air which the Clyde animals experienced was not the main cause of difference in breeding behaviour between these and the Essex animals.

Phagocytosis in the Gonad

The process of phagocytosis in the gonad following spawning has been recorded only twice in ascidians, to my knowledge. Carlisle (1951) observed the formation of corpora lutea in the ovary of *Ciona intestinalis* (L.) after ovulation had been artificially induced by injection of mammalian gonadotrophin, and Pérès (1952) described phagocytosis in the testis of the same species during sexual inactivity. Carlisle described the invasion of nearly ripe ova by the surrounding follicle cells, which phagocytosed the yolky material, and subsequently liberated a secretion.

In *Dendrodoa grossularia* essentially the same process occurs after natural ovulation. The inner follicle cells, which lie in the protoplasm of the ovum immediately internal to the chorion, migrate inwards, multiply apparently with amitotic division of the nucleus, and start to ingest the yolk granules (Fig. 9, *right*). The chorion and outer follicle cells eventually disappear, and a mass of swollen phagocytes is left which contain the remains of the yolk granules. I have not been able to decide whether a syncytium is formed, such as Carlisle described in *Ciona*, but after multiplication, and ingestion of yolk, the phagocytes are certainly closely packed together. Nor have I seen any clear evidence of the production and liberation of a secretion, although this may be formed.

In the testis, of course, the origin of the phagocytic cells is different. They are probably derived from the blood or connective tissue surrounding the testis, since phagocytes are common elements of these tissues in many ascidians. Their cycle of activity begins with a migration into the tissue of the testis. When they have penetrated to the central part of the lobes where residual sperm are situated, they ingest these sperm and perhaps also spermatids. They then withdraw with their contents to the peripheral part of the testis lobes and eventually disappear, perhaps by moving out of the testis altogether although this was not discovered.

How far the processes involving phagocytosis in the ovary and testis can be regarded as comparable depends on whether the function of the phagocytes in the ovary is primarily to remove unshed eggs, or whether this removal is merely a necessary step towards the production of a secretion. In the testis it appears that no more is involved than the removal of unshed germ cells. Also the origin of the phagocytic cells is different in the two cases. The question cannot be answered until we know something about the nature of the supposed secretion formed in the ovarian part of the gonad.

Factors influencing Breeding

Temperature is probably the most important of the factors influencing the breeding of marine invertebrates. In many species there is no fixed

temperature at which spawning occurs, but rather, as Korringa (1941) says of *Ostrea edulis* L., 'the maturation-period of the eggs is a function of time and temperature'. This has been shown to be true, for instance, in *Crassostrea virginica* (Loosanoff & Davis, 1952). Nevertheless, these workers also found that a certain minimum temperature is required before maturation of the gonad can start. Very little is known of the precise effect of other factors, e.g. food, in initiating the reproductive cycle. Once the gametes are mature they may be liberated in different species by a variety of stimuli, of which temperature change is one of the commonest.

When considering the influence of temperature on the breeding of *Dendrodoa grossularia* we must first recognize that the Clyde animals studied, being intertidal in habitat, were subjected to air temperature for varying daily periods. The effect of air temperature on the animals during their period of exposure is unknown. It seems likely, however, to have been much less than the effect of water temperature during submersion, since some of the most important vital activities, e.g. water filtering and feeding, cease during exposure to the air. The evidence from samples taken from points high and low on the shore also suggests that exposure to air was not a major factor affecting the rate of gonad development. It is perhaps reasonable, therefore, to consider only water temperature when dealing with the Clyde animals. Yet it is difficult to find any correlation between the sea-surface temperature and the onset of those changes in the gonad which initiated the breeding cycle. Thus although the water temperature in March was relatively steady between 7·0 and 7·5° C., it was during this period that important and rapid changes were accomplished in both male and female parts of the gonad (Figs. 10, 11). Similar changes in the gonads of the Essex animals had occurred at least a month before (Figs. 7, 8), during a time when the water temperature there rose quickly from 0·0 to 3·5° C. Unless we assume the existence of physiologically differentiated races in the two localities, adapted to different temperature ranges, we must conclude that it was not temperature *level* which determined the onset of those changes in the gonad. Nor was it a rising temperature that started the maturation of the gonad in the Clyde animals, although this could explain the results obtained in the Essex samples.

The first liberations of ripe gametes from the gonads occurred in spring at about the same water temperature, 8·0–9·0° C., in the Essex and Clyde animals. At the approach of winter the liberation of gametes from the gonads ceased when the temperature dropped to about 11·0° C., in both areas.

It is more difficult to discover whether there also exists an upper temperature limit to the liberation of gametes. The answer to this question depends on the explanation adopted for the absence of breeding during July in Essex and the reduced breeding in July and August in the Clyde. Two possible explanations can be considered. The first is that intense spawning in early summer exhausted the animals in Essex so that by July they were spent. If this explanation were right the Clyde animals, having bred with much less

intensity, should still have been capable of undiminished breeding thoughout the summer. But in fact there was a definite reduction of breeding in July and August, and the theory of exhaustion is therefore unsatisfactory.

The second possible explanation is that, in addition to the lower temperature limit which has been demonstrated, an upper temperature limit to the liberation of gametes from the gonad also exists. Histological examination shows that in July the gonads of the Essex animals still had large yolky eggs but these, instead of being shed, were retained and attacked by phagocytes, a process suggesting that an environmental factor suppressed further ovulation. That factor may well have been high temperature.

It appears that, at temperatures over 15·0° C., ovulation becomes progressively less, and above about 20·0 ° C. is totally suppressed.

The present investigation has raised a number of problems concerning the influence of environmental factors on breeding which can probably only be solved by experiment.

Summary

Samples of the ascidian *Dendrodoa grossularia* were studied from a sublittoral habitat in the River Crouch, Essex, and from a littoral habitat on the Isle of Cumbrae, Firth of Clyde. The pattern of annual growth was similar in these areas, and the life-span appeared to be 18 months to 2 years.

Developing eggs are retained in the atrial cavity by brood filaments.

In Essex a first maximum of breeding in spring and early summer was separated from a second maximum in late summer and autumn by a short interval when no breeding occurred. In the Clyde breeding was continuous from early summer to autumn, but was slightly reduced in August.

The histological changes in the gonad are described. Phagocytosis in both ovary and testis followed each period of breeding.

Gametes were shed from the gonad when the sea temperature in spring reached 8·0–9·0° C., and ceased to be shed in autumn when the sea temperature fell below about 11·0° C. Release of gametes appeared to be reduced at temperatures above 15·0° C. and totally suppressed above about 20·0° C.

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J. Mar. biol. Ass. U.K. (1954) 33, 49–60
Printed in Great Britain

CONCENTRATION GRADIENTS AND THEIR SIGNIFICANCE IN *LAMINARIA SACCHARINA* (L.) LAMOUR.

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(Text-figs. 1–5)

In previous publications the author (Black, 1948*a–c*, 1949, 1950*a*) has reported the seasonal variations in chemical composition that occur in certain of the common Phaeophyceae. In the sublittoral and littoral zones it has been found that a correlation exists between the chemical composition and the depth of immersion of the plant (Black, 1950*b*, and unpublished work). Differences in composition, particularly during the summer months, also occur in the same species taken at the same time and depth but from different habitats (Black, *loc. cit.*), and there is also evidence that in the laminarians the stage of development of the plant has a profound influence on the composition. In all the above-mentioned analyses the entire plants have been separated into stipe and frond, dried under controlled conditions, milled, and a well-mixed representative sample of each taken for analysis.

Work on the distribution of iodine and potassium in *Laminaria flexicaulis* (=*digitata*) has been carried out by Spindler (1948), Rinck (1948), Rinck & Brouardel (1949*a*, *b*), and Brouardel & Rinck (1950). Rinck & Brouardel (1949*a*) determined the iodine content of 325 samples of the fresh and dried alga, and found it to vary regularly with the distance along the frond, being at a minimum at the base of the frond where the youngest cells are and then increasing with the length of the frond. A similar variation was also obtained in the water content of different parts of the frond. As with iodine, they found a regular variation in the potassium content, the results indicating a direct relation between the morphological portions of the alga—holdfast, stipe, stipe-frondal zone and frond—and the concentration of the chemical elements. Moss (1948, 1950), in her studies on the structure and chemical composition of *Fucus vesiculosus*, has shown that a concentration gradient exists in the thallus and that the receptacles and sterile tips differ markedly in composition. As anatomical differentiation proceeded from the apex, the region of growth, to the base of the thallus, so did the chemical composition vary: the percentage dry weight and organic nitrogen (expressed as percentage fresh weight) increased while alginic acid decreased.

Recent work by Gerdes (1951) on the distribution of aneurin in the algae has shown a constant decrease in the aneurin content with distance from the

growing point. Sections of *Laminaria saccharina* were analysed from the transition zone, the upper part of the frond, the stipe and the holdfast. The transition zone contained 7·25 p.p.m. and the upper part of the frond 1·31 p.p.m. (dry basis).

Observations on the rate of growth of *L. digitata* and *L. saccharina* in the Dalne-Zelenetzky Gulf were made by Kuznetzov (1946), and Parke (1948) has shown that in *L. saccharina*, from the coasts of Devon and Argyll, there are two periods of growth, (i) a period of rapid growth from January to June/July with the most rapid growth between March/June, and (ii) a period of slow growth from July to December with the slowest period between September and December. Throughout a yearly cycle regular changes in growth rate affected the length, width, and thickness of the frond, while loss of frond tissue from the distal end started when a plant was a few months old and continued throughout its life. According to Parke (1948) there can be present in a *L. saccharina* frond in October a portion of newly formed tissue and tissue at least 7 months old. It is logical, therefore, to expect variations in composition with age. As the stipe of this species is relatively small compared to the frond, and as no variation existed in the dry-matter content along the stipe, only the frond was in each case analysed.

No work appears to have been carried out to show the presence of a concentration gradient in *Laminaria*. Moss (1948, 1950) has studied *Fucus vesiculosus*, but, whereas in *Fucus* the growing region is near the tips of the branches, in *Laminaria* it is at the base of the frond.

This work forms part of a programme of research and development of the Institute of Seaweed Research to whom the writer is indebted for permission to publish. The writer is also indebted to Dr E. T. Dewar for his great care in collecting and sectioning the plants, to W. J. Cornhill for his assistance with the analytical work and to F. T. Walker for his advice and encouragement. An abstract of this paper was read at the 2nd International Biochemical Congress in Paris in July 1952.

Preparation of Samples and Methods of Analysis

For the investigation now to be reported samples were collected and treated as follows.

The initial sample of *L. saccharina* (L.) Lamour. was collected on 19 August 1949 at Shandon in the Gareloch at 4 m. (low water). This was plant 1, which had the dimensions shown in Fig. 1 A. The frond was divided transversely into five equal sections 30·5 cm. long and dried at 25–30° C. for 24 hr.

Plants 2–4 were collected in Kerrera Sound, Oban, Argyllshire, at approximately 4 m. (low water), on the 17 October 1950, 26 April 1951 and 30 May 1951 respectively, and sectioned as shown in Fig. 1. The sections were dried

at 100° C. for 16 hr. Each of the dried sections was then milled in a Christy and Norris no. 8 Laboratory Mill fitted with a 64-mesh screen, and analysed as previously described (Black, 1948*a*; Black & Cornhill, 1951).

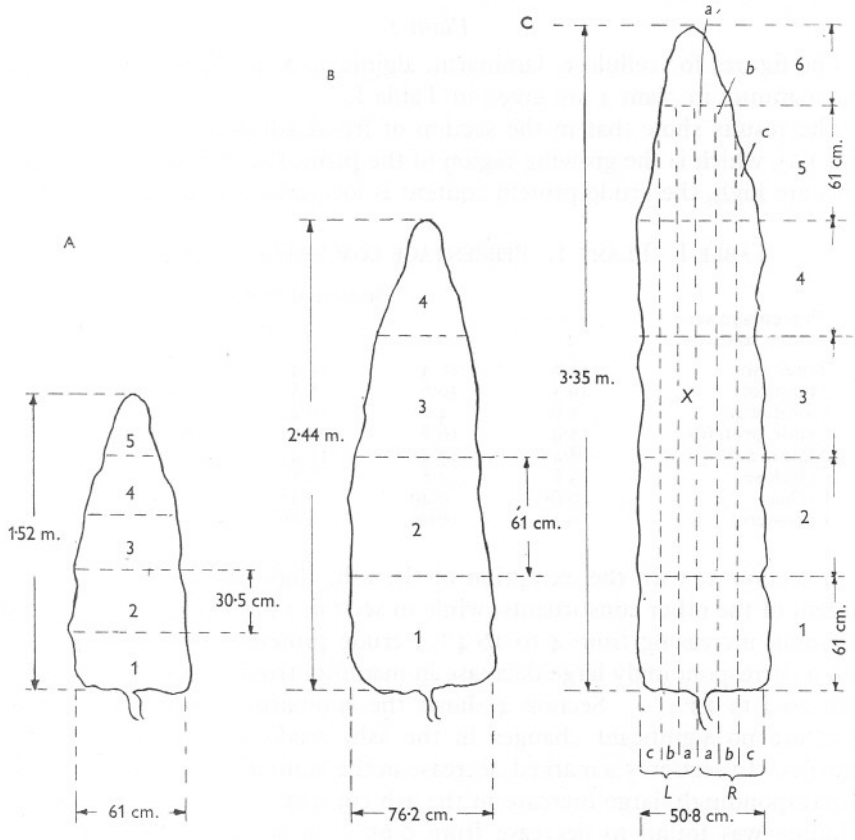

Fig. 1. Method of dividing the fronds investigated. A, plant 1, Gareloch, 19 August 1949. B, plants 3 and 4, Kerrera Sound, 26 April 1951 and 30 May 1951. C, plant 2, Kerrera Sound, 17 October 1950. All were divided into transverse 'sections' numbered from the base. In A there were five sections of 30·5 cm.; in B, four of 61 cm.; in C, six sections of which the first five were 61 cm. long and the terminal 30·5 cm. In C there were, in addition, longitudinal divisions. The frond was first divided into right and left halves (R and L). Each half was divided further into three strips, *a*, *b* and *c*, the first the innermost. $a = b = 7{\cdot}6$ cm., $c = 10{\cdot}2$ cm. (Any of the 30-odd subdivisions of the frond can be specified by a combination of three symbols: thus the unit marked *X* would be called L 3*a*.)

Results

The results obtained are expressed as fresh-weight and dry-weight percentages. When calculated as percentage concentrations in the water present the graphs were parallel to those for the fresh weight and have been omitted.

The calculation of the results on the 'residual dry weight' was also considered, but, as this underwent considerable variation, calculation on the fresh weight, which approximately gives the results in terms of concentration in the water present, was preferred.

Plant 1

The figures for cellulose, laminarin, alginic acid, crude proteins, total ash and mannitol in plant 1 are given in Table I.

The results show that in the section of frond adjacent to the stipe, (1 in Fig. 1 A), which is the growing region of the plant, the mannitol and ash contents are high, the crude protein content is low, and laminarin is absent.

TABLE I. PLANT 1. PERCENTAGE COMPOSITION (DRY BASIS)

Percentage on dry basis	Section of frond				
	1	2	3	4	5
Total ash	28·8	26·4	19·4	20·0	26·0
Mannitol	29·5	30·6	8·8	9·6	10·6
Laminarin	1·0	4·0	16·4	22·0	12·4
Crude proteins	15·4	16·8	20·5	19·6	18·2
Alginic acid	8·4	13·4	13·4	18·3	17·7
Cellulose	3·8	3·8	3·8	3·8	3·8
Iodine	0·65	0·49	0·47	0·42	0·40
Fucosterol	0·10	0·06	0·06	—	—

In section 2, with the exception of the ash, slight increases occur in the content of the other constituents, while in section 3 marked differences occur, laminarin increasing from 4 to 16·4%, crude proteins from 16·8 to 20·5% with a correspondingly large decrease in mannitol from 20·5 to 8·8% and ash from 26·4 to 19·4%. Section 4 shows the laminarin still increasing, while there are no significant changes in the ash, crude proteins and mannitol contents. In section 5 a marked decrease in the laminarin content occurs with a correspondingly large increase in the ash content.

Iodine was found to decrease from 0·65% in section 1 to 0·49% in 2, 0·47% in 3, 0·42% in 4 and 0·40% in 5 (dry basis), while fucosterol decreased from 0·10% in section 1 to 0·06% in section 2, quite a significant difference for this sterol. The alginic acid content increased from 8% in section 1 to 18% in sections 4 and 5.

Plant 2

These results having shown that marked differences in composition occurred along the frond, a more detailed investigation was carried out with plant 2 which was divided into thirty-one areas (Fig. 1 C), one at the tip, with the five transverse sections subdivided each into six longitudinally. The frond being thickened medially the innermost sections were about twice as heavy per unit area as the outer with the percentage water only slightly higher in the central strip.

Dry Matter

As one passes up the axis of the frond there is a marked increase in dry matter (Fig. 2). This reaches a maximum two-thirds of the way up and then decreases towards the eroded tip. It is interesting that although the dry-matter content increases, e.g. from 12·6% in L 1*a* to 31·1% in L 4*a* the fresh weights of the sections remain practically constant (45·5 and 47·0 g respectively). Also sections L 1*a* and R 1*a* contain double the amount of dry matter of the adjoining sections L 1*b* and R 1*b*, but the same percentage dry weight, indicating that they have approximately the same percentage composition but differ in thickness. No significant difference in concentration occurs across the frond.

Mineral Matter

The figures for the mineral matter (total ash) expressed on the dry basis, and on the fresh-weight basis, are given in Figs. 2 and 3. When expressed on the dry basis (Fig. 2) the mineral matter is high in the sections adjoining the stipe (25–35%), then decreases half-way up the frond (19–23%), and then increases slightly. This is also true if the ash is calculated on the 'residual dry-weight' basis. On the fresh basis (Fig. 3) almost the reverse occurs, the mineral matter increasing two-thirds of the way up the frond and then decreasing towards the tip. The mineral matter, therefore, increases as the dry-matter content increases and as the mannitol decreases. Possibly mannitol is being utilized in respiration or for further synthesis and mineral matter is diffusing into the cells to control the osmotic pressure. The concentration of mineral matter across the frond is somewhat irregular.

Mannitol

Although mannitol is unlikely to be the primary product of photosynthesis the value found is usually regarded as indicative of the amount of photosynthesis. In the sections adjacent to the stipe (Fig. 3) the mannitol content is high but increases to a maximum one-third of the way up the frond then decreases towards the tip, falling from 35% to 11·5% of the dry matter.

Laminarin

Laminarin is regarded as a storage foodstuff. It is absent from the stipe of the laminarians and from the frond during the period of rapid growth in the spring. It appears to be formed during the period of slow growth when the nitrate and phosphate content of our inshore waters is very low. Laminarin is almost entirely absent from plants growing in habitats where the above nutrients are available all the year as a result of pollution or upwelling. The results for this carbohydrate are given in Fig. 4. Laminarin is entirely absent from the active growing sections adjacent to the stipe, and increases up the frond to 30·4% in L 4*c* and 34·5% in R 3*b*; in general it increases with the dry-matter content and then decreases to 5% at the tip.

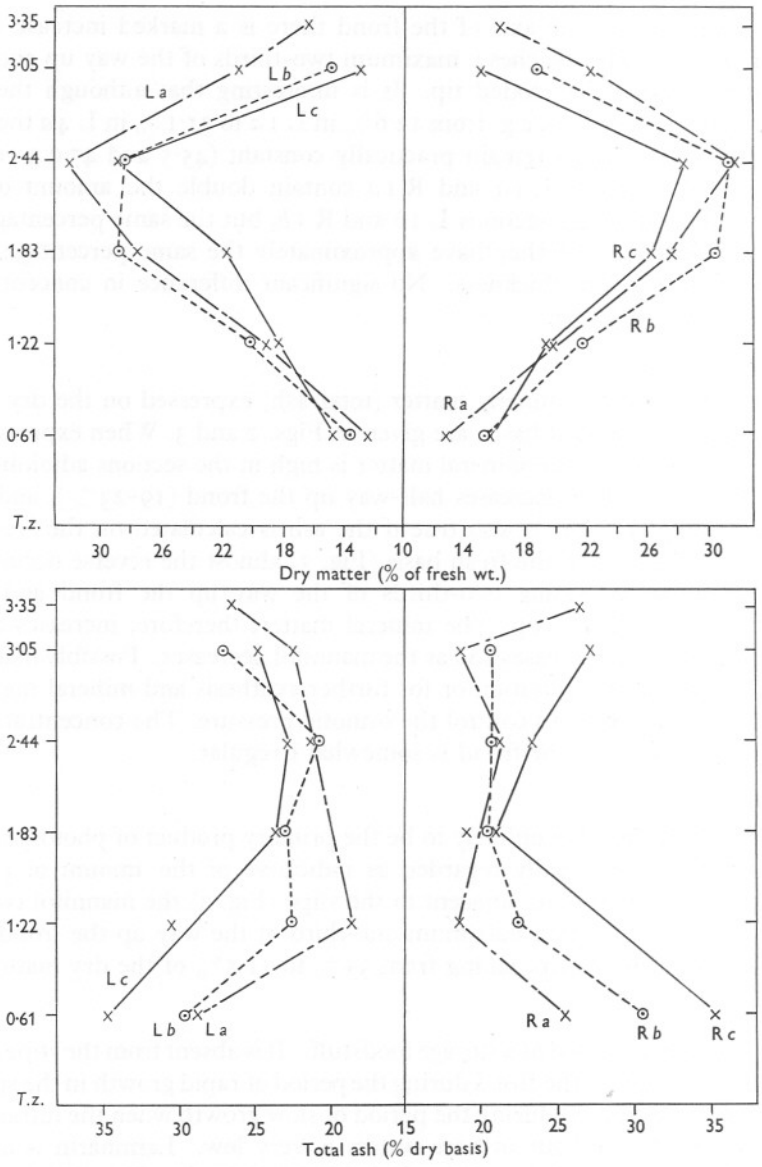

Fig. 2. Plant 2. Variation in (above) *dry-matter content* as percentage of fresh weight, and (below) *total ash* as percentage of dry matter. Each graph line represents one strip of the frond, with one value entered for each section. Left and right sides are graphed separately. *T.z.*, transitional zone at base of frond. Vertical scale *d* represents distance along the frond, from the base, in metres.

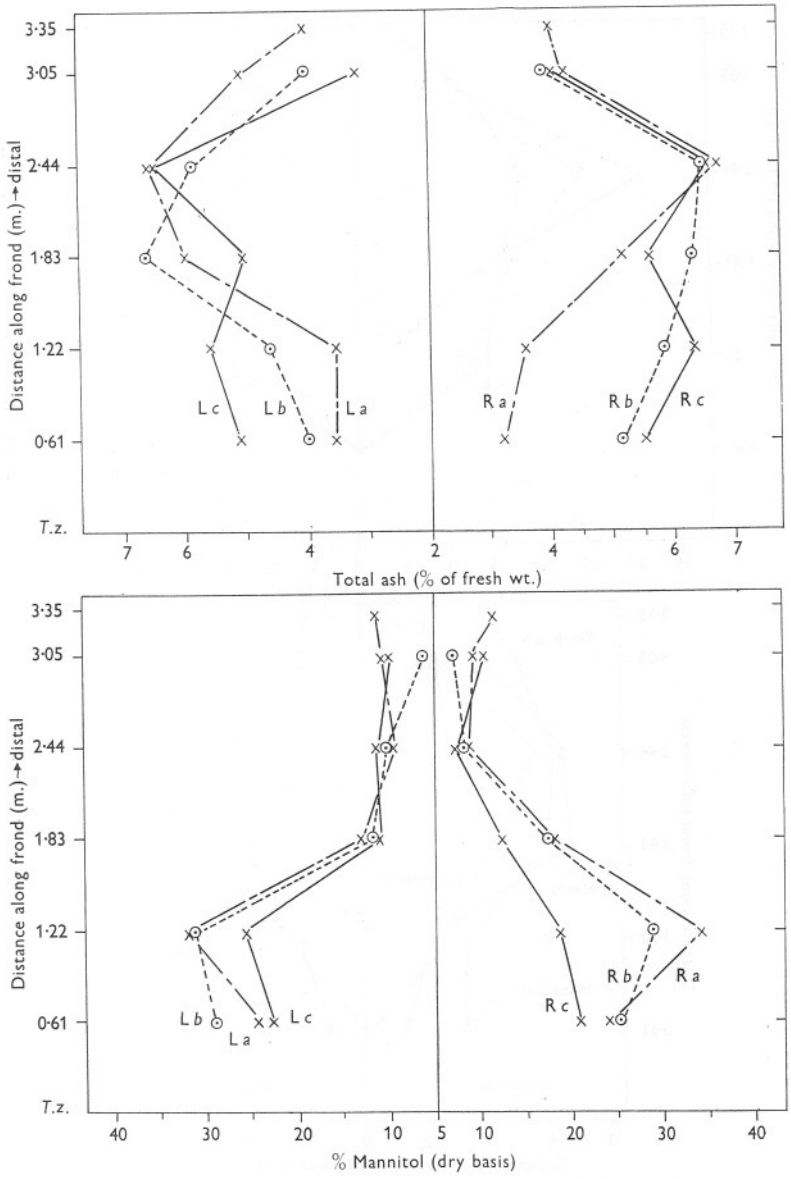

Fig. 3. Plant 2. Variation in (above) *total ash* as percentage of fresh weight, and (below) *mannitol* as percentage of dry matter. Particulars as in Fig. 2.

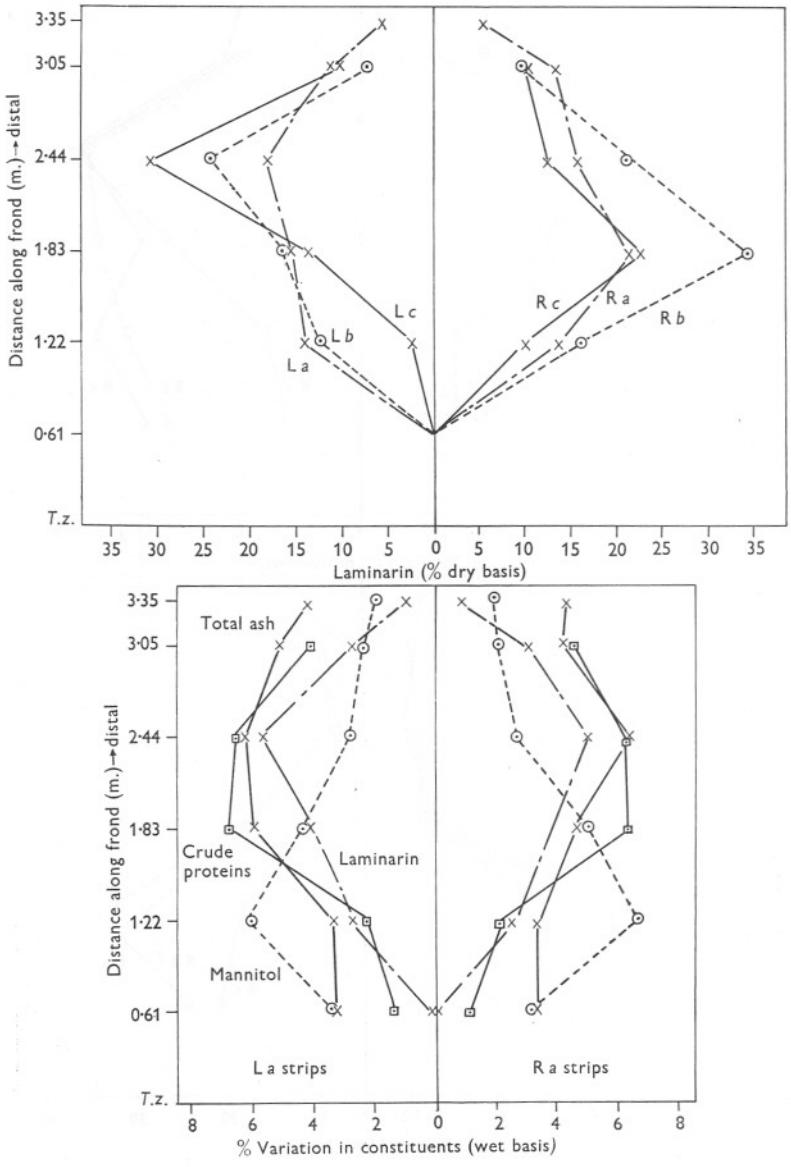

Fig. 4. Plant 2. Above: variation in *laminarin* as percentage of dry matter. Below: percentage variation in four constituents compared, on a wet basis, only the *a* strips considered.

Alginic Acid

The alginic acid content is exceedingly low in the sections L 1*a* and L 2*a* and is of such a low grade (i.e. of low molecular weight) that it is indeterminable by the standard method of analysis. In L 3*a* and L 4*a* values of 13·6 and 13·1 % were obtained, indicating that a higher polymer of alginic acid is no doubt present.

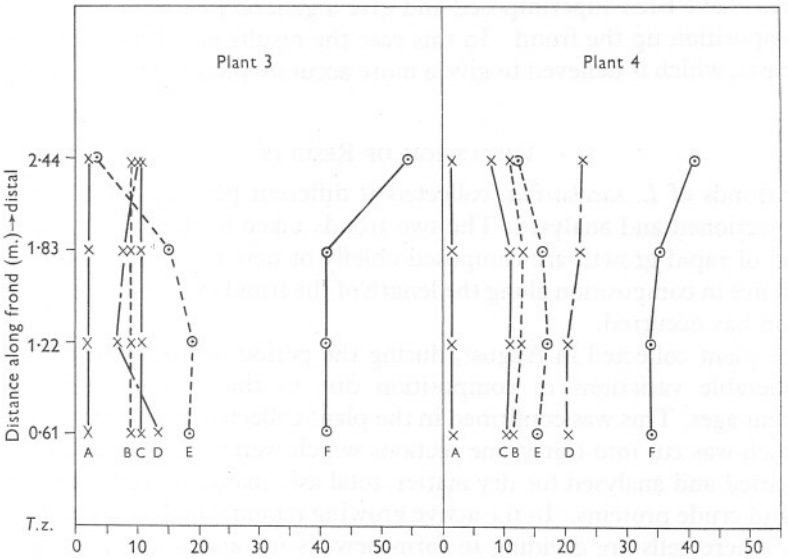

Fig. 5. Plants 3 and 4, illustrating the period of rapid growth. Variation in (A) *laminarin*, (C) *crude protein*, (D) *alginic acid*, (E) *mannitol*, (F) *total ash* (on dry basis); and (B) *dry matter* (on wet basis)

Plants 3 and 4

In order to prove that variations such as those recorded above were improbable during the period of rapid growth in the spring, plants 3 and 4 were taken and sectioned as in Fig. 1 B, and the analytical results are given in Fig. 5. The graphs show clearly that at this time of the year the composition of the frond is constant throughout its length, only at the tip where erosion takes place is there any noticeable change.

In previous publications (Black, 1948*b*, 1950*a*) the composition of the frond of *L. saccharina* is given for a period of 4 years, and the results agree with those of the present investigation. Plants 3 and 4, taken in April and May during the period of rapid growth, are composed mainly of tissue laid down during the preceding 3 months with perhaps only the distal part retained from the previous year, whereas plants 1 and 2 are composed of new tissue adjacent to the stipe and tissue up to 7–8 months old.

Analysis of a frond of *L. cloustoni* in May, when it was possible to differentiate between the new and old growth, showed that the old frond was low in

mannitol but still contained laminarin, while the new frond was appreciably higher in mannitol but contained no laminarin. In April/May the composition of an *L. saccharina* frond will, therefore, depend on whether old growth from the previous year has been retained or cast. From May onwards loss of frond is then due to erosion at the tip.

In Fig. 4 the graphs for the sections representing the central portion (L*a* and R*a*) have been superimposed and give a general picture of the variations in composition up the frond. In this case the results are all expressed on the wet basis, which is believed to give a more accurate picture of the living plant.

Discussion of Results

Four fronds of *L. saccharina*, collected at different periods of the year, have been sectioned and analysed. The two fronds taken in April/May during the period of rapid growth are composed chiefly of new tissue and there is little difference in composition along the length of the frond except at the tips where erosion has occurred.

The plant collected in August, during the period of slow growth, showed considerable variations in composition due to the presence of tissue of different ages. This was confirmed in the plant collected in October, the frond of which was cut into thirty-one sections which were weighed separately and later dried and analysed for dry matter, total ash, mannitol, laminarin, alginic acid and crude proteins. In the active growing region which is proximal to the stipe, where cells are dividing to form new tissue, and practically along the whole length of the frond during the period of rapid elongation, laminarin is absent and the dry-matter content is very low (10–12%) and is composed chiefly of mineral matter (36–40%) and mannitol (20–30%). In the active growing region the proteins are at a minimum, and the alginic acid is of such a low grade as to be indeterminable by the standard method. The results are in good agreement with those of Moss (1948) who found the dry matter and inorganic nitrogen, for example, lowest, and the mineral matter highest in the distal samples (tips) of *Fucus vesiculosus* where the growth is apical.

During the period of rapid growth there is extensive elongation due to cell enlargement with vacuolation. Then the tissue is low in dry matter composed mainly of mannitol and mineral matter and laminarin is absent. In July (Parke, 1948) a period of slow growth commences with the slowest growth between September and December when plants 1 and 2 were collected. June to September is also the period (Black & Dewar, 1949) when the nitrate and phosphate content of our inshore waters is at a minimum. During the period of slow growth, therefore, the stipo-frondal zone would alone represent new cells while the remainder of the frond, although able to photosynthesize, ceases active cell division and accumulates carbohydrates fated to become the food reserve for the winter or for sporogenesis. What Parke described as

mature tissue is, therefore, the storage portion of the frond two-thirds of the way up and containing a reserve of laminarin with proteins; the distal portions represent tissue which has spored or is badly eroded. Iodine and fucosterol are highest in the stipo-frondal (or transition) zone where they are in all probability required for new growth.

The results show that at certain times of the year concentration gradients of carbohydrates, proteins and mineral matter exist up the frond; they may be the result of restricted growth and may not be present in plants from habitats where nutrients are available all the year round and where laminarin has already been shown to be absent (Black, 1950*a*).

There is no evidence of a concentration gradient across the frond although the thickness shows considerable variation.

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J. Mar. biol. Ass. U.K. (1954) 33, 61–63
Printed in Great Britain

ON THE HORMONAL INHIBITION OF MOULTING IN DECAPOD CRUSTACEA

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Recently I have reported experiments which have led me to throw doubt on the universal applicability of the theory of the production of a moult-inhibiting hormone by the eyestalks of the decapod Crustacea (Carlisle, 1953). I suggested that the eyestalk moult-inhibiting hormone might be associated with the existence of a definite moulting season. In animals which possessed such a moulting season moulting would then be inhibited outside this season, but within the season there might well be no such inhibition. The experiments reported here support a modified form of this hypothesis.

Small crabs of the species *Carcinides maenas* (Pennant) were used. All specimens were between 2·5 and 4 cm. in carapace breadth. The sexes were not separated but no females were in berry. The crabs were kept individually in glass jars (4 lb. rock jars or Breffitt jars) of about 3 l. capacity, in 1 l. of sea water. The temperature was $18{\cdot}4 \pm 1{\cdot}5°$ C. The animals were fed once a week on squid flesh and the water changed after feeding. A group of about 250 crabs was selected and divided at random into two groups. In group E (experimental) the left eyestalk was severed at the base and cauterized. In group C (control) the retinal portion of the left eyestalk was destroyed by cautery. Two days later the right eyestalks of group E were removed and the right eyes of group C seared to destroy the retinal portion. The animals which survived these operations (eighty-four of group E and eighty of group C) were then isolated in the Breffitt jars. During the next 49 days six crabs moulted in each group. On the fiftieth day the blood calcium level of the survivors which had not moulted was measured (see Table 1). The death-rate had been almost identical in the two groups.

Four crabs in group C and five in group E showed signs of an approaching moult in the heightened blood calcium level. Except for these nine crabs, in which the blood calcium level was of the order of 100 mg. %, the level ranged between 28 and 41 mg. % in both groups. The mean for group E (excluding the five very high ones) was 33·4 mg. % and for group C 35·2 mg. %. Quite clearly the two groups do not differ in this respect and neither operation precipitated moulting nor even initiated proecdysis, for then the blood calcium level would be enhanced after a full 50 days, whereas this has only happened in a few animals which doubtless had commenced proecdysis without the extraneous stimulus of the operation.

Histological examination showed that the crabs of group C had intact the sinus gland and X organ, which were of course absent in the animals of group E. Now the moult-inhibiting hormone of crabs has been shown to emanate from the X organ-sinus gland complex (e.g. Passano, 1953). Under the experimental conditions I have employed, then, destruction of the seat of the eyestalk moult-inhibiting hormone has not precipitated moulting nor initiated proecdysis. Evidently, then, under these conditions no moult-inhibiting hormone from the eyestalk is interfering with the initiation of proecdysis.

Now according to Broekhuysen (1936) *C. maenas* moults seasonally. It does not moult during the months of February, March and April. This experiment started at the end of May and continued through all June and part of July.

TABLE I. BLOOD CALCIUM LEVELS IN THE BLOOD OF CRABS 50 DAYS AFTER THE BEGINNING OF THE EXPERIMENT

(Expressed in mg./100 g.)

Group E	Group C	Group E	Group C
35·0	41·6	38·7	39·6
31·8	35·2	32·1	39·2
46·8	34·4	30·4	35·6
30·1	32·5	23·2	29·9
29·2	31·8	38·4	35·9
27·4	28·6	38·6	39·8
38·5	36·8		
30·3	38·2	100·7	84·3
32·2	37·5	96·3	94·2
32·7	36·5	109·8	100·3
34·4	36·8	87·4	105·6
32·5	26·4	93·7	

That is to say it lay wholly within the moulting season. This is the chief difference from the experiments of American workers who have experimented almost entirely on animals outside the moulting season, when they have found clear evidence of the existence of a moult-inhibiting hormone secreted by the X organ-sinus gland complex. This is true of *Carcinides* (Passano, personal communication). But within the moulting season I have found no evidence of a moult-inhibiting hormone produced by the eyestalk. That some factor is inhibiting moulting there can be no doubt, for most of the animals in both experimental groups show blood calcium levels characteristic of anecdysis. Stephens (1951) has produced evidence that a moult-inhibiting hormone is produced in the central nervous system. It is possible that this is the factor responsible for the inhibition of moulting during the moulting season in those species which during this period do not allow one metecdysis to run straight into the next proecdysis.

The conclusion seems to be that whereas outside the moulting season ecdysis may be inhibited by a hormone secreted by the X organ-sinus gland complex of the eyestalk, and removal of the eyestalk or of this complex leads to the initiation of proecdysis, in the moulting season removal of the eyestalk

has no such effect and there is no evidence of the secretion of a moult-inhibiting hormone by the eyestalk. Moulting may, however, be inhibited during this period by some factor which emanates from some other centre than the eyestalk.

My thanks are due to my friend Dr P. F. R. Dohrn, of the Naples Zoological Station, in collaboration with whom I began investigations into the problem of moulting in Crustacea and who has been unfailing in his help and encouragement.

SUMMARY

During the moulting season in *Carcinides maenas* ablation of the eyestalks does not initiate proecdysis. Blood calcium levels, however, indicate that during this period moulting is partially inhibited by some factor emanating from some other centre than the eyestalk.

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J. Mar. biol. Ass. U.K. (1954) 33, 65–68
Printed in Great Britain

THE EFFECT OF MAMMALIAN LACTOGENIC HORMONE ON LOWER CHORDATES

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If a male dogfish is held in the hand and its belly stroked firmly backwards with the ball of the thumb a fluid can be expressed from the cloaca. In my experience this fluid in a fresh animal invariably contains sperm, whatever the season of the year. After a period of inanition, however, sperm eventually disappears from the cloacal fluid. Such an animal is a convenient test object for gonadotropic activity by a method which closely parallels the toad-pregnancy test of Galli Mainini (1947).

In a former paper I have shown that ascidians respond to an injection of chorionic gonadotropin by ovulation and release of sperm into the water (Carlisle, 1951). Anterior lobe pituitary gonadotropic extract (Praephyson) has the same effect in equivalent doses, when tested on *Ciona intestinalis*.

Materials used

The anterior lobe gonadotropic hormone used was the German Praephyson (Chemische Fabrik Promonta, Hamburg), stated to be assayed at 15 m.u. luteinizing hormone per ml. Reassayed by me on mice by the Ascheim-Zondek test it titred 70 i.u./ml.

Chorionic gonadotropin was extracted from human pregnancy urine by Dr W. M. S. Russell and myself, and assayed by the Ascheim-Zondek test and by the *Xenopus* ovulation test at 110 i.u./mg. This material was dissolved immediately before use at the rate of 1 mg./ml. of distilled water, yielding a solution containing 110 i.u./ml.

The lactogenic hormone was extracted from human *post-partum* urine. Urine was taken 1–7 days *post partum*, at which time there is the highest content of lactogenic hormone. After adjusting the pH to approximately the isoelectric point of the hormone (pH *c.* 5·6) 5% by volume of a saturated solution of benzoic acid in acetone was added slowly with stirring. Micro-crystals of benzoic acid are thus formed which supply a large surface for the adsorption of the hormone and other proteins. The mixture was left in a refrigerator overnight. The benzoic acid with its adsorbed proteins was then filtered off. The filtrate was then re-extracted in the same way. The combined fractions of benzoic acid were washed with distilled water and then the benzoic acid washed out with several changes of absolute acetone, in which the proteins are insoluble. The proteins are thus left behind as a precipitate,

free of benzoic acid. The precipitate is dissolved in 50% acetone, and any remaining insoluble material discarded. The dissolved material was precipitated by raising the acetone concentration above 92% and filtered off.

This precipitate was then purified and crystallized by the dilute acetone method of White, Bonsnes & Long (1942).

This method of crystallization has apparently failed in the hands of a number of workers. The method depends, in the last analysis, on the isoelectric precipitation of the hormone, with the precipitation delayed by the addition of acetone to give time for crystals to form, instead of a fast amorphous precipitation. Obviously, then, the final solution must be at the isoelectric point of the hormone at the time of precipitation. In the outline of the method given by White *et al.* the adjustment of the solution to the isoelectric point before the addition of acetone is prescribed, but no mention is made of the necessity of readjusting the pH after the addition of acetone. Acetone, even of Analar grade, frequently departs quite widely from the desired pH, and correspondingly the solution to which this acetone is added is altered in pH away from the isoelectric point. Under these conditions crystallization, or indeed precipitation, is unlikely to occur. It is thus essential to readjust the pH of the mother liquor to the isoelectric point of the hormone after the addition of acetone.

The yield of crystalline lactogenic hormone by this method is approximately 20 mg/l. of urine. It assays at about 25 i.u./mg. as tested by the pigeon-crop method. When tested by the Ascheim-Zondek test for gonadotropic content, 10 mg. administered to each of five mice gave no response. Ten i.u. of chorionic gonadotropin (0·09 mg.) produced a positive response in a parallel test.

Experimental data

An injection of 1 or 2 mg. of chorionic gonadotropin dissolved in distilled water into starved male dogfish—four tests at each level of dosage—stimulated a reappearance of sperm in the cloacal fluid. An injection of 0·5 mg. had no such effect on six animals. Presumably this was below the threshold of activity.

An injection of 2 mg. of lactogenic hormone dissolved in distilled water into each of seven animals stimulated the reappearance of sperm in the cloacal fluid.

An injection of 1 ml. distilled water into each of seven animals had no effect.

An injection of 1·5 ml. praephyson into each of six animals stimulated the reappearance of sperm in the cloacal fluid.

An injection of 0·5 ml. praephyson into each of six animals had no effect.

An injection of 1 mg. of chorionic gonadotropin into each of ten *Ciona* provoked ovulation and sperm discharge. An injection of 1 ml. praephyson into each of five *Ciona* provoked ovulation and sperm discharge.

An injection of 1 mg. lactogenic hormone into each of ten *Ciona* provoked ovulation and sperm discharge.

An injection of 0·1 mg. chorionic gonadotropin into each of ten *Ciona* had no effect.

An injection of 1 ml. distilled water into each of ten *Ciona* had no effect.

The results of these tests are listed in Table I. The positive effect in dogfish was as already stated, that in *Ciona* was ovulation and sperm discharge.

TABLE I

(C.G. = chorionic gonadotropin, L.T.H. = lactogenic hormone, each dissolved in distilled water.)

Injection				
Substance	Dose	Subject	No. of individuals	Effect
C.G.	1–2 mg.	Dogfish, ♂	7	+
C.G.	0·5 mg.	Dogfish, ♂	6	o
L.T.H.	2 mg.	Dogfish, ♂	7	+
Distilled water	1 ml.	Dogfish, ♂	7	o
Praephyson	1·5 ml.	Dogfish, ♂	6	+
Praephyson	0·5 ml.	Dogfish, ♂	6	o
C.G.	1 mg.	*Ciona*	10	+
Praephyson	1 ml.	*Ciona*	5	+
L.T.H.	1 mg.	*Ciona*	10	+
C.G.	0·1 mg.	*Ciona*	10	o
Distilled water	1 ml.	*Ciona*	10	o

Comment

Since 10 mg. lactogenic hormone had less gonadotropic effect on mammals than 0·09 mg. chorionic gonadotropin, and since 0·5 mg. chorionic gonadotropin was below the threshold dosage for the dogfish, then the spermatic activity of the lactogenic hormone on the dogfish could not be due to an impurity of gonadotropin contained in the lactogenic hormone, for the assay on mice indicated that 1 mg. of lactogenic hormone must contain less than 0·009 mg. (1 i.u.) of gonadotropic hormone. A similar argument applies to the comparative dosages on *Ciona*. Evidently, then, it must be the lactogenic hormone itself which has had the gonadotropic effect on these two animals. Physiologically speaking, they seem unable to distinguish between the two hormones and respond, apparently, to both of them in the same way. Are we here perhaps at an evolutionary level before the differentiation of a primitive sexual hormone of the pituitary into several different hormones with different functions?

This work was performed in the laboratories of the Department of Comparative Anatomy and Zoology, Oxford, and of the Zoological Station at Naples, while holding a Beit Memorial Fellowship for Medical Research, the Oxford Naples Scholarship, a Senior Demyship of Magdalen College, Oxford, and grants from the Browne Fund of the Royal Society, at various times. I wish to thank Prof. A. C. Hardy, F.R.S., Prof. R. Dohrn, Dr A. E. Needham and Dr W. M. S. Russell.

SUMMARY

Lactogenic hormone of mammals has a gonadotropic effect on dogfish and on *Ciona*, an ascidian. This activity resides in the hormone itself and not in any impurity.

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THE GROWTH RATE OF THE HAKE, *MERLUCCIUS MERLUCCIUS* (L.), IN THE CLYDE AND OTHER SCOTTISH SEA AREAS

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(Plate I and Text-figs. 1–13)

CONTENTS

INTRODUCTION

Hake belonging to the genus *Merluccius* are large fish often growing to over a metre in length; they are predators, being well streamlined and armed with formidable teeth; they feed pelagically on smaller fish of their own and other species. Of the various species distributed round the ocean basins, the European Hake, *M. merluccius* (L.), ranges from Lofoten on the coast of Norway, to Dakar on the African west coast; it is found most plentifully along the edge of the continental shelf of Western Europe but also extends into the Mediterranean and North Sea, while stragglers occasionally range as far as Iceland.

In the Clyde area the hake is common in deep water, being found on the east side of Arran, in Kilbrennan Sound, and in Loch Fyne as far up as Inveraray. Elsewhere, in shallower water, it is less frequent and only smaller specimens are taken. The main fishery for hake is in the area off Tarbert, where they are caught on long lines set in mid water.

The most important papers on the biology of the hake are those of Hickling (1927–33), who made an intensive study primarily of hake from S.W. Ireland, and Belloc (1923, 1929), who worked on the French and North African coasts, while Hart (1948) has published a review in which he has summarized a number of scattered works on the distribution and biology of the species. The present paper describes the results of an investigation on the growth rate of hake in the Clyde area.

Methods and Gear

Since June 1949 trawl hauls have been taken at irregular intervals in the Clyde area from M.F.V. *Calanus*, and the lengths of the hake obtained have been measured to the nearest centimetre below. Most of the hauls have been made in the vicinity of Millport, the majority being taken between Great Cumbrae and Bute. Other hauls have been made farther afield whenever the opportunity arose, and an effort has been made to obtain fish from as many parts of the Clyde area as possible.

The gear used has been of two main types; a small-mesh cotton trawl and a larger-mesh commercial trawl. The specifications of the former have remained relatively constant over the sampling period, whereas those of the large-mesh trawl have changed very considerably. Not only have the proportions and absolute sizes of the various parts changed, but also the mesh size, length of bridles, and size of otter boards have all changed. For this reason no emphasis can be placed on the quantitative aspect of the catches obtained. However, there is strong evidence which suggests that for the size-frequency distribution of the fish, the catches are reliable samples of the population irrespective of the gear used (see p. 74). The sampling of small hake was seriously prejudiced by the loss of the first small-mesh trawl on 10 January 1950, and hake were not again caught with this kind of gear until 15 September 1950.

The Material

Between June 1949 and March 1953, 2250 hake were caught on sixty-five occasions. Details of the hauls are given in Table I and are separated into six populations on the basis of their length distributions.

Population *a* has only been caught during the winter of 1952–53, and consists of forty-six fish believed to have been hatched in 1952.

Population *b* consists of ninety-four fish believed to have been hatched in 1951.

Population *c* first appeared in September 1951 when one fish of 30 cm. was caught. Altogether only fifty-six hake of this population have been caught.

Population *d* is the most important group and has provided the majority of the fish, 1551 having been caught during the sampling period. These fish,

TABLE I. HAKE POPULATIONS *a–f*, CAUGHT IN THE CLYDE AREA FROM JULY 1949 TO FEBRUARY 1953

Date		No. in sample	Mean length	Length range	Standard deviation	Position of haul	Gear
			Population a				
1952							
18. xii.		40	10·0	7–14	1·99	Tarbert	Large mesh
1953							
11. ii.		5	13·0	12–14	—	E. side of Arran	Large mesh
16. ii.		1	16	—	—	Bute Channel	Large mesh
			Population b				
1952							
4. vii.		3	36·0	33–39	—	Bute Channel	Small mesh
4. xii.		1	39	—	—	Bute Channel	Small mesh
18. xii.		13	24·9	22–28	1·90	Tarbert	Large mesh
19. xii.	♀	2	36·5	30–43	—	Strachur to	Large mesh
19. xii.	♂	1	31	—	—	Inveraray	Large mesh
1953							
15. i.		2	37·5	35–40	—	Bute Channel	Small mesh
16. i.	♀	2	33·5	33–34	—	Mountstuart	Large mesh
16. i.	♂	2	34·0	33–35	—	Mountstuart	Large mesh
20. i.		1	36	—	—	S.E. of Pladda	Large mesh
21. i.	♂	2	35·0	—	—	S.W. of Sanda	Large mesh
21. i.	♀	2	33·5	33–34	—	E. of Ailsa Craig	Large mesh
5. ii.		2	38	—	—	Mountstuart	Large mesh
11. ii.	♀	2	37·5	36–39	—	E. side of Arran	Large mesh
16. ii.	♀♂	3	36·3	34–38	—	Bute Channel	Large mesh
18. ii.	♀	33	32·5	28–39	2·59	Off Ardrossan	Large mesh
18. ii.	♂	23	34·2	29–40	2·56	Off Ardrossan	Large mesh
			Population c				
1951							
13. xii.		1	34	—	—	Mountstuart	Large mesh
1952							
14. iii.		4	41·0	40–42	—	Mountstuart	Large mesh
18. xii.	♀	3	56·3	51–60	—	Tarbert	Large mesh
	♂♀	4	50·0	45–53	—	Tarbert	Large mesh
19. xii.	♀	15	48·3	45–53	2·19	Strachur to	Large mesh
	♂	11	47·9	39–54	4·16	Inveraray	Large mesh
1953							
15. i.		1	52	—	—	Bute Channel	Small mesh
16. i.		1	49	—	—	Mountstuart	Large mesh
10. ii.	♀	1	56	—	—	Holy Isle	Large mesh
13. ii.	♀	4	51·0	47–55	—	Tarbert	Large mesh
	♂	3	50·7	50–51	—	Tarbert	Large mesh
		8	55·1	51–59	3·36	Tarbert	Large mesh
			Population d				
1949							
14. x.		137	8·1	6–10	0·99	Bute Channel	Small mesh
17. x.		160	7·6	5–10	1·01	Bute Channel	Small mesh
26. x.		85	7·9	6–10	1·05	Loch Fyne	Small mesh
29. x.		38	9·5	6–13	1·27	Bute Channel	Small mesh
15. xi.		43	11·7	9–14	1·21	Bute Channel	Small mesh
22. xi.		17	12·2	10–15	1·52	Wemyss Bay	Small mesh
		125	12·1	8–18	1·70	Bute Channel	Small mesh
28. xi.		28	12·2	6–16	2·33	Bute Channel	Small mesh
1950							
18. i.		3	15·7	14–19	—	Bute Channel	Large mesh
19. i.		3	16·0	15–17	—	Bute Channel	Large mesh

TABLE I (*cont.*)

Date	No. in sample	Mean length	Length range	Standard deviation	Position of haul	Gear
			Population d (cont.)			
1950						
25. i.	1	15	—	—	Mountstuart	Large mesh
27. i.	5	16·8	14–19	—	Mountstuart	Large mesh
7. ii.	9	17·4	14–22	2·88	Mountstuart	Large mesh
10. ii.	5	20·0	19–21	—	Mountstuart	Large mesh
15. ii.	1	22	—	—	Mountstuart	Large mesh
17. ii.	8	15·9	12–20	2·70	Loch Fyne	Large mesh
20. ii.	6	18·0	16–21	1·79	Bute Channel	Large mesh
20. ii.	4	18·0	16–19	—	Mountstuart	Large mesh
27. ii.	1	18	—	—	Mountstuart	Large mesh
9. iii.	17	16·5	13–21	2·24	Kilbrennan Sound	Large mesh
21. iii.	13	17·5	15–19	1·39	Ailsa Craig	Large mesh
28. iii.	46	19·1	15–25	2·20	Inchmarnock Water	Large mesh
31. v.	3	20·0	19–21	—	Mountstuart	Large mesh
15. ix.	32	27·6	18–34	3·42	Mountstuart	Small mesh
15. ix.	37	27·9	21–34	2·93	Mountstuart	Large mesh
20. xi.	23	34·8	27–41	3·43	Mountstuart	Large mesh
1951						
15. i.	3	39·7	37–42	—	Mountstuart	Large mesh
7. ii.	6	34·0	25–42	5·83	Knock Castle	Large mesh
12. ii.	99	36·6	30–44	3·09	Mountstuart	Large mesh
21. ii.	41	37·2	27–44	3·79	Mountstuart	Large mesh
18. iv.	24	39·1	28–45	3·51	Mountstuart	Large mesh
23. v.	26	38·2	33–48	3·52	Mountstuart	Large mesh
23. v.	10	39·1	31–45	4·48	Knock Castle	Large mesh
11. vi.	29	40·4	33–47	3·50	Mountstuart	Large mesh
28. vi.	7	40·1	36–44	2·91	Mountstuart	Large mesh
28. vi.	19	41·0	36–48	1·17	Bute Channel	Large mesh
10. vii.	17	40·5	35–47	3·47	Mountstuart	Large mesh
14. viii.	16	41·3	34–47	3·48	Mountstuart	Large mesh
28. viii.	2	40·5	35–46	—	Mountstuart	Large mesh
11. ix.	4	46·8	45–50	—	Mountstuart	Large mesh
12. ix.	2	38·5	33–44	—	Mountstuart	Large mesh
15. x.	5	49·6	45–54	—	Bute Channel	Large mesh
21. xi.	5	47·0	42–50	—	Mountstuart	Large mesh
22. xi.	3	51·3	49–55	—	Bute Channel	Small mesh
1952						
8. i.	1	55	—	—	Mountstuart	Small mesh
12. ii.	9	55·2	52–60	3·03	Mountstuart	Small mesh
20. ii.	6	55·7	51–61	3·50	Bute Channel	Small mesh
14. iii.	8	53·0	49–59	2·93	Mountstuart	Large mesh
8. iv.	6	55·3	50–63	5·16	Mountstuart	Large mesh
14. iv.	6	53·8	52–59	2·71	Bute Channel	Small mesh
23. v.	1	50	—	—	Mountstuart	Large mesh
4. vii.	2	56·5	53–60	—	Bute Channel	Small mesh
11. xii. ♀♂	14	68·9	63–75	3·24	Off Corrie, Arran	Large mesh
11. xii. ♀♂	3	51·7	51–53	—	Off Corrie, Arran	Large mesh
18. xii. ♀♂	16	68·1	61–75	3·98	Tarbert	Large mesh
18. xii. ♀♂	18	63·3	55–72	4·30	Tarbert	Large mesh
19. xii. ♀♂	59	62·6	53–72	4·43	Strachur to	Large mesh
19. xii. ♂	20	59·3	51–67	4·63	Inveraray	Large mesh
1953						
15. i.	1	66	—	—	Bute Channel	Small mesh
22. i. ♀	3	68·3	67–69	—	Kilbrennan Sound	Large mesh
10. ii.	2	68·5	68–69	—	E. side of Arran	Large mesh
11. ii. ♀	6	71·0	65–75	3·74	E. side of Arran	Large mesh
11. ii. ♀♂	2	69·5	69–70	—	E. side of Arran	Large mesh
13. ii.	50	69·5	60–77	4·50	Tarbert	Large mesh
13. ii. ♂	27	63·7	58–69	3·24	Tarbert	Large mesh
13. ii.	122	67·8	57–77	3·78	Tarbert	Large mesh
16. ii.	1	68	—	—	Bute Channel	Large mesh

Date		No. in sample	Mean length	Length range	Standard deviation	Position of haul	Gear
				Population e			
1949							
6. vii.		6	18·3	14–22	3·01	Inchmarnock Water	Large mesh
4. x.		4	31·5	30–33	—	Bute Channel	Large mesh
22. xi.		1	34	—	—	Wemyss Bay	Small mesh
2. xii.		4	30·3	26–33	—	Off Ardrossan	Large mesh
1950							
25. i.		14	38·6	33–45	3·76	Mountstuart	Large mesh
27. i.		53	37·6	31–47	3·95	Mountstuart	Large mesh
7. ii.		19	40·4	32–46	4·00	Mountstuart	Large mesh
10. ii.		58	38·2	27–51	4·65	Mountstuart	Large mesh
15. ii.		56	39·7	33–47	3·34	Mountstuart	Large mesh
17. ii.		12	44·4	39–50	3·73	Loch Fyne	Large mesh
20. ii.		6	41·0	36–50	4·82	Bute Channel	Large mesh
20. ii.		46	38·6	28–48	4·48	Mountstuart	Large mesh
27. ii.		42	39·0	31–46	3·26	Mountstuart	Large mesh
21. iii.		29	36·7	30–47	3·91	Ailsa Craig	Large mesh
31. v.		14	40·9	31–46	4·15	Mountstuart	Large mesh
15. ix.		7	46·6	41–52	4·35	Mountstuart	Small mesh
15. ix.		11	46·5	38–52	4·52	Mountstuart	Large mesh
20. xi.		4	50·5	47–55	—	Mountstuart	Large mesh
1951							
15. i.		2	51·5	47–56	—	Mountstuart	Large mesh
12. ii.		3	52·7	49–60	—	Mountstuart	Large mesh
21. ii.		6	51·5	47–55	2·81	Mountstuart	Large mesh
11. vi.		1	61	—	—	Mountstuart	Large mesh
10. vii.		2	61·0	60–62	—	Mountstuart	Large mesh
15. x.		1	61	—	—	Bute Channel	Large mesh
11. xii.		1	62	—	—	Fairlie Channel	Small mesh
1952							
11. xii.	♀	3	83·3	83–84	—	Off Sannox, Arran	Large mesh
11. xii.	♂	3	70·3	68–72	—	Off Sannox, Arran	Large mesh
18. xii.	♀	2	84·0	82–86	—	Tarbert	Large mesh
18. xii.	♂	3	75·0	73–78	—	Tarbert	Large mesh
19. xii.	♀	5	75·8	71–80	—	Strachur to	Large mesh
19. xii.	♂	1	73	—	—	Inveraray	Large mesh
1953							
22. i.	♀	2	76·0	75–77	—	Kilbrennan Sound	Large mesh
10. ii.	♂	1	82	—	—	Off Holy Isle	Large mesh
11. ii.	♀	2	84·5	82–87	—	Off Sannox, Arran	Large mesh
13. ii.	♀	7	80·0	74–86	4·04	Tarbert	Large mesh
13. ii.	♂	7	73·3	71–77	1·98	Tarbert	Large mesh
13. ii.		34	77·9	72–88	4·28	Tarbert	Large mesh
16. ii.	♀	1	77	—	—	Mountstuart	Large mesh
				Population f			
1949							
6. vii.		2	41·5	38–45	—	Inchmarnock Water	Large mesh
6. vii.		2	62·5	62–63	—	Inchmarnock Water	Large mesh
1950							
19. i.		1	90	—	—	Bute Channel	Large mesh
17. ii.		6	60·8	56–65	3·31	Loch Fyne	Large mesh
17. ii.		2	71	—	—	Loch Fyne	Large mesh
17. ii.		10	80·0	75–87	3·94	Loch Fyne	Large mesh
20. ii.		1	58	—	—	Mountstuart	Large mesh
27. ii.		1	54	—	—	Mountstuart	Large mesh
1953							
22. i.		4	103·0	93–110	—	Kilbrennan Sound	Large mesh
11. ii.		1	92	—	—	Off Sannox, Arran	Large mesh

which are believed to have been hatched during the summer of 1949, were first caught during October of that year, when they had reached a mean length of 8 cm., and ranged from 5 to 10 cm. The catches obtained at this time are of very great interest as hake of this size had not been caught before in any quantity.

Post-larval hake of under 2 cm. are well known; they have been described by Schmidt (1907) and reported from the Plymouth area (Clark, 1920; Russell, 1930–7; Corbin, 1948), and from S.W. Ireland (Schmidt, 1909; Hickling, 1933). However, very little indeed is known about the adolescent post-larval stage; Hickling (1933) only records nineteen fish of 10 cm. and less, which he himself caught in 1932. During the autumn of 1949, 452 young hake of this size were caught in the Clyde area. The good catches of this year class ended in January 1950 owing to the loss of the small-mesh trawl, although some of these fish have been caught almost every month up to the end of the sampling period.

Population *e* consists of 473 fish which hatched in 1948, growing to 18 cm. by June 1949 and 78 cm. by February 1953.

Population *f* consists of all fish longer than those that fit into population *e*. There is no evidence that they are a homogeneous collection, and they have been listed together only for convenience; they do not contribute materially to the results.

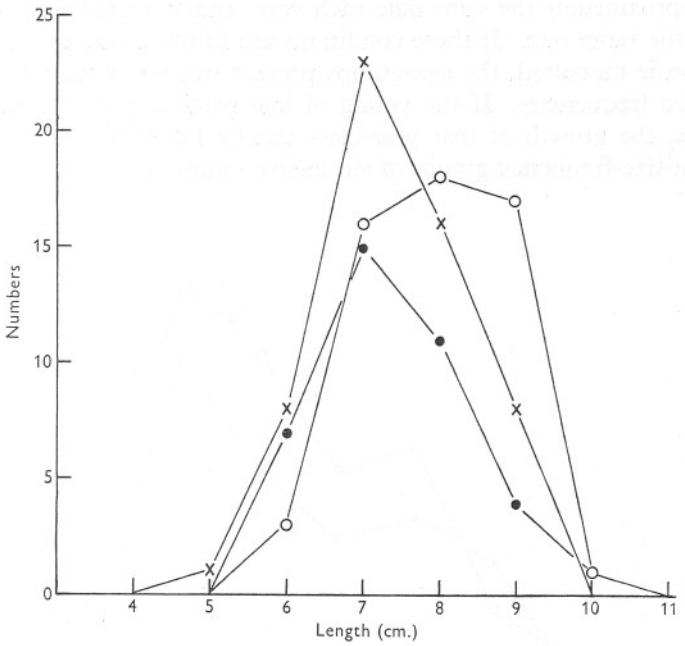
Reliability of the Samples

If fish measurements are to be used for the determination of growth rate it is essential that the catches should give an adequate indication of the size-frequency distributions of the populations. To test the reliability of the samples three series of hauls were considered and the means within each series compared; in no comparison was a significant difference found.

Series I is of three hauls taken with the small-mesh trawl in Bute Channel on 17 October 1949. The data are given in Table II, and Text-fig. 1; there is no significant evidence that it was not the same population that was sampled in each haul.

Series II compares two hauls taken with the large-mesh trawl off Inveraray on 19 December 1952. The data are given in Table III and Text-fig. 2; again the same population has been sampled in both hauls.

Series III, caught on 15 September 1950, and given in Table IV and Text-fig. 3, shows that the two kinds of gear used may be relied upon to give comparable samples from the same population, provided the fish are large enough not to be selected by the size of mesh. The large-mesh trawl caught thirty-seven fish with a mean length of 27·9 cm., while the small-mesh gear obtained thirty-two with a mean length of 27·6 cm.



Text-fig. 1. Length-frequency distributions of hake taken in series I. ×, haul I; o, haul II; ●, haul III.

TABLE II. SERIES I SIZE-FREQUENCY DISTRIBUTION, ILLUSTRATED IN TEXT-FIG. 1

Length (cm.)	Number of hake		
	Haul I	Haul II	Haul III
5	1	—	—
6	8	3	7
7	23	16	15
8	16	18	11
9	8	17	4
10	—	1	1
Total	56	55	38
Mean	7·4	7·9	7·4

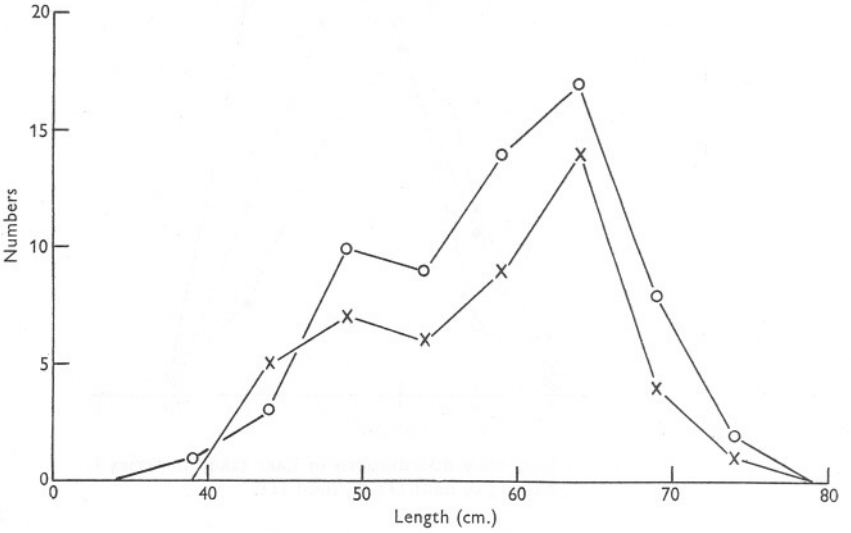
GROWTH RATE

The growth rate of the Clyde hake has been determined by Petersen's method, based on size-frequency distributions, and confirmed by examination of the otoliths of the fish caught during December 1952 and January and February 1953.

From Size-Frequency Distributions

Petersen's method of age determination and deduction of the growth rate depend upon two conditions: first, that the fish spawn during a limited period

and at approximately the same date each year; and secondly, that they grow at about the same rate. If these conditions are fulfilled and a sample of the population is measured, the age-groups present can be found by inspection of the size frequencies. If the young of one particular year are especially numerous, the growth of that year-class can be followed over a period of time from size-frequency graphs of successive samples.

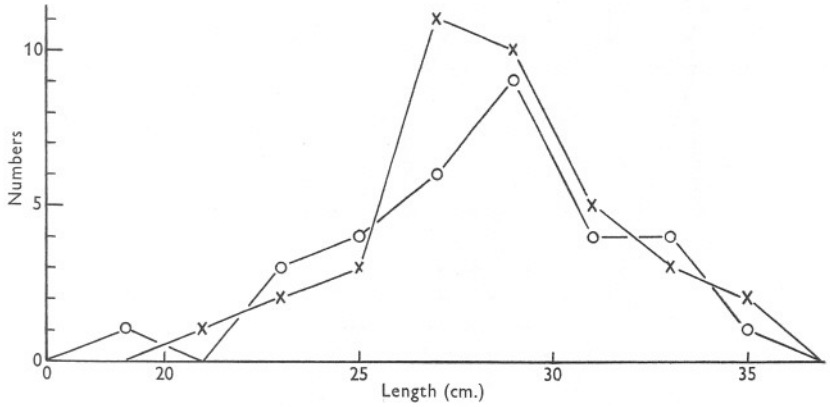


Text-fig. 2. Length-frequency distributions of hake taken in series II. ×, haul I; o, haul II.

TABLE III. SERIES II SIZE-FREQUENCY DISTRIBUTION, ILLUSTRATED IN TEXT-FIG. 2

Size-groups (cm.)	Number of hake		Size-groups (cm.)	Number of hake	
	Haul I	Haul II		Haul I	Haul II
37–41	—	1	62–66	14	17
42–46	5	3	67–71	4	8
47–51	7	10	72–76	1	2
52–56	6	9	Total	46	64
57–61	9	14	Mean	58·1	58·9

The data given for each population in Table I have been summed for each month and are plotted in the graph in Text-fig. 4. It can be seen that there was never, during any month, an overlap of the ranges of any two populations except during December 1952 and January and February 1953, when a special effort had been made to obtain as many hake as possible, some 580 being caught. Because these monthly samples are considerably larger than the others, their ranges do overlap and have therefore not been added to Text-fig. 4; for these months, only the mean lengths and standard deviations



Text-fig. 3. Length-frequency distributions of hake taken in series III. ×, Large mesh; o, Small mesh.

TABLE IV. SERIES III SIZE-FREQUENCY DISTRIBUTION, ILLUSTRATED IN TEXT-FIG. 3

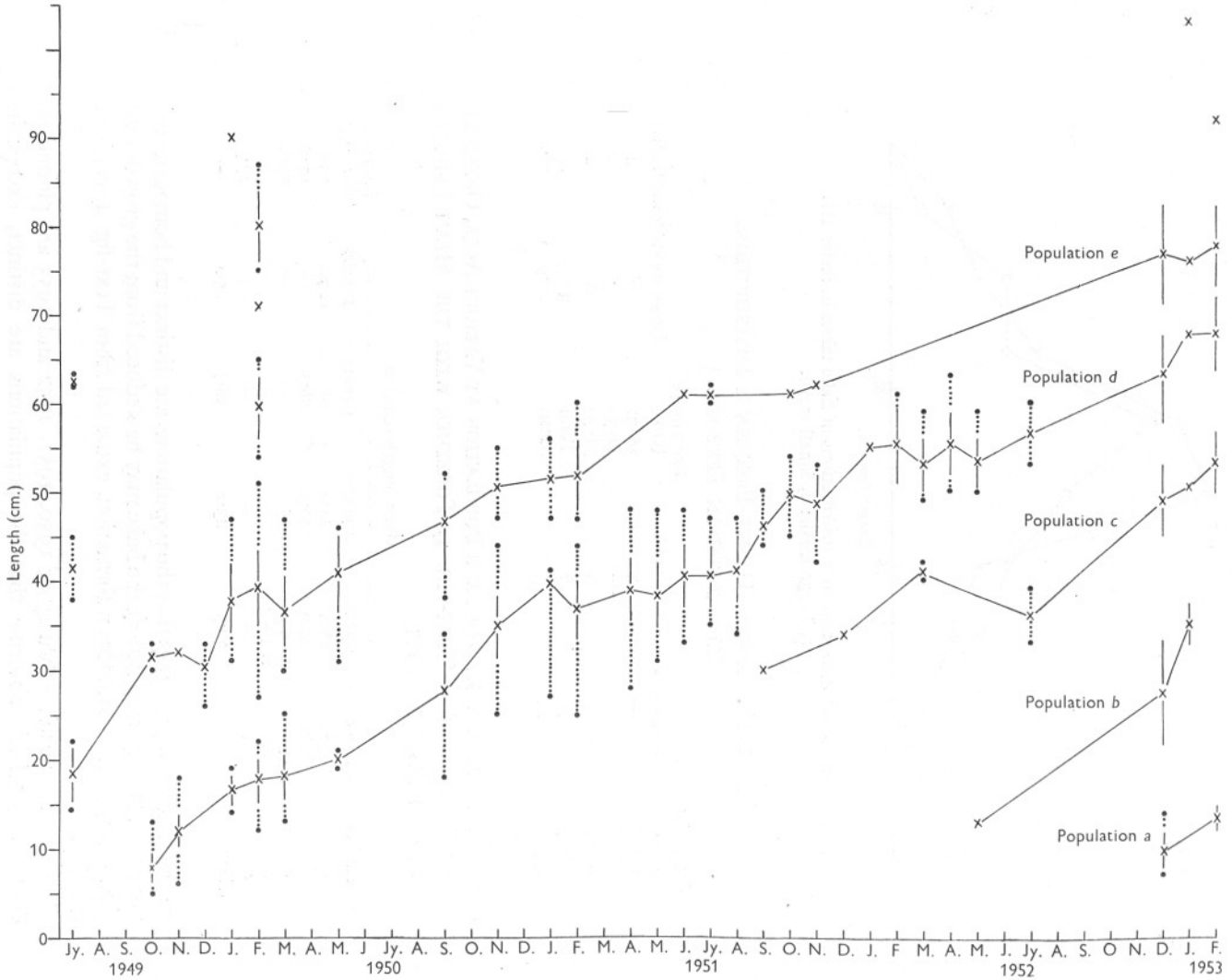
Size groups (cm.)	Large mesh	Small mesh	Size groups (cm.)	Large mesh	Small mesh
18–19	—	1	30–31	5	4
20–21	1	—	32–33	3	4
22–23	2	3	34–35	2	1
24–25	3	4	Total	37	32
26–27	11	6	Mean	27·9	27·6
28–29	10	9			

TABLE V. MEAN LENGTHS OF EACH POPULATION AT VARIOUS AGES, OBTAINED BY INSPECTION OF TEXT-FIG. 4, TOGETHER WITH THE MEAN LENGTHS DURING FEBRUARY 1953

		Mean length (cm.) at				
Population	Hatched	1 year	2 years	3 years	4 years	Length, Feb. 1953
e	1948	18·3	44·0	61	*c.* 70	77·8
d	1949	24·0	40·5	56·5	—	67·8
c	1950	*c.* 28	*c.* 45	—	—	53·3
b	1951	*c.* 18–20	—	—	—	33·6
a	1952	—	—	—	—	13·5
Mean	—	22·3	43·2	58·7	70	—

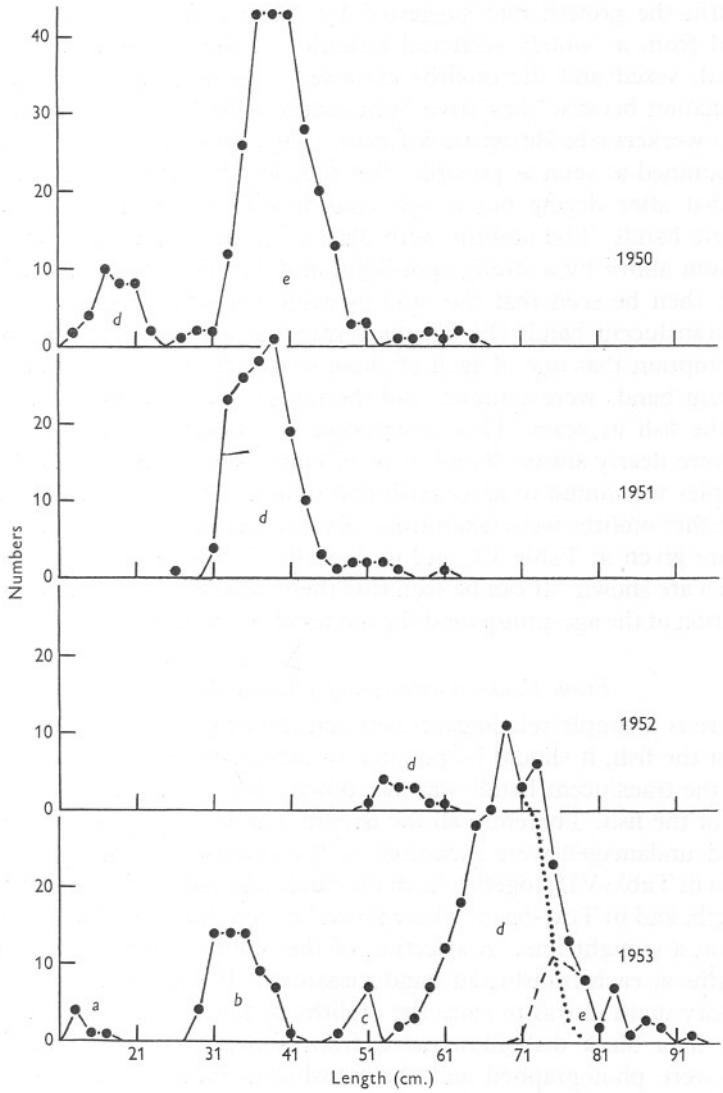
are given. The graph shows that the populations are distinct and homogeneous groups, and the growth rate of the hake may be deduced from the growth rate of each population, and this information, extracted from Text-fig. 4, is given in Table V.

The data for each February of 1950, 1951, 1952 and 1953 are plotted in Text-fig. 5, which confirms that the populations are distinct, except in February 1953 when population *e* (marked with a dashed line) was masked



Text-fig. 4. Graph showing the mean (×), ± one standard deviation (——), and the range (.....) of each hake population plotted against the month of capture.

by population *d*, but this was discovered only from the results of otolith readings. With large samples of relatively large fish, it is considerably more



Text-fig. 5. Length-frequency diagram of hake caught each February of 1950, 1951, 1952, and 1953. The letters *a–e* refer to the populations shown in Table I.

difficult to pick out the various age-classes, as, with increasing age, there is a greater possibility of overlap, and with increasing numbers, there is a greater possibility of catching the extremes of range.

From direct Otolith Readings

In December 1952 it was decided that corroborative evidence was needed to confirm the growth rate suggested by the size frequencies. Hake were obtained from as widely scattered localities in the Clyde area as possible, measured, sexed and the otoliths extracted. Otoliths were chosen for age determination because they have been found to be the most satisfactory by previous workers (see Birtwistle & Lewis, 1925; Hickling, 1933). The otoliths were examined as soon as possible after they had been extracted since it was found that after drying out it was considerably more difficult to see any concentric bands. The otoliths were placed in water in a black dish illuminated from above by a strong spot-light, and examined with the naked eye. It could then be seen that the otoliths exhibited opaque zones with intervening translucent bands (Pl. I); the centre was almost always opaque. On the assumption that one of each of these structures is formed annually, the translucent bands were counted, and the number found was taken to be the age of the fish in years. This assumption is reasonable since fish of equal length were nearly always found to be of equal age, and the age structure of the samples was found to agree with that obtained by other methods.

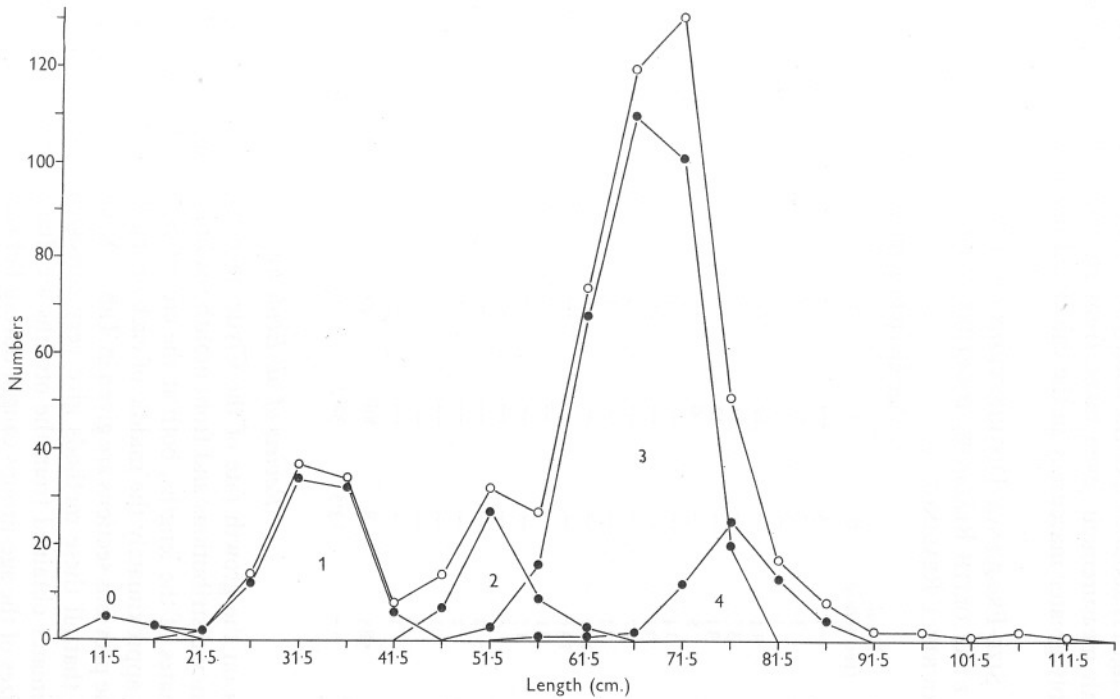
Altogether otoliths were taken from 585 fish and the results of their examination are given in Table VI, and in Text-fig. 6, where the size frequencies of the fish are shown. It can be seen that there is close agreement between the distribution of the age-groups and the modes of the total size-frequency graph.

From Back-calculation of Otolith Readings

If there is a simple relationship between the length of the otolith and the length of the fish, it should be possible to calculate the size of the fish when each of the translucent bands was laid down; that is once a year throughout the life of the fish. Therefore all the overall lengths of the otoliths that were extracted undamaged were measured to the nearest millimetre; the results are given in Table VII, together with the range and mean of the corresponding fish length, and in Text-fig. 7, where it can be seen that the points lie approximately on a straight line. A selection of the otoliths were re-examined and the lengths at each translucent band measured. It was found that the most satisfactory method was to store the otoliths in alcohol and examine them in creosote in a black dish illuminated from above. Using this method the otoliths were photographed and the translucent bands measured from the image of the negative placed in an enlarger.

It was found sometimes that the rings, though easily counted, were not necessarily clear on the long axis of the otolith; for this reason readings were not obtained from all the otoliths examined.

From these measurements the sizes of the fish during the formation of each ring were calculated, with the results given in Table VIII. In considering them



Text-fig. 6. Length-frequency diagrams of the various hake age-groups, 0–4 (●), as indicated by their otoliths, and of all the hake examined (o).

it must be remembered, first, that for each size of otolith there is often a considerable range in size of fish (see Table VII); and secondly that the otolith length is about 0·04 of the length of the fish, so that any inaccuracy in the otolith measurement (even taken from an enlarged picture) leads to a considerably greater inaccuracy in the calculated fish length.

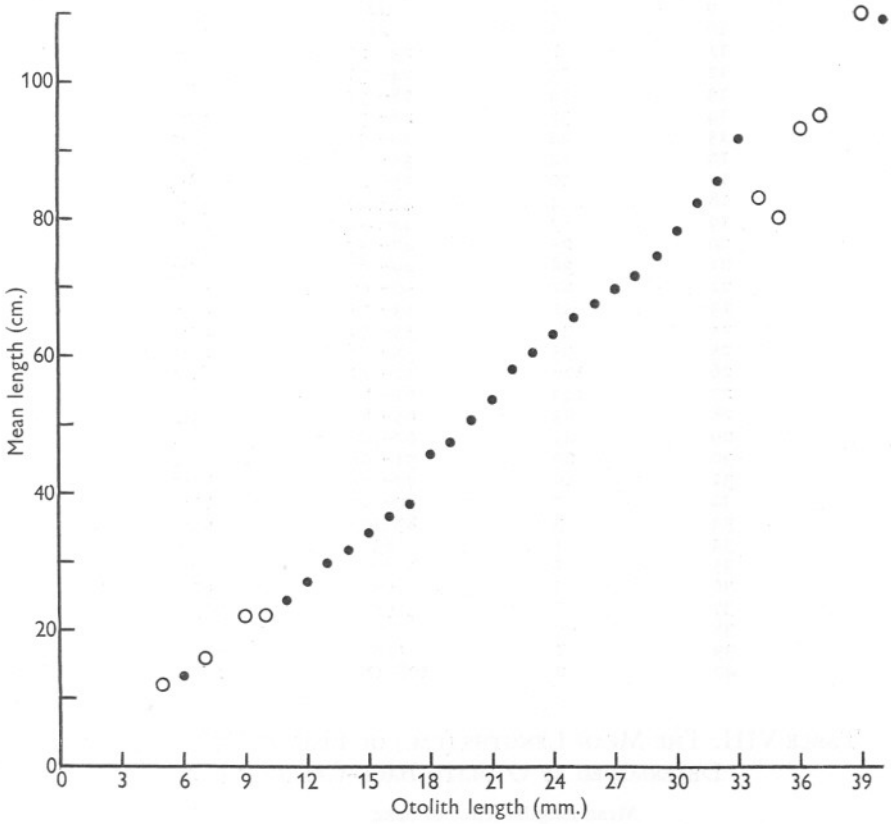
TABLE VI. SIZE-FREQUENCY DISTRIBUTIONS OF HAKE AGE-CLASSES, DETERMINED BY OTOLITH READINGS, TOGETHER WITH TOTAL SIZE-FREQUENCY AND DOUBTFUL READINGS

Size-groups (cm.)	Total frequency	Age-group frequencies					Illegible and uncertain
		0	1	2	3	4	
9– 13	5	5	—	—	—	—	—
14– 18	3	3	—	—	—	—	—
19– 23	2	—	2	—	—	—	—
24– 28	14	—	12	—	—	—	2
29– 33	37	—	34	—	—	—	3
34– 38	34	—	32	—	—	—	2
39– 43	8	—	6	—	—	—	2
44– 48	14	—	—	7	—	—	7
49– 53	32	—	—	27	3	—	2
54– 58	27	—	—	9	16	1	1
59– 63	74	—	—	3	68	1	2
64– 68	120	—	—	—	110	2	8
69– 73	131	—	—	—	101	12	18
74– 78	51	—	—	—	20	25	6
79– 83	17	—	—	—	—	13	4
84– 88	8	—	—	—	—	4	4
89– 93	2	—	—	—	—	—	2
94– 98	2	—	—	—	—	—	2
99–103	1	—	—	—	—	—	1
104–108	2	—	—	—	—	—	2
109–113	1	—	—	—	—	—	1
Total	585	8	86	46	318	58	69
Mean	—	13·5	32·7	51·8	66·5	76·0	—

Review of all Evidence

The data on the growth rate of the Clyde hake have been obtained from size-frequency distributions and from otolith readings; and each method has given estimates of the lengths, both at the end of each of the first 4 years of life, and at approximately the middle of each of the first 5 years. Data summarizing the previous sections are given in Table IX, and in Text-fig. 8. It will be noticed that all these methods give very consistent results. The slightly smaller estimates obtained from the otoliths are due to several factors: first, the mean sizes of the age-groups caught during February 1953 (*a* in Table IX) apply to the last of the 3 months during which otoliths were extracted (*c*); and secondly, the estimates for each population given in Table V (*b* in Table IX) were obtained by inspection from Text-fig. 4, while the possibilities of errors in otolith back-calculation (*d*) have already been stressed; and thirdly, it is not certain what date approximates to the mid-point of each growth-year for Clyde

hake. The otoliths of only one hake, a male of 25 cm. caught on 21 January 1953, have suggested a growth rate which does not agree with that outlined in this paper. The otolith showed three very clear translucent bands which were quite unlike subsidiary rings. Unfortunately, the fish was not kept after the otolith was removed so it was impossible to determine what had caused the slow growth.



Text-fig. 7. The relationship between the length of hake and the length of their otoliths. o, only one otolith examined.

Comparison with Previous Studies

When le Danois (1920) published his summary of the existing knowledge of the hake, it was clear that at that time very little was known about the growth rate. The first work on this was by Belloc (1923), who examined scales from 264 fish obtained from three localities: Morocco, the Bay of Biscay and south of Ireland, and he suggested that there are racial differences in the growth rate in the three regions. Later, he republished this data adding some observations

TABLE VII. OTOLITH SIZE-FREQUENCY WITH ASSOCIATED FISH LENGTHS

Otolith length (mm.)	Frequency	Range of fish length (cm.)	Mean length of fish (cm.)
5	1	12	12
6	8	13–14	13·2
7	1	16	16
8	—	—	—
9	1	22	22
10	1	22	22
11	8	23– 27	24·4
12	13	24– 31	27·0
13	14	28– 31	29·8
14	38	28– 36	31·8
15	44	30– 38	34·3
16	14	35– 40	36·7
17	10	35– 43	38·4
18	4	45– 46	45·5
19	13	44– 51	47·2
20	25	47– 57	50·6
21	38	49– 67	53·4
22	22	50– 67	58·0
23	51	53– 71	60·3
24	75	55– 74	63·1
25	93	55– 76	65·8
26	126	52– 82	67·3
27	143	61– 82	70·0
28	83	65– 80	71·6
29	49	67– 98	74·5
30	23	71– 98	78·1
31	10	77– 88	82·1
32	7	80– 92	85·6
33	3	87–101	91·7
34	1	83	83
35	1	80	80
36	1	93	93
37	1	95	95
38	—	—	—
39	1	110	110
40	2	108–110	109

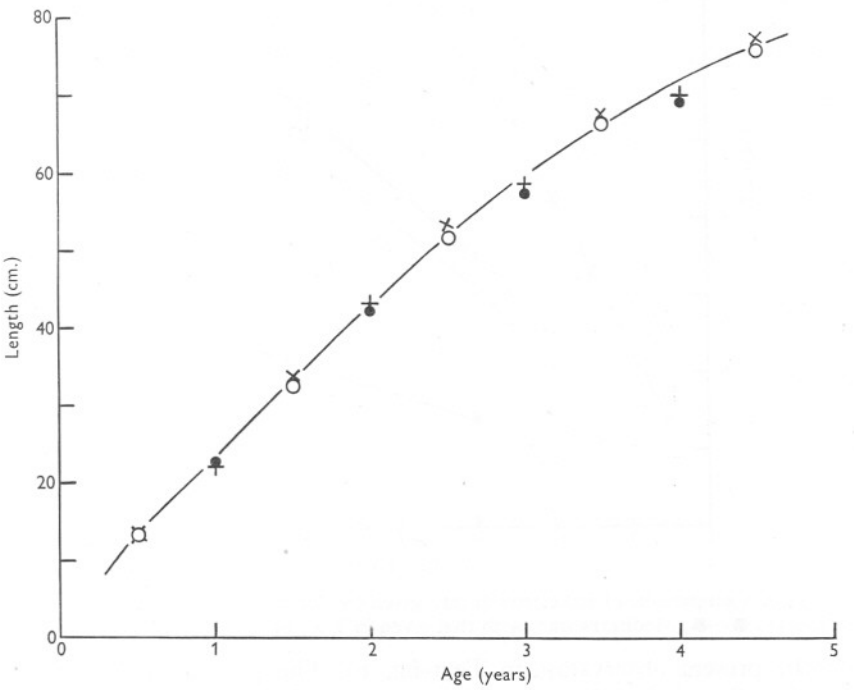
TABLE VIII. THE MEAN LENGTHS (CM.) OF FISH OF VARIOUS AGES AS DETERMINED BY OTOLITH BACK-CALCULATION

Age (years)		Mean lengths (cm.) of hake calculated from otoliths showing: Two translucent bands — No.	Two translucent bands — Length	Three translucent bands — No.	Three translucent bands — Length	Four translucent bands — No.	Four translucent bands — Length	Mean length (cm.)	Grand mean length (cm.)
1	♂	5	22·22	23	22·29	3	22·77	22·14	22·59
	♀	6	22·16	44	22·32	7	24·64	23·04	
2	♂	4	39·04	23	43·49	2	43·09	41·87	42·48
	♀	6	38·08	46	43·44	7	47·77	43·10	
3	♂	—	—	7	54·42	3	58·11	56·26	57·52
	♀	—	—	22	58·22	6	59·34	58·78	
4	♂	—	—	—	—	1	65·94	65·94	69·26
	♀	—	—	—	—	3	72·58	72·58	

on a slow-growing stock found in the Mediterranean (Belloc, 1929). His results are given in Table X and compared with the Clyde results in Text-fig. 9. It can be seen that even the fastest-growing race, those from the Morocco Coast,

TABLE IX. SUMMARY OF ALL EVIDENCE OF MEAN FISH LENGTHS AT DIFFERENT AGES; ILLUSTRATED IN TEXT-FIG. 8

Age (years) ...	0+	1	1+	2	2+	3	3+	4	4+
From length frequencies									
(*a*) Mean length, Feb. 1953	13·5	—	33·6	—	53·3	—	67·8	—	77·8
(*b*) Mean of all populations (Table V)	—	22·3	—	43·2	—	58·7	—	70·0	—
From otoliths									
(*c*) Direct reading (Table VI)	13·5	—	32·7	—	51·8	—	66·5	—	76·0
(*d*) Back-calculations (Table VIII)	—	22·6	—	42·5	—	57·5	—	69·3	—
Mean	13·5	22·4	33·1	42·8	52·5	58·1	67·1	69·6	76·9



Text-fig. 8. Summary of growth of hake from all evidence. +, from length-frequency measurements, mean of all populations; ×, from length frequencies, mean lengths in February 1953; o, from direct otolith readings, mean lengths of each age-group; ●, from back-calculation of otoliths, mean of all populations at each age.

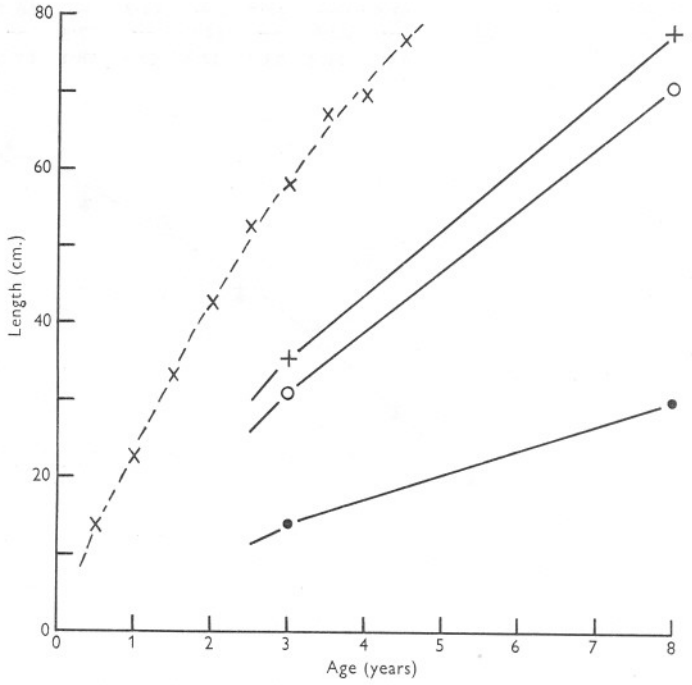
only reach a size of about 79 cm., 9 years after hatching, whereas the Clyde hake reach a comparable size during their fifth year.

The first British work published was that of Birtwistle & Lewis (1925), who studied the hake in the Irish Sea and to the north-west and south-west of

Ireland. These authors used scales and otoliths for age determination; although they found scales very difficult to read, they were forced to use them for all marketable fish. Their results are given in Table XI and compared graphically

TABLE X. MEAN LENGTHS (CM.) OF HAKE OF VARIOUS AGES FROM FOUR REGIONS, ACCORDING TO BELLOC

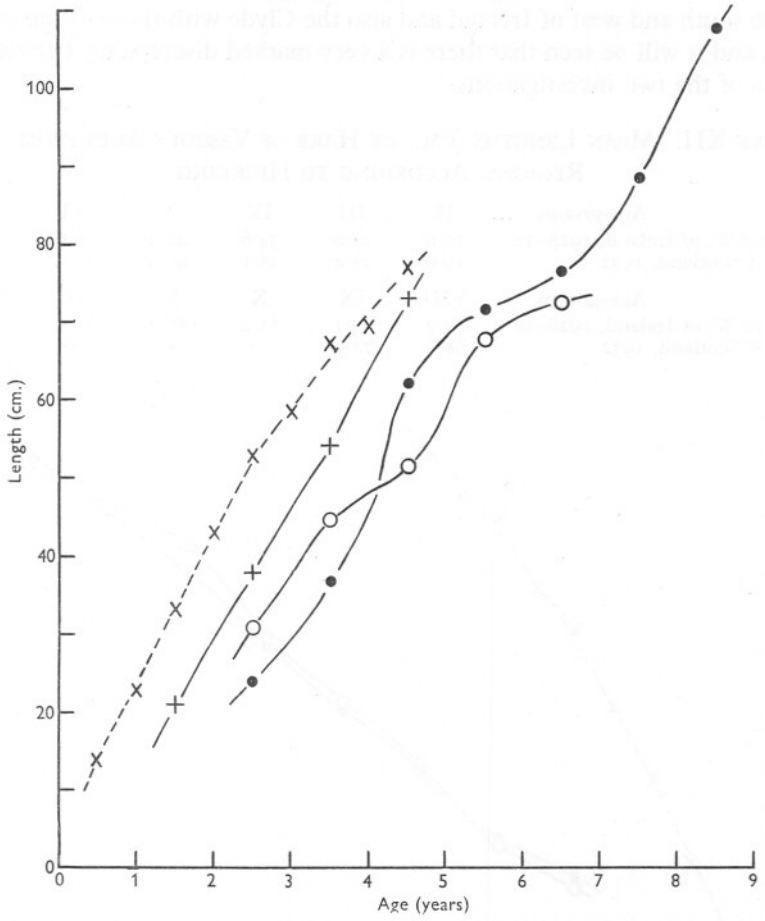
Years after hatching	Morocco	Bay of Biscay	Ireland	Mediterranean
3	35	31	—	14
8	78	71	65	30
13	87	82	75	36



Text-fig. 9. Comparison of hake growth rate given by Belloc (+—+, Morocco; o—o, Bay of Biscay; ●—●, Mediterranean) with that given in Text-fig. 8 for the Clyde (×- - -×).

with the present observation in Text-fig. 10. The growth rates of the fish studied by Birtwistle & Lewis are all slower than that described in the present paper, although the findings for the Irish Sea are quite comparable to those of the Clyde.

In 1933 Hickling published the fourth part of his 'Natural History of the Hake', in which he considers age determination and growth rate. His results are based on the measurements of 54,560 hake, of which he examined the otoliths of 25,930; as he aptly remarks 'We therefore have plentiful material on which to base our results'. These results are shown in Table XII (taken



Text-fig. 10. Comparison of hake growth rate given by Birtwistle & Lewis (+—+, Irish Sea; ●—●, S.W. Ireland; o—o, N.W. Ireland) with that given in Text-fig. 8 for the Clyde (×- - -×).

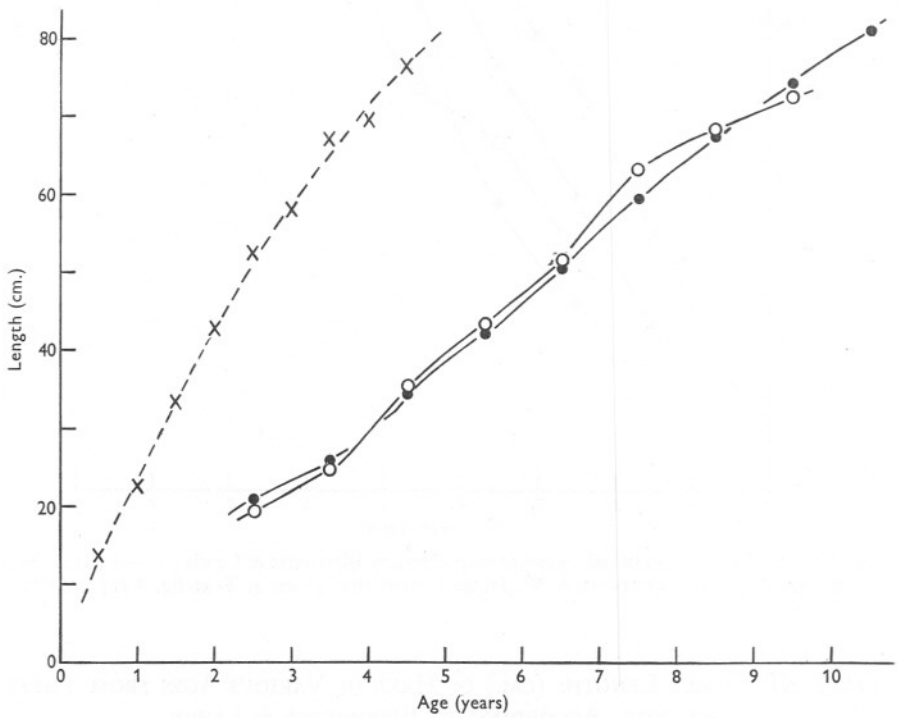
TABLE XI. MEAN LENGTHS (CM.) OF HAKE OF VARIOUS AGES FROM THREE REGIONS, ACCORDING TO BIRTWISTLE & LEWIS

Age	Irish Sea	S.W. Ireland	N.W. Ireland
1	20·3	—	—
2	37·5	23·7	30·7
3	54·0	36·8	44·3
4	72·5	61·9	51·7
5	—	71·8	67·5
6	—	76·3	72·5
7	—	88·3	—
8	—	107·5	—

from his table IX*c*). Text-fig. 11 compares graphically the results of Hickling for the south and west of Ireland and also the Clyde with those of the present work, and it will be seen that there is a very marked discrepancy between the results of the two investigations.

TABLE XII. MEAN LENGTHS (CM.) OF HAKE OF VARIOUS AGES FROM TWO REGIONS, ACCORDING TO HICKLING

Age-groups ...	II	III	IV	V	VI	VII
S. and W. of Ireland, 1928–32	20·9	25·6	34·6	42·1	50·9	59·8
W. of Scotland, 1932	19·6	25·4	35·1	43·2	51·4	63·4
Age-groups ...	VIII	IX	X	XI	XII	XII+
S. and W. of Ireland, 1928–32	67·9	74·1	81·2	86·2	82·9	97·0
W. of Scotland, 1932	68·0	72·9	—	—	—	—



Text-fig. 11. Comparison of hake growth rate given by Hickling (●—●, south and west of Ireland; o—o, Clyde), with that given in Text-fig. 8 for the Clyde (×- - -×).

Hickling (1933) explained the difference between his results and those of Birtwistle & Lewis by suggesting that the latter authors had misread the scales and halved the age of each fish, because they suggested that a check ring appears on the scale when the growth slows down in the winter, and another faint ring is formed when the summer growth recommences, so that two rings

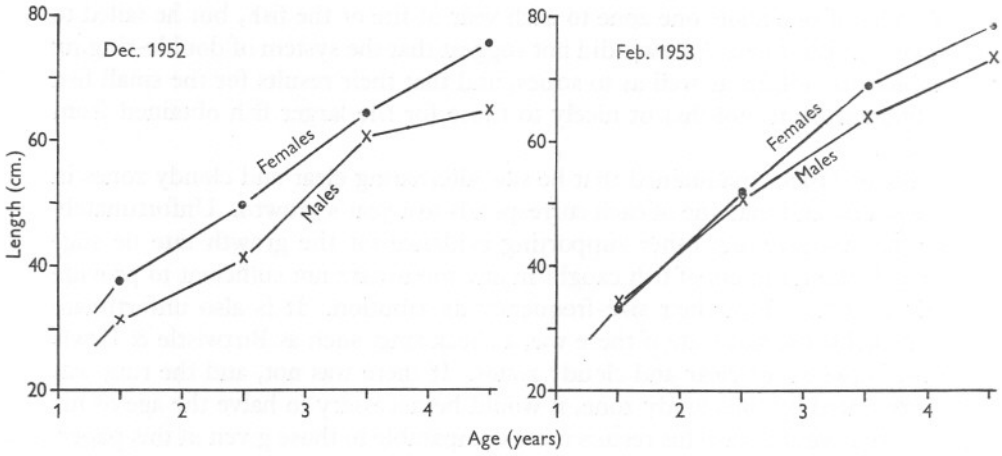
correspond to 1 year's growth. Hickling pointed out that their results agree with his if one allots one zone to each year of life of the fish, but he failed to note that Birtwistle & Lewis did not suggest that the system of double-ringing applies to otoliths as well as to scales, and that their results for the small fish (obtained from otoliths) fit nicely to those for the larger fish obtained from scales.

Belloc (1929) maintained that he saw alternating clear and cloudy zones in the scales, and that one of each corresponds to a year's growth. Unfortunately he did not give any other supporting evidence for the growth rate he suggested; the numbers of fish caught in any month are not sufficient to provide any evidence from their size-frequency distribution. It is also unfortunate that Belloc did not state if there was a check ring, such as Birtwistle & Lewis saw, between his clear and cloudy zones. If there was not, and the ring was represented by the cloudy zone, it would be necessary to halve the age of his fish; this would make his results more comparable to those given in this paper, and to those reported by Birtwistle & Lewis.

Hickling's conclusions, on the other hand, are definitely based on sufficient material, and he provides adequate supporting evidence. Moreover, he illustrates his method of age determination by means of an excellent photograph of four otoliths (his figure 11), and there can be little doubt that his interpretations are correct. Nevertheless, the mean annual increase in length from all sources, is given as 8·7 cm., while elsewhere (footnote on p. 20) an account is given of the only successful hake-marking experiment; the fish grew 11·7 cm. in 8 months which would correspond to an annual increment of 17·5 cm. No explanation is given of this discrepancy.

Sexual Growth-Rate Differences

As reported by other workers (Belloc, 1929; Birtwistle & Lewis, 1925; Hickling, 1933), it has been found that the female hake grow faster than the males. The mean sizes of the age-groups of the two sexes during December 1952 and January and February 1953 are given in Table XIII, and for two of the months the data are shown graphically in Text-fig. 12. In Table XIV comparisons are made of the sizes of the two sexes caught in the same hauls on various dates, and it can be seen that the mean length of the females becomes very significantly larger during the third year. With the 2-year-old fish, although the females are larger, only in one sample is the difference possibly statistically significant. The 1-year-old fish are somewhat anomalous because in both samples the males are the larger. In the bigger haul taken on 18 February 1953, the larger size of the males is very definitely significant. In contrast, from the results of the back-calculations of the otoliths (Table VIII), it appears that it is more usual for both sexes to be of approximately the same size until they enter their third year of life when the large size of the females becomes more clearly recognizable and consistent.



Text-fig. 12. Comparison of growth rates of male and female hake.

TABLE XIII. MEAN LENGTHS (CM.) OF MALE AND FEMALE HAKE OF VARIOUS AGES DURING DECEMBER 1952 AND JANUARY AND FEBRUARY 1953

Age-group ...		1	2	3	4
December	♂	31·0	41·0	60·5	64·7
	♀	37·5	49·6	64·6	75·8
January	♂	35·7	—	—	—
	♀	33·5	50·5	67·7	76·0
February	♂	34·2	50·6	64·3	74·4
	♀	33·1	52·0	69·6	79·9

TABLE XIV. COMPARISON OF MEAN LENGTHS (CM.) OF MALE AND FEMALE HAKE CAUGHT IN THE SAME TRAWL HAUL

		Males		Females		
Date	Age	No.	Mean	No.	Mean	Difference between mean lengths
16. i. 53	1	2	34·0	2	33·5	—
18. ii. 53	1	23	34·2	33	32·5	Highly signif., $P<0.01$
18. xii. 52	2	4	50·0	3	56·3	—
19. xii. 52	2	11	47·9	15	48·3	Not signif., $P=0.4$
13. ii. 53	2	3	50·6	4	51·0	—
11. xii. 52	3	3	51·7	14	68·9	—
18. xii. 52	3	18	63·3	16	68·7	Highly signif., $P<0.01$
19. xii. 52	3	20	59·3	59	62·6	Highly signif., $P<0.01$
16. i. 53	3	2	69·5	6	71·0	—
13. ii. 53	3	27	63·7	50	69·5	Highly signif., $P<0.01$
11. xii. 52	4	3	70·5	3	83·3	—
18. xii. 52	4	3	75·0	2	84·0	—
19. xii. 52	4	1	73·0	5	75·8	—
13. ii. 53	4	7	73·3	7	80·0	Highly signif., $P<0.01$

Regional Size Differences in the Clyde Area

In all parts of the Clyde area hake of a given age have been found to have a similar mean size, though a few of the minor irregularities should be noted. The hake caught at the head of Loch Fyne between Strachur and Inveraray, on 19 December 1952, were consistently smaller than those of the same age caught just north of Tarbert the day before (Table XV). It is indeed very remarkable to find hake so plentiful (115 were caught in 1½ hr.) in such a land-locked position as Inveraray; it must be remembered that typically the species is found on the edge of the continental shelf.

TABLE XV. COMPARISON OF HAKE CAUGHT OFF TARBERT AND INVERARAY

Age ...	1	2		3		4	
Sex ...	♂+♀	♂	♀	♂	♀	♂	♀
Tarbert, 18. xii. 53	24·9	50·0	56·3	63·3	68·1	75·0	84·0
Inveraray, 19. xii. 53	37·5	47·9	48·7	62·6	59·3	73·0	75·8

TABLE XVI. LENGTH COMPARISON OF HAKE CAUGHT OFF MOUNTSTUART AND IN BUTE CHANNEL

	20. ii. 50		28. vi. 51	21 and 22. xi. 51
Population ...	*e*	*d*	*d*	*d*
Bute Channel	41·0	18·0	41·0	51·3 (Small Mesh)
Mountstuart	38·5	18·0	40·1	47·0 (Large Mesh)

Often the depth at which the fish were caught is responsible for differences in the sizes obtained. As a general rule the larger fish are found at the greater depth. This may be seen by comparing hauls from the Mountstuart ground (*c.* 50 m.) with some from the middle of Bute Channel (*c.* 80 m.) 1½ miles away (Table XVI). This depth effect is seen also by comparing the Mountstuart catch of 15 February 1950 (see Table I) when fish of 39·7 cm. mean size were caught, with the Loch Fyne catch 2 days later, at a depth of *c.* 200 m., when the mean size was 44·4 cm.

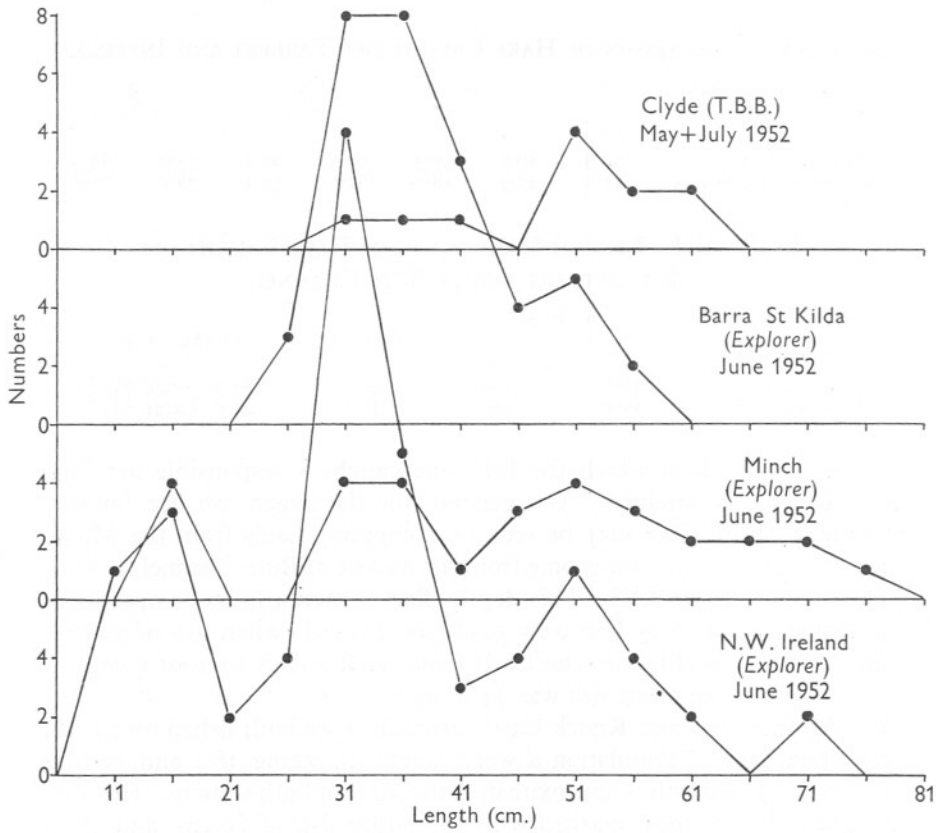
The Mountstuart and Knock Castle grounds were both fished on 23 May 1951, when hake of Population *d* were caught averaging 38·2 and 39·1 cm. respectively. The depth is approximately the same at both stations. However, the depth effect is most marked with the larger fish of 80 cm. and above. In only one instance, when one fish was obtained, have populations averaging 80 cm. and more been caught elsewhere than in the deep water of Loch Fyne and on both sides of Arran.

Growth Rate in other Scottish Areas

Through a kind invitation from the Marine Laboratory of the Scottish Home Department it was possible to join the F.R.S. *Explorer* on a west

Scotland cruise during June 1952, and thanks are due to Dr B. B. Rae for permission to use the hake length data that were obtained.

Hauls were taken at stations scattered round the north and west Scottish coasts, together with a few in the Moray Firth area and the Irish Sea. The hake lengths, measured to the nearest centimetre, have been grouped into 5 cm. classes and the length distributions summed for seven regions. These data are given in Table XVII, and Text-fig. 13, where Clyde figures for May-plus-July are also plotted for comparison.



Text-fig. 13. Length-frequency diagrams of hake from three of the Scottish areas (F.R.S. *Explorer*, June 1952), and from the Clyde during May and July 1952.

The size frequencies suggest that there are 5 year-classes present; the means of each have been calculated and are given in Table XVIII.

The yearly increments obtained from Tables XVIII and IX are given in Table XIX; the contrast between all these results and the mean increment of 8·7 cm./year suggested by Hickling (1933) is very marked.

TABLE XVII. LENGTH (CM.) FREQUENCY DISTRIBUTIONS OF HAKE FROM SEVEN SCOTTISH AREAS. F.R.S. *EXPLORER* DATA

Length (cm.)	North Sea	N. Scotland	Barra-St Kilda	Minch	N.W. Ireland	Clyde	Irish Sea
10–13	—	—	—	—	7	—	—
14–18	—	—	—	4	9	—	—
19–23	—	—	—	—	2	—	—
24–28	—	—	3	—	4	—	—
29–33	1	—	14	4	22	—	—
34–38	1	1	14	4	11	—	—
39–43	—	—	9	1	3	—	—
44–48	—	1	4	3	4	—	—
49–53	—	3	5	4	7	—	—
54–58	1	—	2	3	4	—	1
59–63	3	—	—	2	2	—	—
64–68	—	—	—	2	—	1	—
69–73	3	—	—	2	2	1	—
74–78	1	—	—	1	—	—	—
79–83	2	—	—	—	—	—	—
84–88	—	—	—	—	—	1	—

TABLE XVIII. MEAN LENGTHS (CM.) OF PRESUMED AGE CLASSES FROM F.R.S. *EXPLORER* DATA

Age classes ...	1	2	3	4	5
North Sea	—	32·5	59·7	72·0	81·5
N. Scotland	—	37	50·7	—	—
Barra-St Kilda	—	34·7	50·6	—	—
Minch	16·7	33·9	52·4	71·2	—
N.W. Ireland	14·4	32·2	49·9	66·7	—
Clyde	—	—	—	70·0	84
Irish Sea	—	—	58	—	—

TABLE XIX. PRESUMED YEARLY INCREMENTS (CM.) CALCULATED FROM TABLES XVIII AND IX

	2nd year	3rd year	4th year	5th year
From Table XVIII				
North Sea	—	27·2	12·3	9·5
N. Scotland	—	13·7	—	—
Barra-St Kilda	—	15·9	—	—
Minch	17·2	18·5	18·8	—
N.W. Ireland	17·8	17·7	16·8	—
Clyde	—	—	—	14·0
From Table IX				
Clyde	20·4	15·3	11·5	—

DISCUSSION

The results given in this paper, together with those of previous workers, suggest that the growth rate may be more variable than indicated by Hickling.

It seems that the methods of Birtwistle & Lewis were quite justified and that there are marked regional variations in the growth rate, though whether these are sufficient to be considered as 'variations ethnique' (Belloc, 1923) is

open to doubt, since there is no evidence that there are not long-term variations among the same stock; indeed three authors have considered the fish from south of Ireland and have come to very different conclusions. The 'law' propounded by Belloc (1929) is that 'Le croissance du merlu varie avec la latitude. Ce poisson crôit d'autant plus rapidement qu'il vit à une latitude plus faible', and this he attributes to the water temperature, though the variations in the growth during individual years he attributes to the availability of suitable food. Hickling does not actually consider regional differences in the rate of growth, but regional differences in size at a given age, the southern fish being larger than the northern, and this he explains as being due to earlier spawning in the south. Apparent differences in growth rate in individual years were also noted, but were dismissed as being due to inconsistent sampling. A definite explanation of the fast growth rate shown in this paper cannot be given, and it can only be suggested that the rate of growth may change over a number of years. In view of this, and the recent revival of interest in the biology of the hake (see le Gall *et al.*, 1952) it is hoped to continue the present work in the Clyde area in an attempt to pick out any possible long-term growth-rate fluctuations.

Summary

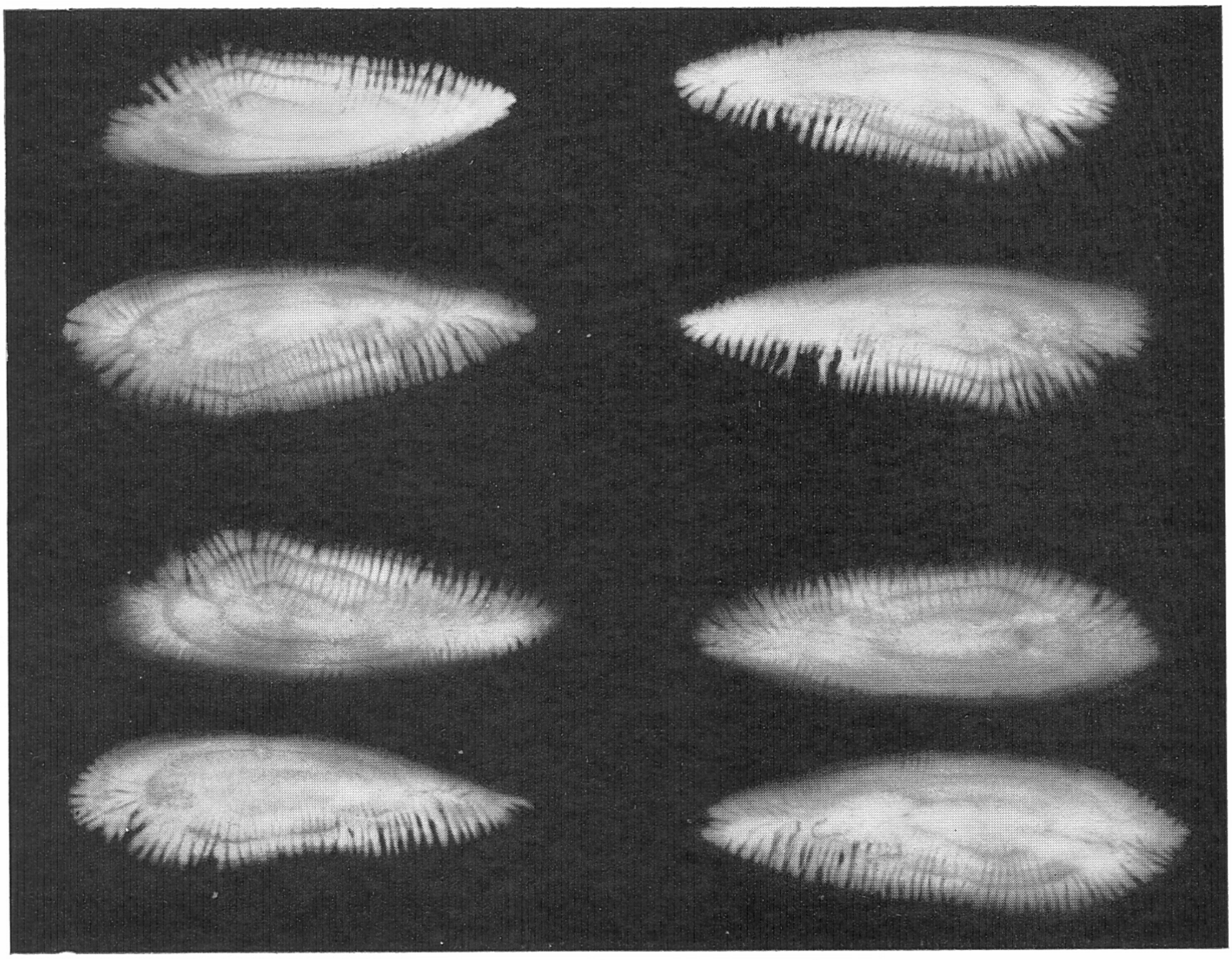
The growth rate of the hake found in the Clyde area has been studied from the inspection of the size-frequency distributions of trawl-caught fish and from an examination of 585 otoliths obtained during the winter of 1952–53. The results indicate that the annual increments decrease from over 20 cm. per annum during the first years to about 10 cm. at about 4 years of age.

These results are compared with those of other workers and found to be considerably greater than has been previously suggested; the nearest comparison being to those investigated in Manx water. It appears that the growth rate can vary considerably.

As reported by previous authors, the females have been found to grow faster than the males, though the difference does not become very pronounced until the third year of life.

The largest fish have been found in the deeper water of Loch Fyne, to the west of Arran and in Kilbrennan Sound, and this depth effect has also been noted elsewhere. The hake of all age-groups from the head of Loch Fyne have been found to be smaller than those off Tarbert, but except for these minor variations, the growth rate and size distribution has been found to be similar throughout the Clyde area.

A small amount of data on hake growth rate in other Scottish areas is given and these suggest that the growth rate noted in this paper is not confined to Clyde hake.



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EXPLANATION OF PLATE I

Otoliths of presumed three-year-old Clyde-caught hake. From top to bottom, left: ♂, 60 cm.; ♂, 63 cm.; ♀, 71 cm.; ♀, 71 cm.; right: ♀, 71 cm.; ♀, 70 cm.; ♀, 69 cm.; ♀, 72 cm.

J. Mar. biol. Ass. U.K. (1954), 33, 97–122
Printed in Great Britain

BREEDING AND GROWTH OF WHITING (*GADUS MERLANGUS* L.) IN ISLE OF MAN WATERS

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(Plate I and Text-figs. 1–6)

CONTENTS

INTRODUCTION

The whiting (*Gadus merlangus* L.) is an abundant food fish in waters to the west and south-west of the Isle of Man. As the stock is at times subject to intensive fishing it seemed desirable to study this species and possibly ascertain the effect of commercial exploitation on the biology of the fish.

The spawning period of the whiting in the Irish Sea has been investigated by Scott (1913, 1914) and Bal (1941); they deduced from counts of pelagic eggs that the spawning time extended from February to May or June, and that peak spawning occurred in April. The present investigation on seasonal changes in gonad condition of adult Manx whiting supports their conclusions.

Age and growth determinations have not previously been made on whiting from the Irish Sea.

This work was started at the suggestion of the late Prof. J. H. Orton, F.R.S., who gave much advice and encouragement. I am indebted to Dr J. W. Jones and Mr J. S. Colman for criticizing the manuscript.

Material and Methods

The whiting examined were caught between December 1948 and December 1951; few fish were taken between June and November in any one year. Three or four samples per month were taken in the seven months from December to June, which include the pre-spawning and main spawning period. The number of fish examined for gonad condition was over 3000, and the number aged (fish caught in 1950 and 1951) was a little over 2000, of which about half were '1 group' fish. The length-range of the fish sampled was 7–52 cm. and the age-range 0–8 years. Very few fish over 4 years old were taken, however.

The fish were taken by seine net and otter trawl. The cod-end mesh of the seine and one trawl was 1 in. bar, 4 in. circumference (Steven, 1950), another trawl had a cod-end mesh of $2\frac{1}{2}$ in. bar. The larger mesh was not used after March 1949 because it did not catch small whiting. In November 1950 a cover of shrimp netting, 90 rows per yard, was fitted to the cod-end of the trawl in use and thereafter only this net was used. The covered trawl effectively retained very small fish.

Hauls were made in depths between 20 and 44 fathoms (36·6–80·5 m.) to the west and south-west of the Isle of Man. The bottom deposit in 20 fathoms was sandy mud and shell, giving way to sandy mud and then soft mud as the depth increased.

Immature '0-group' fish were taken inshore by making hauls at night with a fine-meshed beach seine in Port Erin Bay in the south of the Isle of Man.

Seasonal Changes in Gonad Condition

Methods

Trawl hauls were of short duration, and all the whiting from a haul were examined. When more than 600 fish were taken by seining, random samples of 100–200 fish were examined, otherwise complete catches were used. All fish were examined fresh, i.e. within 24 hr. of capture.

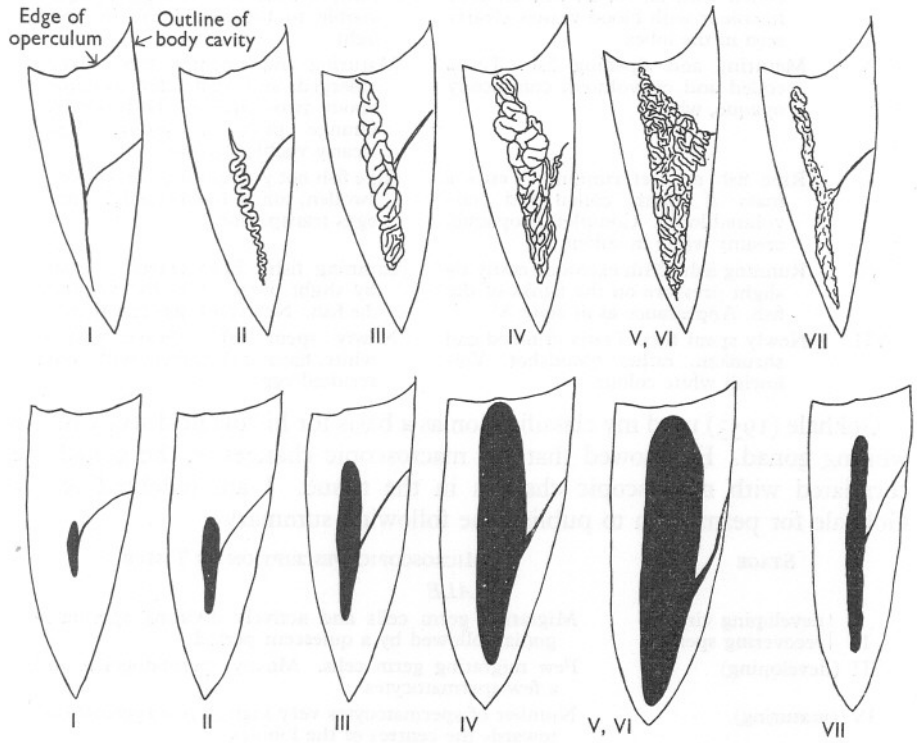
Each fish was measured to the nearest whole centimetre below its actual 'total length' (i.e. lengths from X cm. up to but not including $X+1$ cm. were all recorded as X cm.; the mid-point of the length group was taken as $X{\cdot}5$ cm.). Total length is defined as the length from the point of the upper jaw to the

end of the longest fin ray of the tail fin, which was drawn into line with the main axis of the fish.

Sex was determined macroscopically and a 'gonad stage' assigned according to an arbitrary classification into seven stages, similar to that devised by Hjort (1910) for international herring research.

Classification of Gonad Condition

Bull (1928) used a five-stage maturity scale for whiting, modified from Graham's (1924) classification of cod gonads. Bull's classification has been amplified to avoid the use of intermediate stages, and extended to include



Text-fig. 1. Changes in size of gonad in relation to the body cavity at different stages of maturity. Upper row: testis; lower row: ovary.

a separate stage for newly spent gonads of both male and female whiting. The typical appearance of the gonad at each of the maturity stages employed in this investigation is described below. The relation between gonad size and size of the body cavity, and the shape of the gonad at each stage is shown in Text-Fig. 1.

Stage	Male	Female
I	Immature virgin fish. Testis a very thin narrow translucent ribbon lying along an unbranched blood vessel	Immature, virgin fish. Ovaries very small, not more than one-fifth of the length of the body cavity, usually less than 2 cm. long. Elongated sausage shape. Whitish, translucent. Eggs microscopic
II	Maturing virgins or recovered spents in resting condition. Testis slightly lobed and lightly coiled. Opaque white at anterior end, transparent at posterior at first, later uniformly white	Maturing virgins or recovered spents in resting condition. Ovary not more than one-third length of body cavity, torpedo-shaped. Colour varying from wine-coloured, translucent, to dull orange
III	Maturing and ripening fish. Length of testis about three-quarters length of body cavity. Testis more strongly coiled with fat lobes, whitish grey in colour with blood vessels clearly seen in the lobes	Maturing and ripening fish. Ovary not more than one-half length of body cavity. Colour pink, pinkish buff, or flesh colour. Eggs opaque, visible to the naked eye in good light
IV	Maturing and ripening fish. Testis coiled and convoluted, completely opaque, white	Maturing and ripening fish. Ovary enlarged and distended occupies about two-thirds of body cavity. Orange pink in colour. Eggs clearly visible, opaque
V	Ripe fish not yet running. Testis a mass of tightly coiled and convoluted lobes. Completely opaque, creamy white in colour	Ripe fish not yet running. Ovary very swollen, tunica bursts easily. Some eggs transparent
VI	Running fish. Milt extruded easily by slight pressure on the flanks of the fish. Appearance as at stage V	Running fish. Eggs extruded easily by slight pressure on the flanks of the fish. Nearly all eggs transparent
VII	Newly spent fish. Testis crinkled and shrunken, rather bloodshot. Yellowish white colour	Newly spent fish. Ovary pinkish white, flaccid, shrunken, with some residual eggs

Gokhale (1953) used my classification as a basis for histological study of the whiting gonad. He showed that the macroscopic changes in the gonad are correlated with microscopic changes in the tissue. I am indebted to Dr Gokhale for permission to publish the following summary:

Stage	Microscopic Description of Tissue
	MALE
I {developing virgin} II {recovering spent}	Migrating germ cells and actively dividing spermatogonia, followed by a quiescent period
III (developing)	Few migrating germ cells. Mostly spermatogonia and a few spermatocytes
IV (maturing)	Number of spermatocytes very high; a few spermatozoa towards the centres of the lobules
V (full) VI (running)	Spermatogenesis complete; number of spermatozoa progressively greater
VII (spent)	Lobules almost empty; relict sperm being resorbed. Some migrating germ cells along the interlobular wall
	FEMALE
I {developing} II {virgin}	Large number of oogonia, presynaptic and post-synaptic oocytes
III (developing)	Oocytes larger: number of nucleoli increased. No oolemma in early stages but indications of oolemma forming in late stage III

IV (maturing)	Marked increase in size of oocytes. Yolk vesicles present in oocytes; oolemma present. Some immature ova undergoing degeneration
V (full) VI (running)	Eggs much grown. Some follicles empty; mature eggs lying in central cavity
VII (spent)	Few mature eggs remain, those present undergoing resorption; formation of corpora lutea. Large cavities in the tissue resulting from breakdown of interovular tissue. Loose granulosa cells and blood cells in tissue. Tunica thicker than previous stage
VII–II (spent-recovering)	Resorption of eggs nearly complete. Yellow patches as remnants of corpora lutea; formation of cell pearls. Oogonia and oocytes occupy the rest of the ovary. Tunica thick
II (recovered)	As virgin stage II, except that tunica thick

Gokhale's work showed that the spent-recovering and recovered spawner stages could have been distinguished from the developing virgin stage II in the female whiting, since the yellow patches described for stage VII–II are visible to the naked eye, and the thick tunica in stage II (recovered) can be felt between the fingers. No reliable macroscopic difference was found to distinguish maturing virgins from recovered spents in the male.

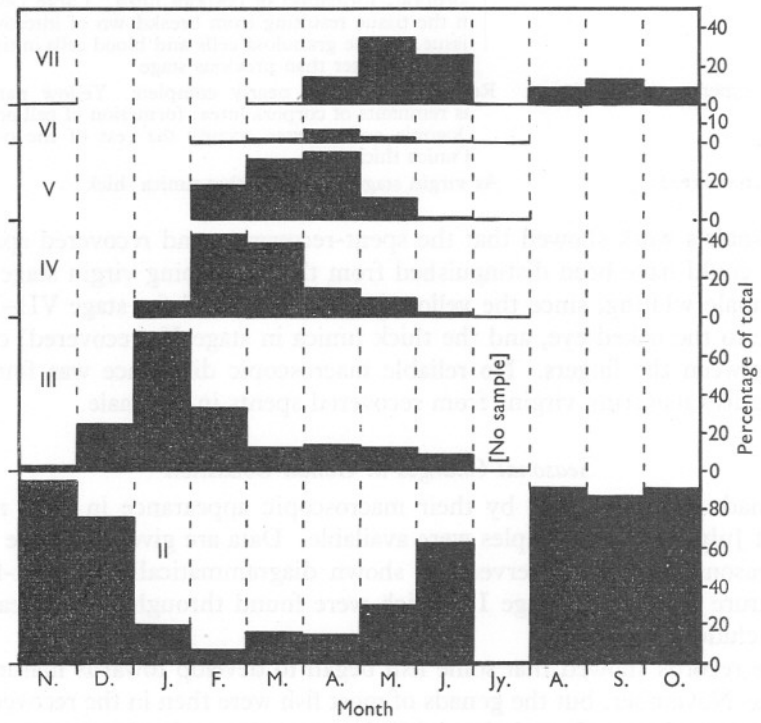
Seasonal Changes in Gonad Condition

Gonads were classified by their macroscopic appearance in each month except July, when no samples were available. Data are given in Table I and the seasonal changes observed are shown diagrammatically in Text-fig. 2. Immature virgin fish (stage I), which were found throughout the year, are not included in the table.

The records showed that some fish began to develop towards maturity as early as November, but the gonads of most fish were then in the recovered or resting state (stage II) and showed little or no change in size or appearance until January. From January until April there was a steady advance towards maturity, and in April ripe fish (stage V) were dominant. In May and June spent and recovering fish (stages VII and II) were the most common. Of the few fish examined from August to October the greater part were recovering or recovered from spawning, a few were spent, and none had maturing gonads. The large range in gonad condition in each month from February to June shows that there is wide variation in the time of the year at which different fish mature.

Running fish (stage VI) were taken between February and June inclusive; the spawning season thus extends over at least 5 months of the year. The small number of fish caught in gonad stage VI suggests that the actual spawning of an individual fish lasts only a short time, so that the chances of catching fish at this stage are low. The intensity of spawning in each month throughout the period is shown in Table II by a comparison of the number of ripe and running fish with the total number of potential spawners (i.e. fish with gonads

developing towards maturity, or spent). Most of the fish spawn or are ready to spawn in March and April and spawning is virtually over by June. A very few spent fish, however, were found in the early winter (Table I), which suggests that there may be a small amount of late spawning.



Text-fig. 2. Monthly percentage of adult fish with gonads at each maturity stage.

Spent gonads appear to recover rapidly in the whiting, as by June most of the fish had gonads in the resting stage (II). This was reflected in the marked fall in the number of spent fish caught in June (Table I). 'Recovery' implies the complete resorption of residual eggs and sperm and the change in general appearance from the shrunken, rather bloodshot spent gonad to the firm, clear gonad described above for stage II.

Size and Age at First Maturity

The smallest Manx whiting found in an advanced state of maturity, i.e. stages V, VI, or VII, were a male of 19 cm. total length and a female of 21 cm.

No female fish in age-group 1 was found with gonads more mature than stage II. Ripe fish were commonly found in age-group 2; on the other hand, no immature virgin fish were found in age-group 3. It therefore appears that

TABLE I. NUMBER OF FISH IN EACH GONAD STAGE IN EACH MONTH (IMMATURE VIRGINS NOT INCLUDED)

(Figures in brackets are values derived from very small samples. The predominating gonad stage is in bold type.)

Month		Gonad stage II	III	IV	V	VI	VII	No. of fish examined
Nov.	No. of fish	64	2	—	—	—	1	67
	% of total	**95·5**	3	—	—	—	1·5	—
Dec.	No. of fish	40	13	—	—	—	—	53
	% of total	**75·5**	24·5	—	—	—	—	—
Jan.	No. of fish	19	68	7	—	—	—	94
	% of total	20	**72**	7·5	—	—	—	—
Feb.	No. of fish	16	73	92	39	1	—	221
	% of total	7	33	**41·5**	17·5	0·5	—	—
March	No. of fish	121	88	273	228	13	7	730
	% of total	16·5	12	**37·5**	**31**	2	1	—
Apr.	No. of fish	49	45	50	117	24	47	332
	% of total	15	13·5	15	**35**	7	14	—
May	No. of fish	130	51	44	48	12	144	429
	% of total	30	12	10	11	3	**33·5**	—
June	No. of fish	87	12	3	1	1	36	140
	% of total	**62**	8·5	2	1	1	25·5	—
July				No sample				
Aug.	No. of fish	10	—	—	—	—	1	11
	% of total	(91)	—	—	—	—	(9)	—
Sept.	No. of fish	20	—	—	—	—	3	23
	% of total	(87)	—	—	—	—	(13)	—
Oct.	No. of fish	10	—	—	—	—	1	11
	% of total	(91)	—	—	—	—	(9)	—

TABLE II. MONTHLY PERCENTAGE OF RIPE AND RUNNING FISH IN THE ADULT STOCK THROUGHOUT THE SPAWNING PERIOD

Month	Jan.	Feb.	Mar.	Apr.	May	June
No. of fish examined	94	221	730	332	429	140
No. of ripe and running fish	0	40	241	141	60	2
% of total	0	18	33	42·5	14	1·5

around the Isle of Man female whiting generally spawn for the first time at the end of their second year of life, and by the end of the third year (fish which are just entering age-group 3) all normal females are mature. Male whiting may spawn at the end of their first year of life: evidence of spawning or maturity was found in approximately 4% of the age-group 1 male fish taken during the spawning season. Practically all males of age-group 2 spawn.

Spawning Habits

No evidence was obtained of segregation of ripe fish into spawning shoals. From February until July fish in all stages of maturity were found together

over all the area investigated. There are no records in the literature that special spawning shoals are formed. Ripe fish continue to feed.

The larger fish matured earlier in the season than the small fish of spawning size, and presumably spawned earlier (see Table III). In February and March, for example, the maturing females between 20 and 30 cm. long, most of them fish of age-group 2 ripening for the first time, were on average in a less advanced stage of gonad development than the females over 30 cm. long, most of which had spawned at least once before. Similar differences in time of ripening can be demonstrated among male fish of different length groups.

Records of gonad development in fish of known age suggest that fish in age-group 3 spawned earlier than did those in age-group 2, but the evidence is not conclusive; it would appear that size is more important than age in determining the time of maturation.

TABLE III. NUMBER OF FISH AT EACH STAGE OF GONAD MATURITY, SHOWN FOR DIFFERENT LENGTH-GROUPS

(Data for February and March 1949 and 1950. The most common stage of maturity is shown in heavy type.)

		Gonad stage						No. of
		II	III	IV	V	VI	VII	fish
% of female fish at each stage in Feb.	length 20–30 cm.	14	**54**	25	7	0	0	72
	length > 30 cm.	0	28·5	**51**	20·5	0	0	49
% of female fish at each stage in Mar.	length 20–30 cm.	24	25	**26**	23	1	0·5	181
	length > 30 cm.	1	5·5	30	**61**	1	1·5	124

Sex Ratio

Of 3118 fish sexed, of length range 12–52 cm., 52·8% were male, 47·2% female. Fulton (1892) found that 32·2% of the whiting he examined were male, 67·8% female. The average size of Fulton's fish was greater than that of mine, and presumably he had more large fish; it will be shown later that the larger fish are predominantly female.

Abnormalities in Gonads

Two female fish were observed, each with one ovary developing normally and the other atrophied. One fish was 27 cm. long with the normal ovary in gonad stage III, the other 30 cm. long with the normal ovary ripe, gonad stage V. No internal parasites which might have caused the abnormality were found in either fish.

Hermaphrodite whiting are rare (Desbrosses, 1948): none were seen among 3118 Manx whiting.

Spawning of Whiting in Different Regions

The spawning behaviour of the Manx whiting is intermediate between that of fish from the northern part of the geographical range of the species and fish from the southern part of the range. This is shown in relation to the spawning

season, the time of maximum spawning activity, and age and length at first maturity.

Spawning of the whiting occurs progressively later as the latitude increases. Table IV shows that there is a variation of 4 months in the onset of spawning in different regions of western Europe. Desbrosses (1945) showed that variation in spawning time is closely connected with sea temperature; in the Irish Sea gonad development is greatest when sea temperatures are low, in February and March, and spawning is at a maximum in April when sea temperature starts to rise. From Table IV it appears that in many areas maximum spawning occurs as the temperature rises after the winter minimum.

Age and length at maturity increase from south to north of the species range. The data for Manx whiting agree closely with those for Scottish fish (Fulton, 1892, 1901) and are a good fit in a table prepared by Desbrosses (1945) to show the geographical variation.

Age and Growth

Methods

Age was determined by means of otoliths, of which less than 1% had to be rejected as unreadable. Some scales were also examined, but the interpretation of many of these was doubtful because secondary growth rings were common and difficult to distinguish from true growth rings.

From March 1950, otoliths were taken from the whiting caught for the study of gonads. Adequate samples of offshore fish were obtained from March to June 1950, and from January to June 1951. Most of the samples were taken at a time of year when there is little growth in length of the fish.

Fresh fish were measured and sexed as described on p. 98. Otoliths were then removed in the following way: the fish was laid on its side, the blade of a strong scalpel was slipped under the operculum and drawn forward dorsally towards the point of the snout, thereby cutting through the skull. The otolith thus exposed was removed, undamaged, by means of forceps. It was found convenient to remove both large otoliths and place those from each fish in a separate labelled envelope. The envelopes containing otoliths from each sample were stored together in a sealed jar of spirit.

Otoliths were prepared for examination by removal of the remains of the sacculus (if present), followed by a wash in clean 95% spirit and immersion for a few minutes in pure creosote until the rings showed clearly (Johnston, 1938). Thick otoliths were found to be more easily read after the convex surface had been ground slightly with carborundum (Menon, 1950).

The otoliths were examined in a matt black dish by reflected light. It was found that any magnification confused rather than clarified the reading (cf. Hickling, 1933), and all age assessments were made after examination with the naked eye.

Table IV. Spawning Period of the Whiting in Different Localities

Region	Approx. latitude	Authority	Method of assessment	Extent of spawning period	Month of maximum spawning	Mean sea temp. at time of max. spawning (° C.)	Annual range of sea temperature (° C.)
Iceland	63–65° N.	Schmidt (1909)	Running fish and presence of eggs	May (or earlier)–Mid July	—	8 (June)	Feb. 5–6° to Aug. 10°
Iceland	63–65° N.	Ehrenbaum (1936)	Presence of eggs	—	End of May	7·5	Feb. 5–6° to Aug. 10°
Shetland	60–61° N.	Damas (1909)	Eggs and young larvae	Up to Sept.	—	—	Feb. 6° to Aug. 12°
Scotland	56–58° N.	Fulton (1892)	Ripe fish	Mar.–Aug.	Apr.	6	Feb. 5° to Aug. 12°
Scotland, E. (Firth of Forth)	56° N.	Williamson (1895)	Abundance of eggs	Mar.–June	Apr.	6	Mar. 5° to Aug. 12°
Scotland, W. (Firth of Clyde)	56° N.	Williamson (1899)	Abundance of eggs	Feb.–Aug.	Apr.	8	Feb. 7° to Aug. 12°
Ireland, W.	52–56° N.	Holt (1891, 1893)	Abundance of eggs	Mar.–June	Mar., Apr.	9	Feb. 9° to Aug. 14°
Irish Sea (Manx Waters)	54° N.	Scott (1913, 1914)	Abundance of eggs	Feb.–May	Early Apr.	9	Mar. 7° to Aug. 14°
Irish Sea (Manx Waters)	54° N.	Bal (1941)	Abundance of eggs	Feb.–June	Apr.	9	
Irish Sea (Manx Waters)	54° N.	Present investigation	Ripe and running fish	Feb.–June	Apr.	9	
Heligoland	54° N.	Heinke & Ehrenbaum (1900)	Abundance of eggs	Jan.–July	Up to May	9·5 (May)	Feb. 3° to Aug. 16·5°
Heligoland	54° N.	Heinke (1905)	Running fish Abundance of eggs	Mar.–June Jan.–May	Mar., Apr., May	3·5, 6, 9·5	
Plymouth area	50° N.	Cunningham (1889)	Presence of eggs	Feb. onward	—	—	Mar. 8·5° to Aug. 15·5°
Plymouth area	50° N.	Clark (1920)	Abundance of larvae	Feb.–July	—	—	
French Atlantic coast	46·5–48° N.	Desbrosses (1945)	Running fish	Jan.–June	Feb., Mar.	10, 9·5	Mar. 9·5° to Aug. 15·5°

Temperature data from Bureau du Conseil (1933), Danish Met. Institute (1917), Proudman, Lewis & Dennis (1937).

Description of the Whiting Otolith

The otolith of the whiting is an elongate structure, rounded or obliquely truncated anteriorly and tapering to a point posteriorly. The shape changes considerably with age (see Pl. I). The margin is crenellated. The surface which lies towards the brain is slightly convex in transverse section; the outer surface is flattened. Whiting otoliths are thinner and flatter than those of most other gadoids (Scott, 1906).

When examined against a matt black background by reflected light, alternate light (opaque) and dark (translucent) concentric zones can be seen (Pl. I). The core of the otolith may be translucent or opaque, and outside this is an opaque zone in which one or more very narrow translucent bands are often visible. This zone is bounded by a clearly marked, though often complex, translucent band: this will be called the first band and the region inside it called the centre. Alternate opaque zones and translucent bands are visible outside the first band up to a total of seven of each. The larger the fish, the greater is the number of zones and bands which may be seen.

The periphery may be formed of an opaque zone or a translucent band, dependent largely on the season of capture. Since the edge may be very thin, it is sometimes difficult to distinguish whether in fact the edge is opaque or translucent (Wallace, 1907; Jones & Hynes, 1950), but with practice it becomes possible.

The relative width of the translucent bands and opaque zones varies considerably in otoliths from different fish (see Pl. IB), but when there are more than three bands on an otolith these bands are narrower and more crowded towards the edge.

The two large otoliths from the same fish nearly always have a similar appearance and give identical readings; about 1% of the total number of fish examined had one typical otolith and one completely crystalline, showing no alternating bands and zones. In one male fish, 33 cm. long, the left otolith was larger than the right, and had at the edge a translucent band that was not present on the right otolith. This was the only discrepancy of the kind in 1088 comparisons of this nature.

Interpretation of Seasonal Changes in the Otolith

The otoliths of 0-group fish taken in July are completely opaque or show a small translucent core with an opaque peripheral area, i.e. only the centre of the otolith has been laid down. In August most of the otoliths show a very narrow translucent edge. Further growth takes place by the addition of opaque material so that the otolith shows a central core bounded by a translucent line, and an opaque edge. The translucent line may become overlaid in older otoliths but can be revealed by grinding the convex surface of the otolith.

The translucent band recognized as the first annual band is broader than

the translucent line formed in August, and is more clearly marked, although it may be of a double or complex nature, i.e. there may be one or two opaque lines within the translucent band. The annual band is laid down at the edge of the otolith in February, March and April (cf. Desbrosses, 1948). By June most of the otoliths show new growth, at the edge of the otolith, which is opaque. Thus the first annual band is formed when the fish has lived one summer and one winter after hatching, and is complete when the fish is about 1 year old. The actual age of fish in the same age-group may, however, vary by several months because there is a long spawning period, which means that a fish hatched early in the season is already 4 months old by the time the last spawned eggs hatch.

The second and subsequent translucent bands are usually laid down at the edge of the otolith between January and May. Otoliths may have a translucent edge in any month of the year but there is a well-marked maximum in February and March. Thompson (1926) found that the 'winter ring' of narrow sclerites on scales was laid down in February and that new growth started in June; this shows some correspondence with the state of the otolith.

Age estimates were made from the number of bands and zones on the otolith as follows:

Otolith with no translucent band	Age-group 0
Otolith showing centre and translucent edge	Age-group 1
Otolith showing centre, translucent band and opaque edge	Age-group 1
Otolith showing centre, translucent band, opaque zone and translucent edge	Age-group 2
Otolith showing centre, two translucent bands with opaque zone between, and opaque edge	Age-group 2

and so on up to age-group 8. In short, the fish was allotted to the age-group corresponding to the number of translucent bands seen on the otolith, irrespective of whether the outermost translucent band was at the edge or within an opaque edge.

Age and Growth as shown by Otolith Readings

Age determinations made by means of otolith readings showed that fish in all age-groups up to 8 were present in the area investigated. Age-groups 1 to 3 were well represented, but older fish were scarce. Ellis (1950) has suggested that older whiting in the North Sea migrate to deep water. A similar migration might account for the scarcity of fish over 3 years old in the samples examined; I have no evidence on this point, since this investigation was limited to depths of 20–45 fathoms.

The oldest female fish was 52·5 cm. long and belonged to age-group 8. The largest male was 47·6 cm. long but the otolith was not readable beyond the seventh band because the outermost markings were confused and indistinct; the fish was certainly more than 7 years old. Four males and four females in age-group 7 were taken. It is possible that older fish would be found in deep

water, but the age limit of 8 years agrees fairly closely with the observations of Saemundsson (1925), Desbrosses (1948) and Knudsen (1950).

The length distribution of the fish in each age-group is shown in Text-fig. 3. Numerical data are given separately for the two years 1950 and 1951 in the Appendix. The range in length and the mean length at each age-group are shown in Table V. Mean lengths at age-groups 5, 6 and 7 are probably not very reliable because the number of fish in these groups was small.

TABLE V. MEAN LENGTH AND LENGTH RANGE OF WHITING AT EACH AGE-GROUP IN 1950 AND 1951

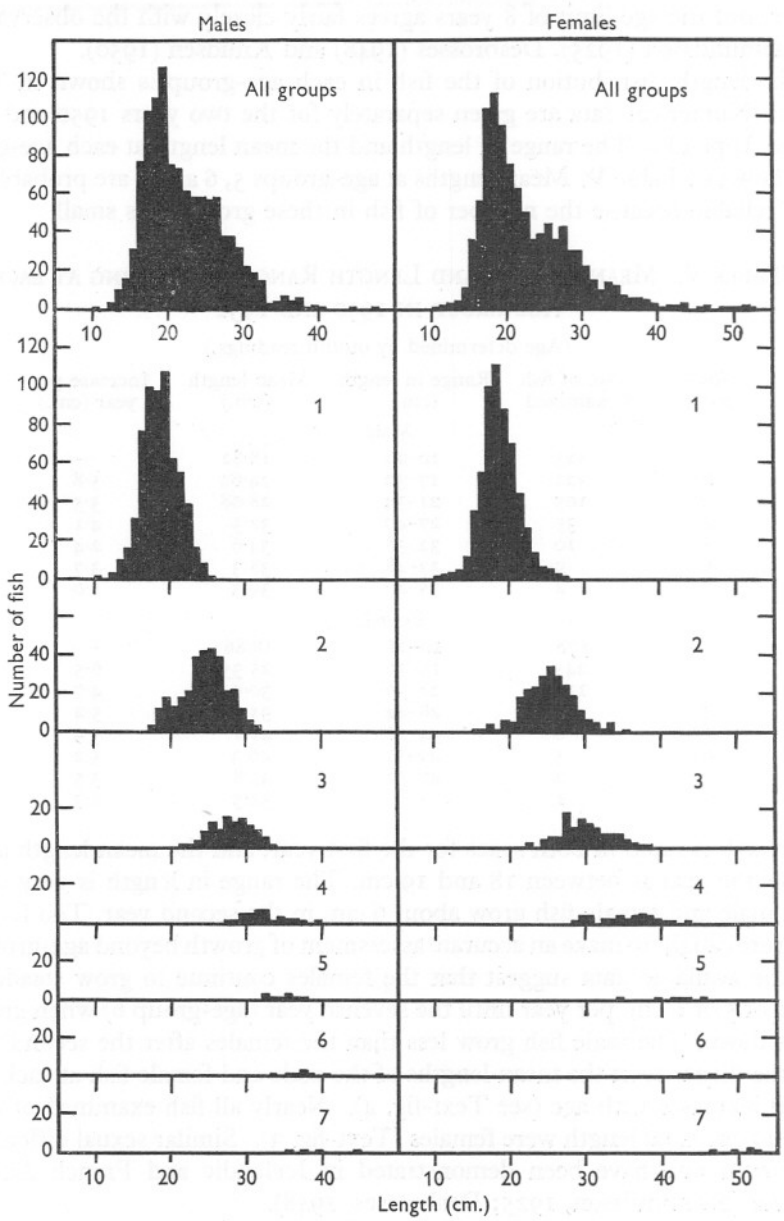
(Age determined by otolith readings.)

Age-group	No. of fish examined	Range in length (cm.)	Mean length (cm.)	Increase per year (cm.)
		Male		
1	525	10–26	18·82	—
2	322	17–32	24·62	5·8
3	105	21–34	28·08	3·5
4	35	27–40	32·3	4·1
5	10	32–38	34·6	2·4
6	9	33–48	38·3	3·7
7	4	35–46	39·3	1·0
		Female		
1	578	10–28	18·86	—
2	245	15–36	25·35	6·5
3	110	22–39	30·0	4·7
4	37	28–44	35·2	5·2
5	6	34–46	41·1	5·9
6	5	42–51	46·3	5·2
7	4	46–52	49·8	3·5
8	1	—	52·5	2·7

Growth is rapid in both sexes for the first year, and the mean length at the end of the year is between 18 and 19 cm. The range in length is very great. Both male and female fish grow about 6 cm. in the second year. Too few old fish were caught to make an accurate assessment of growth beyond age-group 4, but the available data suggest that the females continue to grow steadily at a rate of 5 or 6 cm. per year until the seventh year (age-group 6) when growth slows down. The male fish grow less than the females after the second year, and the disparity in the mean lengths of the male and female fish at each age-group increases with age (see Text-fig. 4). Nearly all fish examined of more than 40 cm. total length were females (Text-fig. 3). Similar sexual differences in growth rate have been demonstrated in Icelandic and French Atlantic whiting (Saemundsson, 1925; Desbrosses, 1948).

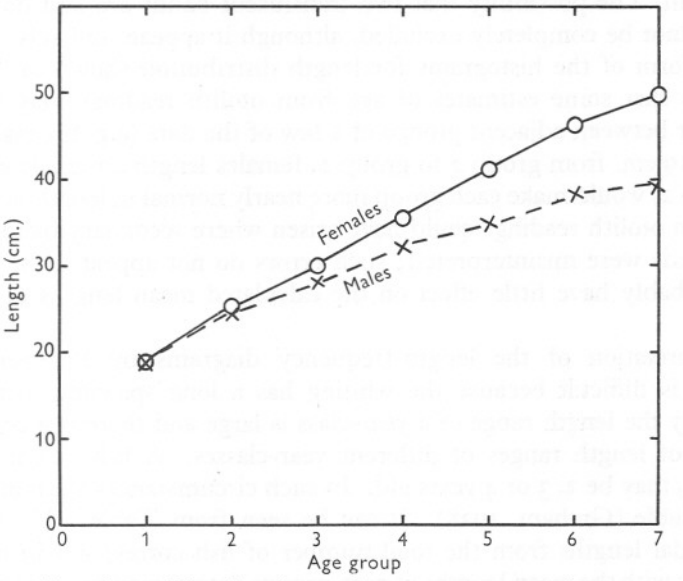
DISCUSSION

The age determinations from otolith readings were based on the assumption that growth rings on the otolith are laid down annually. There is no absolute proof available that this is true (Graham, 1928); seasonal records of the state

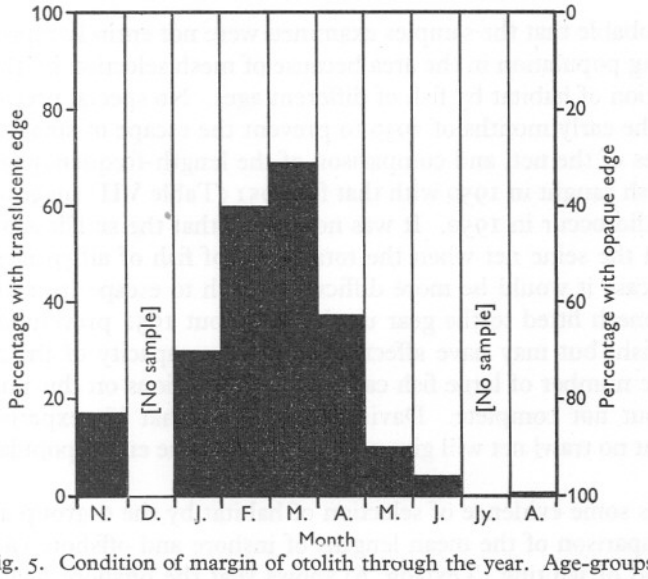


Text-fig. 3. Age and length distribution of offshore whiting, 1950 and 1951. Age determined by otolith readings. The numbers 1–7 refer to the different age-groups.

of the edge of the otolith were incomplete. Records made in 1950 are shown in Text-fig. 5; those for 1951 showed a very similar pattern. The number of



Text-fig. 4. Mean length at each age-group, 1950 and 1951.



Text-fig. 5. Condition of margin of otolith through the year. Age-groups 2–7.

otoliths with a translucent edge (i.e. those in the course of laying down a translucent year band) shows a peak in February and March when the sea temperature around the Isle of Man is lowest, but there is no complete change-over

from translucent edge to opaque edge, data are available for only seven months of the year, and the number of specimens examined in November and August was small. The possibility that two translucent bands are laid down in one year cannot be completely excluded, although it appears unlikely.

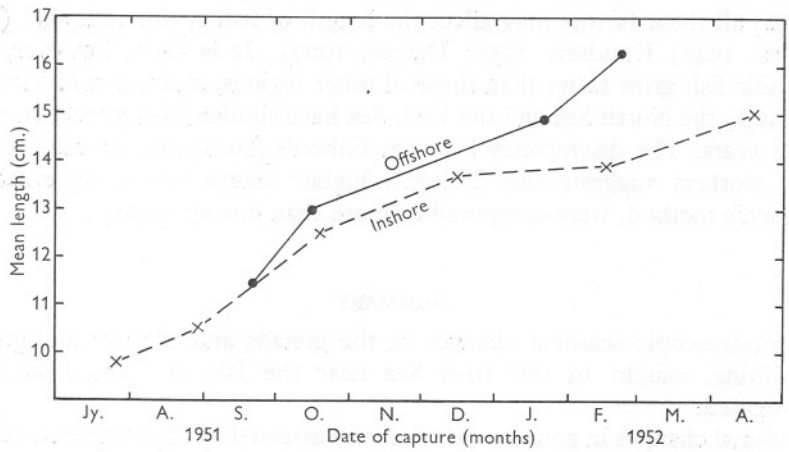
The form of the histograms for length distribution shown in Text-fig. 3 suggests that some estimates of age from otolith readings may be wrong. Transfer between adjacent groups of a few of the data (e.g. for males, length 18 and 19 cm. from group 2 to group 1, females length 27 cm. from group 3 to group 2) would make each group more nearly normal in length distribution. Errors in otolith readings could have arisen where secondary bands and true year bands were misinterpreted; such errors do not appear to be numerous and probably have little effect on the calculated mean lengths of each age-group.

Interpretation of the length-frequency diagrams by Petersen's (1895) method is difficult because the whiting has a long spawning period; consequently the length range of a year-class is large and there is a considerable overlap of length ranges of different year-classes. A fish 30 cm. long, for example, may be 2, 3 or 4 years old. In such circumstances the method is not very reliable (Graham, 1928). It can be seen from Table VII (Appendix) that modal lengths from the total number of fish correspond in only a few instances with the mean lengths of year-groups determined by otolith readings.

It is probable that the samples examined were not entirely representative of the whiting population in the area because of mesh selection by the nets used, and selection of habitat by fish of different ages. No special precautions were taken in the early months of 1950 to prevent the escape of small fish through the meshes of the net, and comparison of the length-frequency table for age-group 1 fish caught in 1950 with that for 1951 (Table VII) suggests that mesh selection did occur in 1950. It was noticeable that the smallest whiting were present in the seine net when the total catch of fish of all species was large, in which case it would be more difficult for fish to escape from the cod end. The fine mesh fitted to the gear used throughout 1951 prevented the escape of small fish, but may have affected the fishing capacity of the net so as to reduce the number of large fish caught. Investigations on this point are proceeding but not complete. Davis (1934) states that his experiments show clearly that no trawl net will give a true sample of the entire population on any ground.

There is some evidence of selection of habitat by the 0-group and 1-group fish. Comparison of the mean lengths of inshore and offshore catches of the 1951 brood of whiting (Text-fig. 6) shows that the offshore fish were larger than those inshore. Great care was taken to avoid mesh selection by the gear used, and samples were numerically large. Scattered observations in earlier years fit well with the 1951–52 data shown in the figure (unpublished work at

Port Erin). Most of the small 1-group whiting caught offshore in spring were at the shallowest limit of the fishing ground (20–25 fathoms). 1-group fish appeared sporadically in sandy bays inshore throughout the summer and autumn. The area between the bays and the offshore fishing ground is too rough for net fishing and there is therefore insufficient evidence to assess the relation between the depth of water and the size of the 1-group fish, or to determine the movements of the fish. The relation and interchange between the inshore shoals and the offshore stock is complex and requires more study, but it seems likely that there is a movement of some young fish away from inshore waters throughout the winter months from September onwards. The offshore stock is probably recruited gradually from the inshore shoals; and the larger fish move offshore in greater numbers than do the smaller fish.



Text-fig. 6. Mean length of inshore and offshore whiting of 1951 brood.

Old fish (age-groups 5–7) were usually taken in the deepest water fished (40–45 fathoms), and the largest whiting are most commonly caught by local fishermen in deeper water. Ellis (1950) found a migration of older whiting to deep water in the North Sea. Thus the largest fish of the higher age-groups may not have been represented in the samples examined, and the calculated mean length for these age-groups may be too low.

The graphs (Text-fig. 4), expressing the increase in length of whiting with age, are based on data from samples collected in the two years 1950 and 1951. A more accurate and direct statement of the growth of the fish would be obtained by sampling given year-classes in each year throughout their life span; this would involve a long-term programme. Short-term investigations, such as the present one, cannot give due weight to annual variations in growth; such variations, particularly in the growth of 0-group fish (Knudsen, 1950),

are probably the cause of anomalies in growth calculations from age analysis. Table V, for example, shows irregularities in the annual increase in length, particularly between age-groups 2 to 3 and 3 to 4, and similar irregularities are shown in the data of Damas (1909) and Desbrosses (1948).

The interpretation of Text-fig. 4 (p. 111) as a growth curve of whiting in the Irish Sea must therefore be made with reservation; variations from the curve are to be expected if data are collected in different years, and if the fish examined are caught at different depths.

The results of previous work on age and growth in the whiting are set out in Table VI. Direct comparisons between investigations are unjustified, since few workers have shown separate growth rates for male and female fish, and samples were taken in different seasons, different years, and from different depths; all these factors may affect the length of fish at different ages (Desbrosses, 1948; Knudsen, 1950; Damas, 1909). It is clear, however, that Icelandic fish grow faster than those of other regions, and that whiting from Denmark, the North Sea and the Irish Sea have similar growth rates over the first 4 years. The discrepancy between Fulton's (1901) figures and those of other workers suggests that Fulton's higher length series, separated by Petersen's method, were composed of more than one age-group.

Summary

The macroscopic seasonal changes in the gonads and the age and growth of whiting, caught in the Irish Sea near the Isle of Man, have been investigated.

Seasonal changes in gonad condition were assessed by applying an arbitrary classification of maturation into seven stages which are described.

Development of gonads from a 'resting' state to the 'full' (ripe) condition takes 3–4 months.

The whiting of this area have a long spawning period extending from February to June, with a peak in April.

Recovery from spawning is rapid.

The male whiting of this area may spawn for the first time at the end of their first year, but most fish spawn for the first time at the end of their second year or beginning of their third year of life, i.e. in age-group 2. Nearly all age-group 3 fish spawn.

The smallest spawning fish found were a male of 19 cm. and a female of 21 cm. length.

Few abnormalities in gonads were found.

Age determinations were made by means of otolith readings.

The translucent year-band in the otolith is laid down in age-group 1 fish in February, March or April, and in older fish between January and May.

TABLE VI. LENGTH WITH AGE OF WHITING FROM DIFFERENT LOCALITIES

Region	Authority	Method of age determination	Months in which samples taken	Mean length in cm. at each age-group (figures in brackets based on less than 5 fish)														
				1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8
Iceland	Saemundsson (1925)	Scales	Aug.	18·1	—	27·0	—	39·3	—	46·6	—	51·4	—	54·7	—	59·2	—	(62·5)
S. Norway	Damas (1909)	Measurements	Sept.	—	24	—	—	—	—	—	—	—	—	—	—	—	—	—
Skagerrak	Nybelin (1946 unpublished) (cited by Desbrosses)	Otoliths	Mar.	18·9	—	25·4	—	28·7	—	33	—	—	—	—	—	—	—	—
N. Denmark	Knudsen (1950)	Measurements, scales and otoliths	July	17·5	—	24·2	—	29·9	—	—	—	—	—	—	—	—	—	—
S. Denmark	Knudsen (1950)	Measurements, scales and otoliths	July	17·3	—	24·6	—	29·9	—	32·5	—	—	—	—	—	—	—	—
North Sea 54–59° N.	Damas (1909)	Scales and otoliths	Nov.	—	20·2	—	23·9	—	30·0	—	33·7	—	37·3	—	(39)	—	—	—
N. North Sea	Carr (1909)	Scales	Mar.	—	—	25·6	—	27·9	—	29·6	—	—	—	—	—	—	—	—
N. North Sea	Fulton (1901, 1902)	Measurements	Oct.–Dec.	—	23·7	—	31·3	—	46·9	—	53·4	—	—	—	—	—	—	—
N. North Sea	Thompson (1926)	Scales	—	13·5	—	21·5	—	28	—	—	—	—	—	—	—	—	—	—
Irish Sea	This investigation	Otoliths	Spring (Jan.–June)	♂ 18·8	—	24·6	—	28·1	—	32·2	—	34·6	—	38·3	—	(39·3)	—	—
				♀ 18·9	—	25·4	—	30·0	—	35·2	—	41·1	—	46·3	—	(49·8)	—	(52·5)
English Channel	Thomson (1904)	Scales	Winter	7–18	—	29–33	—	32–42	—	34–46	—	49	—	—	—	—	—	—
Gulf of Gascony	Desbrosses (1948)	Otoliths	Winter	♂ 18·1	—	23·4	—	30·3	—	40	—	(38·2)	—	(46)	—	—	—	—
				♀ 19·0	—	26·7	—	35·2	—	45·6	—	49·3	—	(59)	—	—	—	—

The maximum age of the fish examined was 8 years.

The offshore stock in the area investigated consisted mainly of fish in age-groups 1, 2, and 3, with lengths lying between 15 and 35 cm.

There is a difference in growth rate between male and female fish; the females grow faster and reach a greater maximum length.

Both male and female fish reach a length of about 19 cm. at the end of the first year. Thereafter female whiting increase in length 5 or 6 cm. per year for five years, after which the rate of growth slows down. Males grow about 6 cm. in their second year; the rate of growth then declines and is very small by the sixth year.

The limitations of the methods employed are discussed.

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EXPLANATION OF PLATE I

Otoliths of whiting ×3. A, top to bottom: ♂, 9 cm., July 1950, centre laid down; two ♂♂, 11 cm., August 1950, new growth outside centre; ♂, 18 cm., May 1950, concave and convex surfaces; ♂, 22 cm., May 1950, showing centre and first year band of extreme complexity. B: ♂, 39 cm., age-group 7 showing broad translucent bands with some 'doubling' of bands; ♀, 50 cm., age-group 6, a thick otolith with a keel-like process at the posterior end. C: ♀, 49 cm., age-group 7 showing centre, seven translucent year bands and a narrow opaque edge; the translucent bands show dark.

APPENDIX

TABLE VII. NUMBER AND LENGTH OF FISH IN EACH AGE-GROUP 1950 AND 1951

Length (cm.)	No. of fish in each age-group 1	2	3	4	5	6	7	Total
				♂ Whiting, 1950				
12	1	—	—	—	—	—	—	1
13	3	—	—	—	—	—	—	3
14	6	—	—	—	—	—	—	6
15	13	—	—	—	—	—	—	13
16	27	—	—	—	—	—	—	27
17	45	—	—	—	—	—	—	45
18	52	5	—	—	—	—	—	57
19	52	11	—	—	—	—	—	63
20	32	9	—	—	—	—	—	41
21	23	13	—	—	—	—	—	35
22	22	10	—	—	—	—	—	32
23	9	16	1	—	—	—	—	26
24	4	19	—	—	—	—	—	23
25	—	21	8	—	—	—	—	29
26	—	17	4	—	—	—	—	21
27	—	10	9	—	—	—	—	19
28	—	17	10	—	—	—	—	27
29	—	8	8	2	—	—	—	18
30	—	5	7	2	—	—	—	14
31	—	2	9	6	—	—	—	17
32	—	—	3	6	3	—	—	12
33	—	—	—	—	—	1	—	1
34	—	—	—	3	—	—	—	3
35	—	—	—	1	3	1	1	6
36	—	—	—	2	1	—	—	3
37	—	—	—	1	1	3	1	6
38	—	—	—	—	—	1	—	1
39	—	—	—	1	—	—	1	2
40	—	—	—	—	—	1	—	1
Total	289	163	59	24	8	7	3	553
Mean length	19·05	24·75	28·75	33·0	34·75	37·2	37·5	

TABLE VII (*cont.*)

♀ Whiting, 1950

Length (cm.)	No. of fish in each age-group							Total
	1	2	3	4	5	6	7	
12	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—
14	4	—	—	—	—	—	—	4
15	12	—	—	—	—	—	—	12
16	14	—	—	—	—	—	—	14
17	41	4	—	—	—	—	—	45
18	55	—	—	—	—	—	—	55
19	37	4	—	—	—	—	—	41
20	30	2	—	—	—	—	—	32
21	25	12	—	—	—	—	—	37
22	11	14	1	—	—	—	—	26
23	10	13	—	—	—	—	—	23
24	4	16	1	—	—	—	—	21
25	4	14	2	—	—	—	—	20
26	—	11	1	—	—	—	—	12
27	—	6	5	—	—	—	—	11
28	—	10	4	—	—	—	—	14
29	—	5	7	2	—	—	—	14
30	—	3	7	2	—	—	—	12
31	—	1	6	—	—	—	—	7
32	—	1	5	1	—	—	—	7
33	—	3	2	1	—	—	—	6
34	—	—	6	4	1	—	—	11
35	—	1	2	2	—	—	—	5
36	—	—	2	4	—	—	—	6
37	—	—	—	4	—	—	—	4
38	—	—	1	4	—	—	—	5
39	—	—	—	2	1	—	—	3
40	—	—	—	1	—	—	—	1
41	—	—	—	—	—	—	—	—
42	—	—	—	—	1	1	—	2
43	—	—	—	1	1	1	—	3
44	—	—	—	—	—	—	—	—
45	—	—	—	—	1	—	—	1
46	—	—	—	—	—	1	1	2
47	—	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—	—
49	—	—	—	—	—	—	1	1
50	—	—	—	—	—	1	—	1
51	—	—	—	—	—	—	2	2
52	—	—	—	—	—	—	—	1 (8 yr.)
Total	247	120	52	28	5	4	4	461
Mean length	19·30	24·98	30·85	36·0	41·1	45·75	49·75	

Length (cm.)	No. of fish in each age-group							Total
	1	2	3	4	5	6	7	
				♂ Whiting, 1951				
10	1	—	—	—	—	—	—	1
11	—	—	—	—	—	—	—	—
12	2	—	—	—	—	—	—	2
13	7	—	—	—	—	—	—	7
14	10	—	—	—	—	—	—	10
15	18	—	—	—	—	—	—	18
16	50	—	—	—	—	—	—	50
17	55	3	—	—	—	—	—	58
18	46	7	—	—	—	—	—	53
19	56	7	—	—	—	—	—	63
20	30	4	—	—	—	—	—	34
21	32	5	1	—	—	—	—	38
22	17	11	1	—	—	—	—	29
23	7	23	2	—	—	—	—	32
24	4	23	8	—	—	—	—	35
25	1	22	5	—	—	—	—	28
26	—	32	5	—	—	—	—	37
27	—	11	7	1	—	—	—	19
28	—	5	3	1	—	—	—	9
29	—	4	7	3	—	—	—	14
30	—	1	4	3	—	—	—	8
31	—	1	—	1	—	—	—	2
32	—	—	2	1	—	—	—	3
33	—	—	1	—	1	—	—	2
34	—	—	—	—	1	—	—	1
35	—	—	—	1	—	—	—	1
36	—	—	—	—	—	1	—	1
37	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—
41	—	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—	—
43	—	—	—	—	—	—	—	—
44	—	—	—	—	—	—	1	1
45	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—
47	—	—	—	—	—	1	—	1
48	—	—	—	—	—	—	—	—
49	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—
Total	336	159	46	11	2	2	1	557
Mean length	18·63	24·49	27·22	30·5	34	42	44	
Mean length 1950 + 1951	18·82	24·62	28·08	32·24	34·6	38·3	39·25	

TABLE VII (*cont.*)

Length (cm.)	No. of fish in each age-group							Total
	1	2	3	4	5	6	7	
				♀ Whiting, 1951				
10	1	—	—	—	—	—	—	1
11	2	—	—	—	—	—	—	2
12	3	—	—	—	—	—	—	3
13	4	—	—	—	—	—	—	4
14	7	—	—	—	—	—	—	7
15	23	1	—	—	—	—	—	24
16	41	1	—	—	—	—	—	42
17	59	—	—	—	—	—	—	59
18	56	1	—	—	—	—	—	57
19	52	2	—	—	—	—	—	54
20	40	3	—	—	—	—	—	43
21	19	6	—	—	—	—	—	25
22	15	8	1	—	—	—	—	24
23	3	10	—	—	—	—	—	13
24	2	13	3	—	—	—	—	18
25	1	20	3	—	—	—	—	24
26	2	18	4	—	—	—	—	24
27	1	18	13	—	—	—	—	32
28	—	9	6	1	—	—	—	16
29	—	6	8	2	—	—	—	16
30	—	3	5	2	—	—	—	10
31	—	4	4	—	—	—	—	8
32	—	—	2	1	—	—	—	3
33	—	2	5	1	—	—	—	8
34	—	—	1	—	—	—	—	1
35	—	—	2	—	—	—	—	2
36	—	—	—	—	—	—	—	—
37	—	—	1	1	—	—	—	2
38	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—
41	—	—	—	1	1	—	—	2
42	—	—	—	—	—	—	—	—
43	—	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—
47	—	—	—	—	—	—	—	—
48	—	—	—	—	—	1	—	1
49	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—
Total	331	125	58	9	1	1	—	525
Mean length	18·53	25·71	29·26	32·6	41	48	—	
Mean length 1950+1951	18·86	25·35	30·0	35·2	41·1	46·3	49·75	

J. Mar. biol. Ass. U.K. (1954) 33, 123–127
Printed in Great Britain

NOTES ON THE EARLY STAGES OF THE COMMENSAL POLYNOID *ACHOLOË ASTERICOLA* (DELLE CHIAJE)

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(Text-fig. 1)

Little is known about the development of polynoids. A large number of unidentifiable polynoid trochophores and nectochaetes have been described by Nolte (1936) and others. As Thorson (1946) states, Nolte has done little to clear up the confusion that prevails in this group and his 'numerous figures, all more or less incorrect, and accordingly difficult to recognize, merely serve to further obscure our picture'.

A search of the literature with the aid of Hartman (1951) has so far revealed only one clearly identifiable member of the family whose early larval stages have been described. Sars (1845*a*, *b*), making some of the earliest observations on polychaete life histories, described under the name *Polynoë cirrata* Fab. the early stages of *Harmothoë imbricata* (L.). Further studies in some detail on this or a closely allied species were made in turn by Müller (1851), McIntosh (1900) and Izuka (1912). The certainty of identification of the parent in this case stemmed from the fact that the early stages, with the exception of those studied by Müller, were not taken in the plankton but were observed attached to the brooding parent after release from it. In this polynoid the eggs are carried in large masses beneath the elytra, and larvae leave the parent as actively swimming trochophores. This method of carrying the young has also been observed in *H. imbricata* by Saemundsson (1918) in Greenland and by M. Pettibone (personal communication) in Puget Sound. Desor (1857) quotes the observations of Sars on the brooding habit, and describes the release of trochophores by *Polynoë squamata* (=*Lepidonotus squamatus* L. ?) on the New England coast. He does not make clear whether the form he studied also brooded its eggs beneath the elytra. However, Fauvel (1916) observed brooding in *Polynoë antarctica* Kinberg, from the Falklands, and MacGinitie & MacGinitie (1949) in *Halosydna brevisetosa* Kinberg from the eastern Pacific. Thus, brooding in polynoids may be commoner than has been suspected.

From October of 1952 until July of 1953, while the author held a Guggenheim Fellowship at the Plymouth Laboratory and was engaged in making studies (Davenport, 1953*a*, *b*) of the response behaviour of commensal annelids

to their hosts, repeated efforts were made to bring to metamorphosis and settlement the young of the polynoid *Acholoë astericola* (Delle Chiaje), commensal with the starfish *Astropecten irregularis* (Pennant). Although in a number of cultures it was possible to carry the larvae through 10–12 days of development, none were taken beyond the trochophore stage; critical experiments to determine the manner of settlement and the method of host-colonization were never possible. In view, however, of the general lack of information concerning polynoid development and the certainty of identification of the young of only one other polynoid species at the present time, it has seemed advisable to describe the stages observed, in spite of failure in bringing the life history to completion.

At no time from October to July was natural spawning observed, in spite of the fact that *Acholoë* were brought into the laboratory on their host starfish continuously during this time and kept under a variety of conditions. Eggs and sperms were obtained by cutting open worms, and fertilizations were made in filtered 'outside' sea water. Dishes were kept at the temperatures of the laboratory circulation (14–16° C.). Cultures were also placed in large plunger jars. Although trochophores were observed to feed on pure cultures of the flagellates *Dicrateria inornata* Parke and *Isochrysis galbana* Parke, after an average of 10 days all dropped to the bottom, became inactive and broke up.

The sequence of events to be described was observed with fertilizations made in the month of November, at which time in any collection the number of adult worms with active sperms or fully formed eggs was low. During the winter the number of such individuals in any collection approached nil. During the months of April and May apparent fertility increased until in July almost all individuals opened had active sperms or fully formed eggs. Thus, it would appear that *Acholoë* is a midsummer or autumn spawner, at least at the latitude of Plymouth.

Eggs freshly removed from the body cavity were pinkish in reflected light and slightly oval, the long dimension averaging approximately 85 μ, with a membrane approximately 1·5 μ in thickness. Very shortly after fertilization the membrane separated from the eggs and there often appeared a slightly raised area or 'bubble' at one pole. From 12 to 14 hr. after fertilization larvae were actively spinning and the beginning of ciliary bands had appeared. The long dimension had by this time increased to about 97 μ.

By the end of 48 hr. many of the oval prototrochophores (Fig. 1A) were actively swimming, rotating on their long axes and moving in the direction of the apical tuft. Their long dimension at this time was approximately 100 μ. The apical tuft was composed of fifteen or more very long cilia fully two-thirds the length of the larva (65 μ), while the clearly evident prototroch consisted of a single band of cilia which were approximately 20 μ long. An opaque central area indicated the beginning of the digestive tract.

By the end of 72 hr. the larvae had considerably advanced, although there was little change in size. Most had developed a single red eye-spot, though in some two had appeared. There was some reduction in size of the apical tuft,

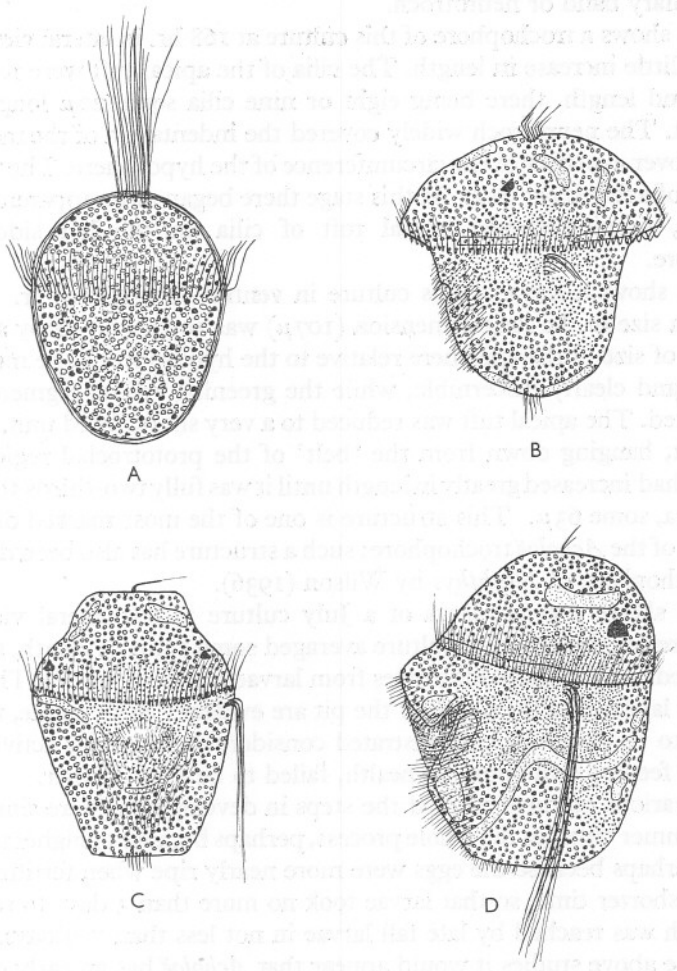

Fig. 1. Early stages of *Acholoë astericola* (Delle Chiaje). A, prototrochophore at 48 hr.; B, trochophore at 168 hr., showing beginning of left lateral tuft; C, trochophore at 216 hr. in ventral view; D, 9-day trochophore of July culture in left lateral view.

still a single-rowed prototroch and as yet no other groups of cilia. Rather large yellowish green pigment bodies had begun to make their appearance in the apical and prototrochal region and in the hyposphere.

By the fourth day one could discern stomach and intestine, in which there was ciliary activity. A small clump of cilia marked the beginnings of the telotroch. Most individuals possessed two eye-spots. At this time the larvae

had begun to assume the typical shape of the early trochophore in which the episphere appears to overhang the hyposphere.

By the sixth day little change had occurred, but one could discern a definite ventral ciliary band or neurotroch.

Fig. 1B shows a trochophore of this culture at 168 hr. in lateral view. There had been little increase in length. The cilia of the apical tuft were reduced in number and length, there being eight or nine cilia some 20μ long sunk in a small pit. The neurotroch widely covered the indentation of the mouth and extended over a quarter of the circumference of the hyposphere. The telotroch consisted of a few short cilia. At this stage there began to be apparent a short, comb-like, scimitar-shaped lateral tuft of cilia on the left side of the trochophore.

Fig. 1C shows a larva of this culture in ventral view at 216 hr. A slight increase in size of the long dimension (107μ) was accompanied by a relative reduction of size of the episphere relative to the hyposphere. The mouth was extensive and clearly discernible, while the greenish yellow pigment bodies were marked. The apical tuft was reduced to a very short, fused unit. The left lateral tuft, hanging down from the 'belt' of the prototrochal region like a scabbard, had increased greatly in length until it was fully two-thirds the length of the larva, some 63μ. This structure is one of the most marked diagnostic characters of the *Acholoë* trochophore; such a structure has also been described in the trochophore of *Nephthys* by Wilson (1936).

Fig. 1D shows a 9-day larva of a July culture in left lateral view. The trochophores of this summer culture averaged some 130μ in length, although they showed no structural differences from larvae raised in the fall. The extent of the oral lappet and the depth of the pit are evident. Such larvae, the most advanced to be observed, demonstrated considerable muscular activity. All, in spite of feeding and apparent health, failed to develop further.

In the various cultures studied the steps in development were similar, but in mid-summer cultures the whole process, perhaps because of higher temperatures or perhaps because the eggs were more nearly ripe when fertilized, took place in a shorter time, so that larvae took no more than 3 days to reach the stage which was reached by late fall larvae in not less than 7–9 days.

From the above studies it would appear that *Acholoë* has an early development that allows the organism to be widely dispersed by ocean currents, and in this respect, in spite of its being an obligate commensal as far as is known, is similar to other polynoids. The problems of settlement and 'colonization' of host remain unsolved. No young worms smaller than 0·5 cm. have ever been observed on *Astropecten*. Do the larvae metamorphose on the correct substrate and later find the host? It is possible that if eggs and sperm can be had from naturally spawning adults, and if problems of temperature and feeding can be met, such questions may ultimately be answered.

SUMMARY

The development of the larva of *Acholoë astericola* (Delle Chiaje), a commensal of *Astropecten irregularis*, is described up to the late trochophore stage. The larva of only one other identifiable polynoid worm had previously been described.

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J. Mar. biol. Ass. U.K. (1954) 33, 129–144
Printed in Great Britain

THE ANATOMY OF THE PROSOBRANCH *TRICHOTROPIS BOREALIS* BRODERIP & SOWERBY, AND THE SYSTEMATIC POSITION OF THE CAPULIDAE

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(Text-figs. 1–4)

The anatomy of some members of the family Calyptraeidae has been adequately described by previous workers (Kleinsteuber, 1913; Giese, 1915; Moritz, 1938), and a considerable amount of information has been published on the feeding and the unusual reproductive activities of *Crepidula* (Orton, 1912; Coe, 1944). Of the anatomy and way of living of the members of those other families of gastropods (Trichotropidae and Capulidae) that Thiele (1929) has united with the calyptraeids in his Stirps Calyptraeacea, much less is known. Orton (1912) and Yonge (1938) have described the feeding mechanism of *Capulus ungaricus*; and the anatomy of *Thyca*, a parasitic member of the same family, has been briefly described by Koehler & Vaney (1912). Nothing seems to be known of the anatomy or way of life of members of the Trichotropidae. Yet an investigation of some of these points seems overdue in the light of Lebour's discovery (1937) that *Capulus* passes through an echinospira larval stage. This stage does not occur in other members of the Calyptraeacea, although present in two other groups of the mesogastropods, the Lamellariacea and the Cypraeacea. The idea at once presents itself that the capulids may perhaps be more accurately classified as members of one or other of these two groups rather than as members of the Calyptraeacea. The work recorded in the following pages was directed towards answering this question, and would suggest that the original classification is the more correct.

The names used are those of Winckworth (1932).

Anatomical observations

Trichotropis borealis Broderip & Sowerby

The family Trichotropidae, of which *Trichotropis borealis* is the sole British representative, is on the whole confined to colder seas. In Britain *T. borealis* is restricted to the northernmost parts of England and to Scotland, and is not littoral. The animals used in this work were collected off Oban by Dr V. Fretter of Birkbeck College, University of London, and it is to her kindness and to that

of Mr T. G. W. Fowler, on whose boat she was working, that I owe the opportunity of examining them. They were found at a depth of 2–3 fathoms on clinker cast overboard from the fire grates of the West Highland boats. The material was fixed in Bouin and proved very difficult to section: I am indebted to Mr C. Best of the Otological Research Unit of the Medical Research Council at the National Hospital for Nervous Diseases, London, for a magnificent series of celloidin sections cut 10 μ thick.

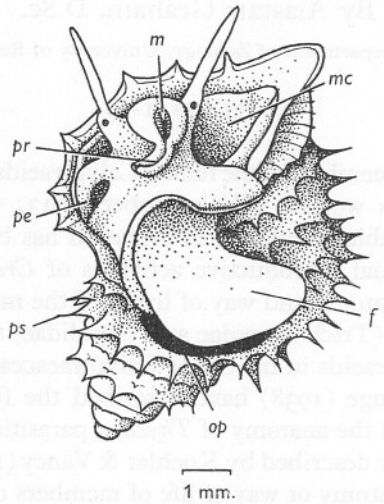

Fig. 1. *Trichotropis borealis*, drawn from a living specimen. *f*, foot; *m*, mouth; *mc*, opening to mantle cavity; *op*, operculum; *pe*, penis recurving into mantle cavity; *pr*, proboscis curving from mouth to right side of foot; *ps*, periostracal spines on shell.

In respect of its external features *T. borealis* presents the usual appearance of a small prosobranch with spirally coiled shell (Fig. 1). The shell itself is well covered with thick periostracum which is drawn into numerous pointed processes (*ps*) set especially along the lines of the spiral ridges running round the whorls. When the head and the foot are extended, it may be seen that the tentacles are long and slender, with the eyes placed about one-quarter of their length from the base. The foot (*f*) is rather abruptly cut off both in front and in the rear with a slight narrowing in the middle, but it is mobile and there does not appear to be any specialization of its anterior end, except for a degree of elongation. The posterior end carries a horny operculum (*op*). The opening into the mantle cavity (*mc*) is large and the edge of the mantle skirt is plain and does not appear to contain marginal glands apart from those concerned with the secretion of the shell. The only other point in the external characteristics of the mollusc is the presence of a short proboscis extending forwards from the mouth (*pr*). Like that of *Capulus*, this proboscis is grooved on the upper side to produce a channel which runs from the tip of the proboscis

along its length to the mouth (*m*) at its base. In normal circumstances the proboscis is kept with its tip turned towards the animal's right so that it lies dorsal to the front end of the foot and rests along the right side of that organ. A similar curvature was noted in respect of the proboscis of *Capulus* by Orton (1912) but denied by Yonge (1938). The surface of the proboscis is not ciliated except on the floor of the groove, and the structure in *Trichotropis* seems to function in the same way as the corresponding organ in *Capulus*. When the process of feeding is investigated by means of carmine powder suspended in sea water it is found that a strong current enters the mantle cavity on the animal's left, that particles from this fall on to and travel across the floor of the mantle cavity and are then carried along the right side of the head and foot, as in *Capulus*. In this way they reach the tip of the proboscis, travel up its groove and at the base of this are licked into the buccal cavity by means of the radula.

Small specimens of *Trichotropis* possess a penis (*pe*), a slightly flattened finger-shaped structure grooved on the lateral side and normally lying recurved in the mantle cavity. It is a solid organ without detectable blood spaces and there are no glands on its surface. From its base the seminal groove may be traced to the genital aperture, an elongated slit lying on the right side of the mantle skirt. Larger specimens lack the penis and are female, so that the animals seem to be successive hermaphrodites and protandrous.

The mouth leads to the buccal cavity, within which lies the buccal mass carrying the radula and strengthened by a pair of cartilages, and into which a pair of salivary glands discharges by very short ducts. The glands are small lobed pouches and manufacture a secretion of almost pure mucus, the epithelium lining the walls being composed of mucous cells alternating with slender supporting cells which are sometimes ciliated. The surface of the buccal cavity is covered with cuticle in most areas, but there is no well developed jaw. The oesophagus is long, running back to the stomach in the visceral hump. The glands of the mid-oesophagus are well developed and the morphologically ventral part of this section of the oesophagus has walls flung into a series of obliquely directed lamellae. The longitudinal dorsal folds, which separate the dorsal food channel from the glands, are long, project deeply into the lumen of the oesophagus and are wrapped one over the other so as to isolate the two parts of the oesophagus more or less completely from each other. The posterior oesophagus is, as is usual in prosobranchs, a simple tube with a ciliated columnar epithelium containing mucous glands.

It leads to the stomach, a not very capacious cavity lying directly underneath the mantle on the outer side of the visceral hump. The oesophageal opening and that of the style sac lie almost side by side at its lower end, and two wide ducts from the digestive gland open to the stomach close to the same point. From one of these the major typhlosole emerges and runs along the style sac; the minor typhlosole, which in the style sac forms the other

boundary of the intestinal groove, dies away on the stomach wall between the two apertures of the digestive gland. A considerable area (perhaps most) of the rest of the stomach wall is covered with cuticle which is raised slightly at one point to form the gastric shield. The epithelium which lines the stomach is tall and columnar, either ciliated or bearing the cuticle; apart from this it is rather featureless. In the style sac there appears the characteristic ciliated epithelium of this part of the molluscan stomach—low columnar cells, with homogeneous cytoplasm and large nuclei, bearing many cilia—with taller, narrower cells and mucous cells in the typhlosoles. No crystalline style is apparent, nor, in view of the existence of well-developed oesophageal glands, is it likely that one exists (Graham, 1939).

The digestive gland occupies the outer half of the more apical whorls of the visceral hump. Its tubules contain two types of cells, one much more abundant than the other, and clearly responsible for most of the digestive activity of the gland. I am not certain what is the function of the other type,

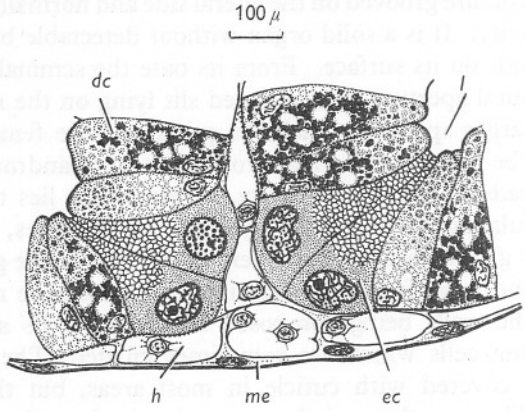

Fig. 2. *Trichotropis borealis*. Part of section across digestive gland. *dc*, digestive cell; *ec*, excretory cell; *h*, interlobular haemocoelic space; *l*, lumen of tubule; *me*, epithelium of mantle.

since I have not had enough animals to allow observation in different physiological states or to permit experiment. The first type, the digestive cell (Fig. 2, *dc*), is a tall club-shaped cell with an inconspicuous nucleus lodged in the narrow base and swelling to a tumid apex bulging into the cavity of the tubule. There are clear signs in the material that this type of cell takes particles of food into food vacuoles for an intracellular digestive process, and I suspect that the distal end of the cell is nipped off with zymogen granules at another stage of the cycle of activity.

The second type of cell (*ec*) is much less abundant and is located in crypts in the tubules especially on the sides which face the mantle (*me*). Its shape is more or less triangular in section with the base broad and a narrow apex

reaching to the lumen of the tubule (*l*). The cytoplasm is not vacuolated, except slightly so near the apex; at the base it is dense and stains rather darkly with iron haematoxylin. The outstanding feature of the cell is the nucleus, which is large, lobed, and contains a prominent nucleolus and numerous granules of chromatin. All these features suggest a high level of metabolic activity, and the broad base which the cell presents to the haemocoelic spaces (*h*) between neighbouring tubules of the gland would indicate that the cell is responsible for some process which involves the transfer of material from blood to cell or vice versa. In other gastropods (see below) this type of cell is often concerned with excretion, and this may be true in *Trichotropis* too, but I have insufficient evidence on the point.

The intestine starts at the distal end of the style sac and runs across the body from left to right to open at the anus, which is situated a little way in from the mouth of the mantle cavity. The whole length is ciliated and glandular with a thin layer of muscle external to the epithelium. Near the stomach the wall is particularly rich in mucous cells and these again become abundant near the anus; in the intervening length of intestine, however, mucous cells are scarce and the glands appear to be manufacturing some type of protein secretion since they stain intensely with such stains as iron haematoxylin. The lips of the anus, which is placed on a short papilla, are drawn out into a filament of some length hanging freely into the pallial cavity.

The faecal pellets are well compacted, short, oval bodies containing sand grains, diatom cases, sponge spicules and vegetable debris—the typical faecal contents of a detritus feeder.

In the nervous system of *Trichotropis borealis* the cerebral ganglia lie close together above the oesophagus and posterior to the salivary glands. Behind them and below are the pleural ganglia, so closely associated with the cerebrals as to form a single dumbbell-shaped mass of nervous tissue on each side. The pedal ganglia with their associated statocysts, however, lie well forward and ventrally so that the cerebropedal and pleuropedal connectives are relatively lengthy. The supra-oesophageal and suboesophageal ganglia lie close to the oesophagus, the former a little posterior to the nerve ring, the latter directly under the right pleural ganglion. It has, in fact, established a zygoneurous connexion with this which is absent on the other side of the body. No dialyneury exists on either side. The visceral ganglia lie ventrally in the visceral hump, both to the left side of the mid-line, one close to the kidney, which it innervates along with the reproductive apparatus, the other near the pericardial wall, to which it sends branches as well as supplying the stomach and the digestive gland.

The osphradium, ctenidium and hypobranchial gland are all well developed structures within the mantle cavity. The osphradium is a bipectinate structure as in many prosobranchs. The gill lies along, rather than across, the mantle

cavity, and the filaments are like those of *Capulus* as described by Yonge (1938). The hypobranchial gland is extensive and rich in secreting cells, of which there appear to be three different sorts, intermingled with ciliated cells. The last are excessively slender cells with wedge-shaped distal ends squashed between the bulging gland cells. Most of the gland cells secrete mucus, but there are also two kinds secreting material which is not of that nature. Mucous cells also occur on the gill filaments and abound on the floor of the mantle cavity so that vast quantities of that substance are available for the trapping of food particles and for their transport to the proboscis. The mantle skirt and the internal parts of the body are largely occupied by a coarsely reticulate connective tissue.

The gonad, a testis in young animals and an ovary in older ones, occupies half of the upper whorls of the visceral hump, lying on the concave inner side of the spiral. It is divided into lobes which converge on a gonadial duct which takes a convoluted path down the visceral hump to the renal and pallial sections of the reproductive tract (Fretter, 1946). In the male phase this section is used as a seminal vesicle and is occupied by masses of mature sperm, unorientated. It is not muscular and is lined by a squamous epithelium which is not ciliated.

Near the ventral end of the visceral hump the gonadial duct joins the renal section of the genital tract, a narrow tube with a ciliated cubical epithelium and some muscle fibres in the walls. It has no pericardial connexion. The renal vas deferens ends by opening into the pallial section, which, even in the male phase, is already showing the rudiments of the female accessory glands, and which may, perhaps, have some prostatic function. The male duct leads on to a ciliated tract which runs along the right (outer) wall of this section. The duct opens to the mantle cavity by a narrow, slit-like aperture and the ciliated tract extends as far as the anterior border of this. For a short distance before it reaches this point, however, it is applied against a similar ciliated tract located on the right body-wall of the snail. Sperm can therefore pass, partly by ciliary action, partly by their own movement, from the genital duct to the seminal groove and so to the penis.

Even in the male phase, as was noted by Giese (1915) in the course of his investigation of the genital tracts of the calyptraeids and of *Capulus*, the beginnings of the later female system are well developed. In *Trichotropis* a receptaculum seminis runs dorsally from the point where the renal and pallial sections of the duct join, in the form of a narrow, blind tube, the inner end of which branches to form a tuft of three short tubules. The main part of the pallial section takes the form of a large glandular pouch within which areas may be distinguished as the albumen and capsule glands. The fundamental histological structure of all these regions is the same—excessively tall and narrow supporting cells, often ciliated, being wedged between gland cells, all of which lie within the basement membrane of the epithelium.

Variation affects only the nature of the secretion elaborated by the glands. In the albumen gland the cells contain spherules which do not stain with iron haematoxylin; while in the capsule gland the granules of secretion usually do take up this stain and there are also, at its inner and outer limits, strips of cells which are exclusively mucous. There is no visible bursa copulatrix, and on copulation it must happen that the penis is inserted directly into the capsule gland.

The oldest animal of which I had sections had still ripe spermatozoa in the spaces of the gonad and in the proximal section of the genital tract, whilst the receptaculum seminis was empty: the animal was therefore still functionally male. Nevertheless, the gonad contained eggs of all sizes, some of which measured 0·3 mm. in diameter and were laden with yolk granules. It appears that this animal was close to the point when change from male to female was imminent, and it would probably have acted as female at the next reproductive phase.

Capulus ungaricus (L.)

The feeding mechanism of this animal has been described by Orton (1912) and Yonge (1938), and the contents of the mantle cavity noted, but otherwise its anatomy is not well known. *Capulus* is limpet-like in shape, with the shell tip tilted backwards like a Phrygian cap. The shell has a wide and round mouth which cannot be closed by an operculum, and it is covered by a thick, brown, periostracal layer, fringed at the edges but smooth elsewhere. The head is not elongated, bears two short, flattened tentacles with eyes at their bases and also a proboscis like that of *Trichotropis* but longer, with the mouth at its base and a groove dorsally. As Yonge (1938) notes, it is mobile and not restricted to one position as that of *Trichotropis* appears to be. The foot is rounded but has a thin anterior part which can be stretched well forward, and it is on to the upper surface of this, as Yonge (1938) has shown, that the food particles from the mantle cavity are led before being licked up by the proboscis. The opening into the mantle cavity is large and the edge of the mantle skirt is simple and devoid of marginal glands apart from the shell gland. The columellar muscle is horseshoe-shaped with the mantle cavity embraced between its two anteriorly directed arms. In this the lobed osphradium and the ctenidium extend obliquely forward from the left arm of the horseshoe, with the space between ctenidium and the right arm of the horseshoe occupied by the hypobranchial gland, the rectum and the reproductive ducts.

The alimentary tract of *Capulus* is simpler than that of *Trichotropis* but in general is similar to it: The oesophageal region, as noted earlier (Graham, 1939), is devoid of glandular equipment apart from mucous cells, and is a relatively simple tube passing to the stomach. The stomach (Fig. 3) lies in a vertical position on the left side of the visceral hump, its morphologically

anterior end almost at the apex of the hump, where the oesophageal aperture is placed, whilst the intestine opens from the style sac ventrally. As in *Trichotropis*, two large ducts lead from the digestive gland, both opening near the point of entry of the oesophagus. From one of these the major typhlosole (t_1) runs to the style sac, the minor typhlosole (t_2) originating on the stomach wall near the oesophageal opening. A gastric shield (*gs*) lies on the right side of the stomach. From the stomach the intestine (*i*) runs across to the anus and is flung into a posteriorly directed loop on the way. It is rich in mucous cells, especially near the anus.

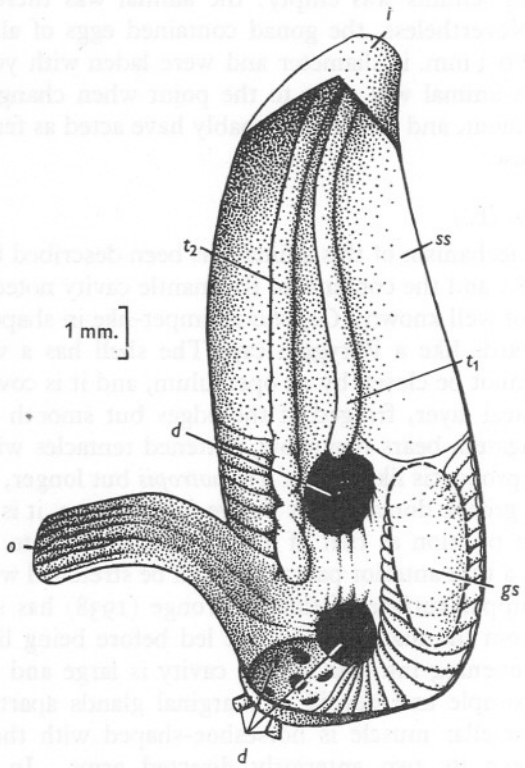

Fig. 3. *Capulus ungaricus*. Dissection of stomach. *d*, ducts of digestive gland; *gs*, gastric shield; *i*, intestine; *o*, oesophagus; *ss*, style sac; t_1, major typhlosole; t_2, minor typhlosole.

In the digestive gland of *Capulus*, as in that of *Trichotropis*, two cell types occur: one digestive, the second with another function. The former is a tall club-shaped cell presenting indications of secretory or phagocytic activity, depending upon the stage in the digestive cycle at which the mollusc has been killed. The second cell is lower, of pyramidal shape, and is tucked into the angles of the tubule with the broad base presented to the intertubular haemocoelic spaces. The cell has dense cytoplasm with a few granules

embedded in it and a large lobed nucleus. It is difficult, in the absence of experimental results, to be any surer of the action of this type of cell in *Capulus* than of its homologue in *Trichotropis*; but the activity is more likely to be secretory or excretory than concerned with the direct manipulation of food particles.

Capulus possesses a nervous system (Fig. 4*A*) built upon similar lines to that of *Trichotropis*, the sole major difference detected between the two being that in addition to the zygoneurous connexion established between the right pleural (*pg*) and the suboesophageal (*sbg*) ganglia, there is a dialyneury (*d*) brought into being by an intermingling of the left pallial nerve (*lpn*) from the left pleural ganglion with the osphradial nerve (*lon*) from the supra-oesophageal ganglion (*spg*): this occurs where the two nerves pass alongside one another over the shoulder of the columellar muscle on their way into the mantle skirt. The two visceral ganglia (*vg*) lie ventrally in the basal part of the visceral hump, as in *Trichotropis*, though perhaps more closely than in that mollusc.

As is well known, *Capulus* is a successive hermaphrodite, each animal passing through a young male phase, when it is provided with a penis and the rudiments of the accessory female glands, to reach a definitive female phase when the penis is lost. The general anatomy of the reproductive system has been previously described by Giese (1915), and I have nothing significant to add to his account of this system: its most important features are the absence of a gonopericardial duct, the presence of a seminal groove from genital pore to penial apex, the lack of penial glands in the male phase, and the development of an albumen gland and receptaculum seminis at the inner end of the capsule gland in the female stage. The receptaculum has the form of a finger-shaped pouch slightly branched at the distal extremity. There is no bursa copulatrix.

Calyptraea sinensis L. and *Crepidula fornicata* L.

The anatomy of these animals agrees in all points with that of the calyptraeids described by Kleinsteuber (1913) and Moritz (1938). It need not, therefore, be elaborated; and it will suffice to mention points of difference between the members of this family, the trichotropids and the capulids. While the general body shape is limpet-like, more of the spiral coiling of the visceral hump has been retained in the calyptraeids than in *Capulus*, although the visceral mass is completely symmetrical in the related genus *Janacus* (Kleinsteuber, 1913). The mantle edge, though still smooth, shows more complexity than in the other families: it is bifurcated and folded over the entrance to the mantle cavity so as to form the food pouch described by Orton (1912) and by Yonge (1938); it is also more extensive, forming a large shelf-like projection round the body of the gastropod. It is no longer simple, but shows a triple folding, the most dorsal part being a simple fold in direct contact with the shell above, lodging the shell gland and covered by an

unciliated cubical epithelium ventrally. The middle fold, by far the largest of the three, contains blood vessels, two circumpallial nerves and a series of large marginal glands which discharge to the ventral side. This fold of the mantle edge has retractor muscle fibres running into it which originate on the shell, and it is covered laterally and medially by an unciliated cubical epithelium, but on its ventral surface by a ciliated columnar epithelium which is rich in mucous cells. The marginal glands are spherical bodies opening by a long, narrow duct lined by squamous cells. The secreting cells are grouped

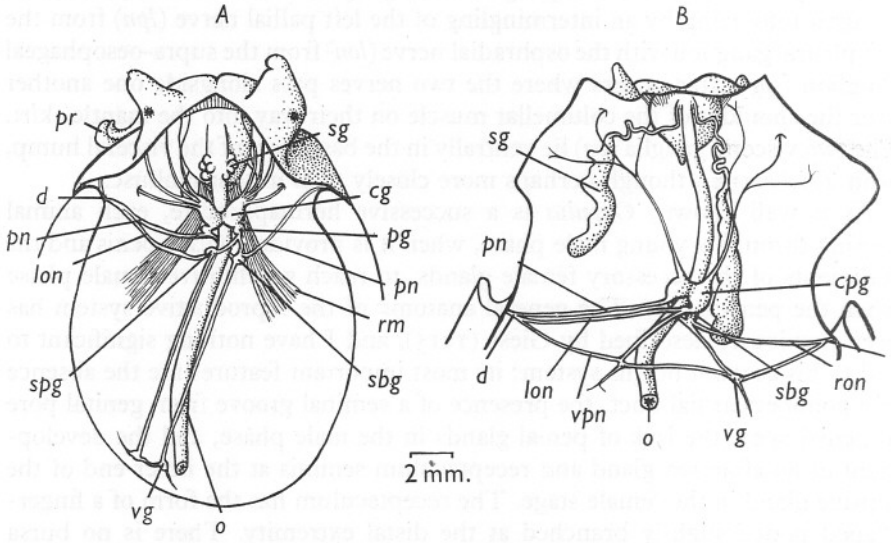

Fig. 4. *A*, dissection to show part of the nervous system of *Capulus ungaricus*. *B*, dissection to show part of the nervous system of *Crepidula fornicata*. *cg*, cerebral ganglion; *cpg*, fused cerebral and pleural ganglia; *d*, point of dialyneury; *lon*, left osphradial nerve from supra-oesophageal ganglion; *o*, oesophagus; *pg*, pleural ganglion; *pn*, pallial nerve from pleural ganglion; *pr*, proboscis; *rm*, retractor muscle of proboscis; *ron*, right osphradial nerve from suboesophageal ganglion; *sbg*, suboesophageal ganglion; *sg*, salivary glands; *spg*, supra-oesophageal ganglion; *vg*, visceral ganglion; *vpn*, pallial nerve from visceral ganglion.

to form the spherical body and are filled with dense cytoplasm. The nature of the secretion is unknown, but it seems to be secreted on noxious stimulation and the glands are therefore repugnatorial. The third fold of the mantle edge is a small one constricted off the medial edge of the second. This triple subdivision of the pallial edge is, as already noted by Odhner (1932), characteristic of the archaeogastropod and bivalve mantle and would seem to be primitive.

The head of a calyptraeid has been elongated in what may be spoken of as the 'neck' region, that is behind the tentacles and penis, and is there provided with a pair of lateral extensions, the cephalic lappets. Their development is

associated with the ciliary food-collecting mechanism of these animals. In relation to that, too, the mantle cavity extends far up the visceral hump on the left side, forcing the shell muscles to lie on the right and preventing them from forming a horseshoe as in *Capulus*. The length of the gill filaments, as described by Orton (1912) and Yonge (1938), has also increased in this connexion and the ctenidial axis (Graham, 1939) has been converted into what Orton (1913) described as an 'endostyle'—an accessory gland producing mucus for the trapping of the food particles. The osphradium is small and pectinate; the hypobranchial gland is well developed.

The alimentary canal (Graham, 1939) is simple. In the buccal cavity the radula is weak and rests on a small buccal mass. The salivary glands (Fig. 4*B*, *sg*) are larger structures than in either trichotropids or capulids. They are tubular, reaching posteriorly from the buccal cavity as far as the level of the nerve ring, the anterior third of this length being occupied by a narrow duct, the rest by gland. In the glandular section three types of cell occur: narrow, ciliated cells squashed between gland cells of two sorts, the more numerous secreting a substance which is not mucus, the second type secreting mucus. There are no oesophageal glands and the stomach contains a crystalline style. The oesophagus enters it at its posterior end and the intestine, which leaves the style sac anteriorly, has a figure-of-eight loop on its course to the anus. The digestive gland contains two types of cell, one digestive and the other excretory. This second type invariably has large vacuoles in the cytoplasm in which lie concretions, often yellow in colour and partly crystalline in nature, though in other individuals the vacuoles may be filled with many minute granules. Since these concretions may also be seen in the faeces, and since the cells in which they lie have broad bases facing the blood spaces in and around the digestive gland, it seems safe to suggest that they consist of waste material. Nothing comparable may be detected in the other cells of the digestive gland, although it is into these that experimentally introduced foods find their way. The excretory matter in the other cells appears therefore to be endogenous rather than faecal in nature. It is possible that no secretion of digestive enzymes takes place from the gland.

The excretory organ is clearly composed of a nephridial gland in close relationship to the pericardial cavity and a large nephridial sac discharging to the mantle cavity.

The nerve ring lies at the posterior end of the 'neck' and therefore some way behind the buccal mass. The dorsal ganglia—an apparently single pair—are formed from fusion of the cerebrals and pleurals, the boundaries between the two being obliterated (Fig. 4*B*, *cpg*). On the right side the supra-oesophageal ganglion lies contiguous to the right pleural, of which it simply appears to be a lobe, whilst the suboesophageal (*sbg*) lies similarly ventrally. Still more ventrally are placed the pedal ganglia. A zygoneury has therefore been established on the right side, and since fusion occurs on the left (near the point

where the nerves pass to the mantle skirt) between the osphradial nerve from the supra-oesophageal ganglion (*lon*), the pallial nerve from the left pleural ganglion (*pn*) and a pallial nerve from the visceral (*vpn*) and, similarly, on the right between osphradial nerves from the suboesophageal ganglion (*ron*) and a pallial branch from the right visceral, there is a very complete link up in the nervous system. The two visceral ganglia (*vg*) lie only a short distance posterior to the nerve ring and also rather far apart, so that the visceral loop has a triangular shape with the base between the two visceral ganglia.

The general plan of the reproductive system of calyptraeids has been described by Giese (1915), and I have nothing to add. The animals are protandrous hermaphrodites, the duct has a gonopericardial canal and the penis has glands at its base and apex.

Discussion

When the anatomical points described above are taken into consideration, it seems that all three families of gastropods may fairly be thought to possess a high degree of similarity of structure.

The shell in at least two of the three families shows a thick periostracal layer which tends to be drawn out into processes. In accord with their mobility the trichotropids preserve the spirally wound visceral hump and shell of the typical prosobranch. They may gather food with their proboscis as well as collect it out of the water current maintained through the mantle cavity. With greater dependence on this current as a source of food less mobility is required and the limpet-like shape of *Capulus* and the calyptraeids is adopted, although that has been achieved in different ways in these two groups, the mantle cavity being elongated in a transverse direction in the capulids (which allows the formation of a horseshoe-shaped columellar muscle) and in a longitudinal direction in the calyptraeids. The evolution of repugnatorial glands is presumably related to the lack of operculum and the immobility of the animals. A similar evolution attends the alimentary tract: *Trichotropis* has an oesophagus which shows the usual prosobranch structure and the animal has no crystalline style. In the calyptraeids there has been developed the style, presumably as a ready source of enzyme in connexion with the microphagous habit; and it seems likely that *Capulus* has evolved in the same way. The oesophageal glands, as is usual in such cases, are lost and the radular apparatus reduced, though there is increasing complexity in the mantle cavity as that part of the body evolves in efficiency as a food collecting apparatus. The fundamental organization of the stomach, with intestinal and oesophageal apertures at opposite ends, two large openings to the digestive glands close to the oesophageal aperture and no spiral caecum, appears similar in all, as does the structure of the gland itself with two contrasting types of cell. In each of the types the intestine runs across from left to right to the anus and is flung into a backwardly directed loop on the way.

In all three groups the nervous system is modified in identical fashion and the only significant difference is in the varying shape of the visceral loop: this is long in *Trichotropis*, moderately long in *Capulus* and distinctly short and triangular in the calyptraeids. But that is, in fact, the kind of change in shape which would accompany a change from the normal prosobranch appearance to the limpet facies and probably has no deeper significance.

The reproductive system is a further example of agreement. All the animals are hermaphrodite; all have the rudiments of the female organs present in the young, male, phase; and all have a seminal groove leading forwards to and along a slender penis which disappears when the animal becomes female. The plan of the glands in the female phase is similar in all three. It seems to be only in *Crepidula* that this special timing of reproductive activity has become the basis of special behavioural patterns.

In their reproduction, too, there seems to be a certain degree of similarity. All appear to lay their eggs in capsules (Thorson, 1935; Lebour, 1937) which may be fastened to bivalve shells (*Trichotropis*), or to stones or shells; they are often incubated by the parent (*Calyptraea*, *Crepidula*, *Capulus*). All, however, differ in the stage at which the young animals escape. In *Crepidula* the eggs give rise to free-swimming veliger larvae; in *Capulus* to free-swimming echinospira larvae; whereas in both *Calyptraea* and *Trichotropis* the veliger is suppressed and the young hatch as crawling miniatures of their parents.

The echinospira larva occurs also in Lamellariacea and in Cypraeacea. To what extent do the members of these groups resemble the Calyptraeacea anatomically? Their anatomical characters have been described by Vayssière (1923), Thiele (1929), Rau (1934) and Fretter (1946, 1951) and may be summarized in the following paragraphs.

There is little tendency for the production of periostracum around the shells of these animals; instead, another tendency becomes obvious—for the mantle to grow over the shell and ultimately enclose it completely. The mantle edge has no special marginal glands but is extended into a respiratory siphon.

The animals are all macrophagous carnivores, provided with jaws and proboscis for the manipulation of their prey, which tends to be predominantly sessile tunicates and coelenterates—as must, inevitably, be the case when such a slow-moving animal as a gastropod mollusc turns carnivore (Graham, 1953). The alimentary canal is well provided with extensive oesophageal glands (Amaudrut, 1898; Fretter, 1951) and the stomach is on the whole simpler than that of the calyptraeacean, as might be expected in view of their food (Graham, 1949).

The foot (Fretter, 1951) is provided with a posterior mucous gland in addition to the ordinary anterior one and in that respect differs from the foot of the calyptraeacean, although there is a tendency in all three groups to lose the operculum. The explanation of the loss is, however, clearly different in

the two cases: in the one it is part of the trend towards a limpet-like way of living; in the others it is part of the trend towards an internal shell.

Other general resemblances between the two groups of gastropods become obvious when their nervous and reproductive systems are compared. In the former there is a considerable degree of concentration, bilateral zygoneury occurring in both Lamellariacea and Cypraeacea and the pleural ganglia being closely adpressed to the cerebrals. In the reproductive system the sexes are separate in Lamellariacea and Cypraeacea and there is no bursa copulatrix in the female. In *Simnia* an open seminal groove runs from the male pore to the penis, but in *Trivia* and *Erato* and in the Lamellariacea the entire male duct is closed except for an accessory opening, perhaps of the nature of a safety valve, which puts the prostate into communication with the mantle cavity. The egg-cases are of relatively delicate construction since they are (in those species that have been observed) given protection by being sunk into the tissues of the ascidians upon which the adult animals normally feed; from them free-swimming echinospira larvae are produced (Lebour, 1935). *Simnia*, though clearly a member of the Cypraeacea in all other respects, is anomalous in its reproductive behaviour since the capsules which it produces are attached to its food on the surface (colonies of *Alcyonium* or *Eunicella*) and the larvae which emerge from them are ordinary veligers.

The picture of an idealized and generalized member of the Cypraeacea or Lamellariacea which emerges from this brief list of their outstanding characters is, clearly, of an animal distinctly different from that of the calyptraeacean. There is, without doubt, a much greater degree of relationship between *Capulus* and the other members of the Calyptraeacea than between it and the members of either the Lamellariacea or Cypraeacea. The echinospira larva is not a stage upon the presence or absence of which weighty arguments can rest: the Cypraeacea and Lamellariacea are akin without appeal to their common possession of that type of larva. *Simnia* is undoubtedly a cypraeacean even though it lacks an echinospira, and *Capulus* is equally a calyptraeacean even though it does possess one.

If this relationship be granted it is also true that the Cypraeacea, the Lamellariacea and the Calyptraeacea may all be regarded as lying close together in the great galaxy of the mesogastropods, and one obvious indication of this is the occurrence of the echinospira larva in all. Its presence, however, should not be taken to indicate more than that.

Summary

The anatomy of *Trichotropis borealis* is described. The salient points are the possession of a spirally coiled shell with an operculum and a suboral proboscis, grooved dorsally, which curves to the right side of the head and collects food particles obtained from the water current in the mantle cavity. The alimentary

canal has small salivary glands and reduced jaws, but well developed glands in the mid-oesophagus and no crystalline style. The stomach is relatively simple with two large ducts opening from the digestive gland. Two main types of cell occur in this gland, one digestive in function, the other believed to be excretory. In the nervous system the cerebral and pleural ganglia are united, the supra- and suboesophageal ganglia have migrated well forwards and a zygoneury and a left dialyneury both occur. The animals are successive hermaphrodites and are protandrous. In the male phase a grooved penis occurs connected by a seminal groove with the genital pore in the mantle cavity. Rudiments of female organs are already present at this stage and later form a capsule gland with an albumen gland and receptaculum seminis. No bursa copulatrix occurs in the female and the penis disappears.

In *Capulus ungaricus* the animal has become limpet-like with a round shell and no operculum. Oesophageal glands have been lost but otherwise the gut and other soft parts conform to the same pattern as those of *Trichotropis*. Of *Crepidula fornicata* and *Calyptraea sinensis* the same may be said. Fusion in the nervous system is more extensive and feeding is carried out entirely by ciliary means; there is no proboscis.

Capulus possesses an echinospira larval stage. Comparison of its anatomy with that of members of the Lamellariacea and Cypraeacea, which (*Simnia* apart) also possess such a larva, shows that it is nearer to the other members of the Calyptraeacea. Possession of the echinospira, therefore, suggests a general relationship of these three superfamilies rather than a closer relationship of *Capulus* to the Lamellariacea and Cypraeacea than to the Calyptraeacea.

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Printed in Great Britain

THE ECOLOGY OF BRITISH SPECIES OF *ENSIS*

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(Text-figs. 1–5)

CONTENTS

INTRODUCTION

In a recent paper describing the distinguishing characters of British species of *Ensis* (Holme, 1951), it was concluded that three valid species occur in British waters: *E. siliqua* (L.), *E. arcuatus* (Jeffreys) and *E. ensis* (L.). This paper is an attempt to analyse the ecological factors controlling the distribution of these species, particular attention being paid to the effect of soil grade.

In addition to those acknowledged in a previous paper, I would like to thank the following for their assistance: Dr A. G. Lowndes, Mr L. Birkett, Mr C. Edwards, and Mr O. D. Hunt. Specimens of *E. minor* were kindly supplied by Dr R. Tucker Abbott, U.S. National Museum.

I am indebted to the Director and Staff of the Station Biologique at Roscoff for facilities provided during a visit to the Station. Dr P. N. J. Chipperfield kindly placed at my disposal the research launch of I.C.I. Paints Division, Brixham, from which the dredgings in Torbay were made. I am grateful to

the Director of the National Institute of Oceanography for facilities provided on R.R.S. *Discovery II*, from which the bottom-sampling station in Aberporth Bay was worked.

The Habitat of *Ensis*

Ensis is found burrowing in sand at low-water mark of spring tides, and also occurs in shallow water offshore. On the beach, it burrows nearly vertically in the sand by means of a powerful foot, but it does not seem to possess a permanent burrow. The short siphons project just above the surface of the sand, when covered by water, and draw in water from just above the bottom. When disturbed, *Ensis* burrows rapidly into the sand, often emitting a jet of water into the air as it starts to burrow. Along a beach at low tide, *Ensis* may be located by the presence of keyhole-shaped depressions in the sand, which become more evident when the surface water has drained away, and also by the jets of water produced when they start to dig. It is possible to survey quite long stretches of beach in this way, only digging when a burrow is seen.

On a number of occasions the animals have been seen to come right out of the sand at low tide, and lie on the surface. This habit is also shared by certain other lamellibranchs living in the same habitat (e.g. *Spisula*, *Donax* and spiny *Cardium*).

On wave-exposed beaches, *Ensis siliqua* may be the only lamellibranch present, but in more sheltered areas the smaller *E. ensis* may be found, together with such species as *Venus striatula* (da Costa), *Donax vittatus* (da Costa), *Tellina fabula* Gmelin, *Gari fervensis* (Gmelin), *Mactra corallina cinerea* Montagu, and *Lutraria lutraria* (L.). The burrowing crab, *Corystes cassivelaunus* (Pennant), and the heart-urchin *Echinocardium cordatum* (Pennant) also occur in these localities.

Ensis arcuatus, which inhabits a coarser grade of soil, may be found with *Dosinia exoleta* (L.), *Tellina donacina* L., and the heart-urchins *Spatangus purpureus* O. F. Müller and *Echinocardium pennatifidum* Norman.

One or other of the species of *Ensis* occurs on practically every beach where there is sand of a sufficient depth and some slight protection from wave action. There may, however, often be none in small patches of sand between rocks: such sands are often highly reducing (see p. 158). *Ensis* does not occur in water of reduced salinity, although its absence from estuaries may sometimes be due to the lack of deposits of a suitable grade. A list of sandy beaches in which *Ensis* has not been recorded is given in Table VII (Appendix).

Methods

In order to eliminate so far as possible the effect of characteristics peculiar to individual beaches, collections were made over as wide an area as possible. On the shore, collections have been made on most of the sandy beaches in S. Devon, S. Cornwall, and the Scilly Isles (Fig. 1). I have also received

samples collected in various other parts of the British Isles. Additional samples have been obtained from certain beaches in Jersey and the north Finistère coast around Roscoff. A list of localities is given in Table VII (Appendix).

Grab or dredge collections are chiefly from Great West Bay (Holme, 1950), off Plymouth (some stations are in Holme, 1953), and in St Austell Bay off the Cornish coast (Fig. 5).

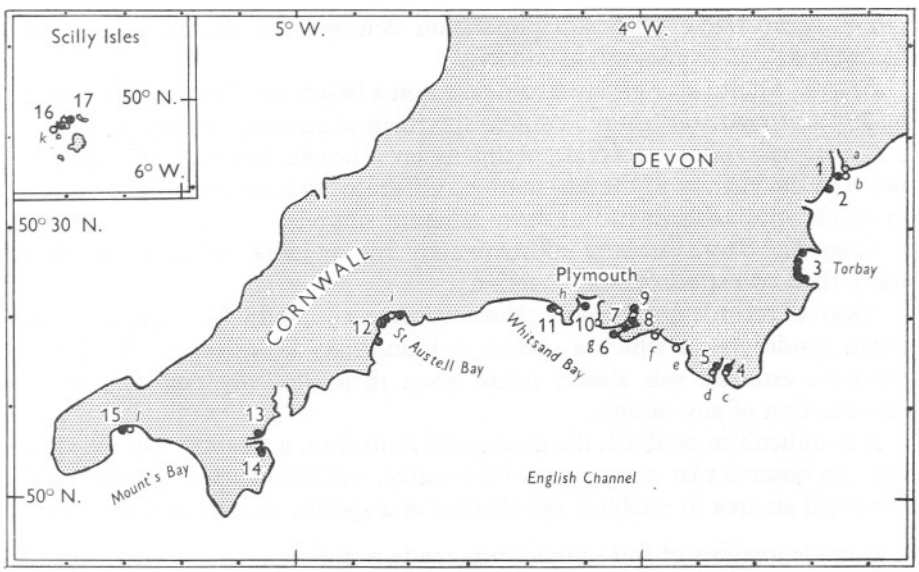

Fig. 1. Position of shore records (●) in Devon, Cornwall, and the Scilly Isles. Localities are numbered as follows: 1, Polesands; 2, Dawlish; 3, Torbay (see Fig. 5); 4, Salcombe, east; 5, Salcombe, west; 6, Cellars beach; 7, below Passage Wood; 8, Yealm sand bank; 9, Thorn Pt.; 10, Drake's Island; 11, Whitsand Bay; 12, St Austell Bay (see Fig. 5); 13, Helford; 14, Flushing Cove; 15, Marazion; 16, Tresco-Bryher channel; 17, Foreman's Island. Negative records (o): *a*, Maer Rocks, Exmouth; *b*, Polesands; *c*, south of Millbay, Salcombe; *d*, North Sands and South Sands, Salcombe; *e*, Borough Island; *f*, Mothecombe; *g*, Bovisand; *h*, Whitsand Bay; *i*, Charlestown (see Fig. 5); *j*, St Michael's Mount; *k*, Bryher.

At each locality a sample of the soil in which *Ensis* was living was taken for grade analysis. On the shore a sample from the top 5 cm. or so of soil was taken, all specimens taken within about a metre of the sample being considered to inhabit the same grade of soil. Samples were taken offshore either with the scoop-sampler (Holme, 1949*b*) or anchor-dredge (Forster, 1953). Where several hauls were made at a station, the soil sample was always taken from that in which *Ensis* was taken. There is little washing out of soil when the scoop-sampler is hauled up; but when using the dredge it was necessary to select a sample from an undisturbed portion of the dredge bag.

No accurate estimates of population density have been made. It is difficult to compare populations on different beaches, as tidal levels have to be taken into consideration. One is only sampling the fringe of the *Ensis* zone when collecting on the beach, and numbers are likely to change considerably over quite short vertical distances, so that the observed density may be much affected by the distance the tide recedes on a particular day. Offshore, the size of the sample, usually 0·5 m.^{2} or less, is too small for an assessment of density.

For comparative purposes, population density was classed as 'scarce', 'occasional', or 'common', as follows:

Scarce. Shore: a single specimen taken on a beach, or at most two or three, widely scattered. Offshore: a single specimen at an isolated station.

Occasional. Shore: several specimens on a beach, but only one need be taken in the vicinity of the soil sample. Offshore: two specimens at a station, or single specimens at two or more adjacent stations.

Common. Shore: density approximately one or more per m.^{2}. Offshore: more than two specimens at a station.

'Scarce' records may be only chance occurrences, and when computing the mean conditions in which a species is found may be disregarded. A conservative estimate was always made when there was any doubt as to the classification of any sample.

It is difficult to establish the absence of *Ensis* from a locality. On the shore one can observe that it is at most very scarce, but the offshore records cover too small an area to establish the absence of a species on a particular ground.

A grade analysis of soil samples was made as follows, the class units being based on the Wentworth system (2, 1, $\frac{1}{2}$, $\frac{1}{4}$ mm. etc.). The methods used and reliability of the results are described by Krumbein & Pettijohn (1938).

Samples were dried before storage, and for analysis were 'broken down' to a convenient size by dividing a flattened-out pile into quarters and taking opposite quarters. This operation was repeated until a sample of about 20 g. was obtained.

This was then treated with 20 vol. hydrogen peroxide on a hot plate for $\frac{1}{2}$ hr. to oxidize organic matter and break up aggregates, and then washed through a B.S.S. gauze sieve, with 0·124 mm. apertures.

The residue on the sieve was dried and screened through a set of B.S.S. sieves of the following apertures: 2·057, 1·003, 0·500, 0·251, 0·210, and 0·124 mm. (dimensions quoted by the manufacturers). The sieves were shaken mechanically by a machine which imparted a short but rapid to-and-fro motion, at the same time tilting them through a small angle to the horizontal by a slowly rotating eccentric. After 10 min. shaking a fairly accurate separation was obtained, and the fraction on each sieve was then weighed.

Material washed through the fine sieve after peroxide treatment was placed in a wide measuring cylinder having two marks, one 10 cm. above the other. Distilled water was added up to the top mark, and the material stirred into suspension. After allowing to settle for 1 min. 56 sec. (at 20° C.) the suspension was siphoned off down to the lower mark, the siphon having an upturned end so as not to disturb material which

had settled. Distilled water was added up to the top mark, and the operation repeated until the siphoned-off material was almost clear. By this means a separation of particles over and under $\frac{1}{32}$ mm. was obtained. The residue in the cylinder was dried and added to that passing the finest of the sieves in the shaker. To the siphoned-off suspension was added a little alum solution and a few drops of acetic acid, to flocculate the particles. After a day or two this was filtered through a weighed filter-paper, washed, dried, and the weight of solid determined.

A separation was thus made at the following approximate particle diameters: 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{8}$ and $\frac{1}{32}$ mm. Large particles, over about 10 mm., were taken out of the sample before analysis, and were not reckoned with when calculating the percentage grade composition of the soil. Besides inorganic particles, the analysis includes shell and other calcareous material.

When more than one sample with the same species was taken from a beach, it was sometimes necessary to reduce the records to a single one to avoid bias to the mean from all collecting grounds in favour of a well-worked beach. When two or more samples did not differ by as much as 10% in any one grade, all but one sample was eliminated. The sample taken last on any one day was usually retained, as this was generally at the lowest level on the beach, and therefore more representative of the typical habitat, which often extends below low-water mark.

The results of mechanical analysis are conveniently expressed as a cumulative curve (Fig. 3, p. 156), with grade size on a logarithmic scale. The cumulative curve for any soil is independent of the class units, provided these are sufficiently closely spaced, and is therefore a more satisfactory method of representation than a histogram, in which the shape of the figure is dependent on the class units employed.

A convenient term for comparing sands which may differ particularly in the percentage of coarser particles is the arithmetic mean (see Krumbein & Pettijohn, 1938, p. 240). The mean of the upper and lower limits of each class is multiplied by the percentage weight in that class. The total of the products is divided by 100 to obtain the arithmetic mean. The method of calculation is shown below:

Grade size (mm.)	Mean grade (mm.)	Percentage in sample	Product
(4)–2	3·0	14·24	42·72
2–1	1·5	10·84	16·26
1–$\frac{1}{2}$	0·75	10·51	7·88
$\frac{1}{2}$–$\frac{1}{4}$	0·375	29·81	11·18
$\frac{1}{4}$–$\frac{1}{5}$	0·225	7·72	1·74
$\frac{1}{5}$–$\frac{1}{8}$	0·1625	16·90	2·75
$\frac{1}{8}$–$\frac{1}{32}$	0·0781	8·38	0·65
$\frac{1}{32}$–0	0·0156	1·60	0·02
		100·00	83·20

Mean = 83·20/100 = 0·832 mm.

The value of the arithmetic mean is clearly more affected by changes in the percentages in the coarser grades than in the finer ones.

Geographical Distribution

Many previous identifications have been incorrect, owing to failure to distinguish *E. arcuatus* as a separate species, and the following notes are therefore based almost entirely on specimens which I have seen. (The records in Ford, 1923, are known to be correct, as I have been able to confirm identifications from specimens in Mr Ford's shell collection.) All three specimens are generally distributed on the Atlantic coast of Europe, and *E. siliqua* and *E. ensis*, at least, occur in the Mediterranean. A list of localities is given below, and a more detailed list of places where living specimens have been taken is given in Table VII (Appendix).

E. siliqua

England: S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Studland Bay, Dorset[1]. **Scotland**: Aberdeen[1]; Fairlie, Ayrshire. **Isle of Man**: Port Erin Bay. **Wales**: Criccieth, Caernarvonshire[1]; Dale, Pembrokeshire; Tenby, Pembrokeshire[1]. **Ireland**: Benderg Bay, Co. Down. **France**: Grève de S. Michel, Finistère. **Italy**: Naples (from specimen department).

E. arcuatus

England: Scilly Isles; S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Pevensey Bay, Sussex[1]; Newton Haven, Northumberland. **Scotland**: Millport, Firth of Clyde (from specimen department). **Isle of Man**: Derbyhaven. **Wales**: Benllech, Anglesey[1]. **Ireland**: Portaferry and Benderg Bay, Co. Down. **Channel Isles**: Bordeaux Harbour, Guernsey; Jersey. **France**: Finistère.

E. ensis

England: S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Pevensey Bay, Sussex[1]; North Sea (52° 45′ N., 02° 44′ E.); off Cumberland coast (approx. 54° 23′ N., 03° 35′ W.). **Isle of Man**: Port Erin Bay. **Wales**: Aberporth, Cardiganshire. **Channel Isles**: Jersey. **France**: Finistère.

The Mediterranean form of *E. siliqua* is apparently smaller than the Atlantic form (Bucquoy, Dautzenberg & Dollfus, 1887–98, as var. *minor*), but specimens from Naples agree with the Atlantic form in the form of the fourth aperture papillae described by Holme (1951). The same authors give a photograph of *E. ensis* from Roussillon, on the French Mediterranean coast (pl. 73, fig. 5, as *E. ensis* var. *minor*; fig. 4 may be a small *E. arcuatus*).

There is insufficient data to fix the northern and southern limits of the three species, but they are not recorded from Iceland by Madsen (1949).

Four[2] species or forms of *Ensis* have been recorded from N. America: *E. directus* Conrad, *E. minor* Dall, *E. californicus* Dall (all listed in Dall, 1900), and the recently described *E. minor megistus* Pilsbry & McGinty (1943).

[1] Empty shells.

[2] A fifth species has recently been described by Stillman Berry (1953).

E. directus resembles *E. arcuatus*, but the shell of the former is broader and more arcuate. In addition, Bloomer (1905) notes that the fourth aperture is bordered by several rows of papillae, whereas in *E. arcuatus* there is only a single row on each side. Pilsbry & McGinty have illustrations of both *E. minor* and *E. minor megistus*. The former closely resembles *E. ensis* in shell form, and may well be the same species. *E. minor megistus* is more slender and parallel-sided than either *E. minor* or *E. ensis*, and the anterior end is obliquely truncated, not rounded as in *E. ensis*. *E. minor megistus* does not correspond with any European species.

I have not been able to see specimens or figures of *E. californicus*.

Apart from the possibility that *E. minor* Dall is the same as *E. ensis*, I have no records of the British species from outside European waters (including the Mediterranean).

Environmental Factors

The distribution of any organism is controlled by a complex of factors, each of which may act at a different point in the life history of the individual. Some factors may be too subtle to be detected by the methods employed, but an analysis of the more readily measurable factors should provide clues to the presence or nature of these other factors.

Any one factor interacts both with the organism and with its environment, and it is often difficult to distinguish whether the action of a factor is direct or indirect. Soil grade, for example, may influence distribution directly (see below), but it also has an indirect effect on the slope and stability of the beach, so affecting tolerance of wave action (see p. 158).

Soil Grade

Analyses of soil grade for the three species are given in Tables I–III. In these tables the samples have been grouped according to population density as 'scarce', 'occasional' or 'common', as defined on p. 148. Fig. 2 shows the percentage in each grade in histogram form, and the distribution of arithmetic means is given in Fig. 4. The mean of 'occasional' and 'common' samples only is shown as a cumulative curve in Fig. 3.

E. siliqua occurs in sands of fairly fine grade, in which particles between 0·21 and 0·0313 mm. preponderate. This species is not confined to sands of any particular grade composition within these limits, but seems to avoid those in which particles over 0·5 mm. form more than about 5% of the total. The percentage of silt (< 0·0313 mm.) is always small, and is usually less than 1%. *E. siliqua* is thus characteristic of ordinary 'clean' beach sands.

E. ensis occurs in soils of a grade similar to that in which *E. siliqua* is found. The histograms showing percentage weights in the different grades are very similar (Fig. 2), as is the distribution of arithmetic means (Fig. 4). Only eight of the *E. ensis* samples also contain *E. siliqua*, so the coincidence of the

TABLE I. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS SILIQUA* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.) >2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Polesands	*47*	0·236	0·4	1·0	1·3	15·2	14·9	64·8	2·3	0·05
Saltern Cove	*53*	0·245	0	0·2	2·1	33·1	11·9	40·6	11·9	0·22
Salcombe, E.	*63*	0·646	3·1	17·4	25·5	10·2	5·3	22·7	15·0	0·76
Drake's Is. (C)	*172*	0·620	7·9	15·9	7·2	5·1	3·3	21·1	37·5	1·95
Drake's Is. (B, C)	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Drake's Is.	*176*	0·103	0·1	0·1	0·3	1·7	1·3	15·4	79·7	1·36
Whitsand Bay	*117*	0·276	0	0·1	1·0	44·2	22·2	31·0	1·5	0·07
Porthpean	*61*	0·701	11·1	10·6	5·8	21·5	12·1	34·7	4·0	0·31
Helford River (C)	*41*	0·268	3·3	1·1	0·9	5·3	5·9	58·9	22·5	2·20
Flushing Cove	*58*	0·110	0	0·1	0·5	6·0	1·8	10·2	78·1	3·35
Mean		0·348	2·6	4·8	4·9	18·0	9·0	33·0	26·7	1·22
OCCASIONAL										
Dawlish	*74*	0·304	5·0	0·6	0·3	1·2	1·3	75·4	15·9	0·33
Salcombe, E. (B)	*6*	0·254	0·1	0·8	2·7	28·8	16·2	40·8	10·4	0·25
Par	*66*	0·287	0·2	0·1	3·2	44·9	14·3	31·8	5·2	0·38
Pentewan	*136*	0·222	0	0·3	1·3	18·9	17·9	57·1	4·4	0·16
Helford River	*38*	0·183	1·1	0·2	0·2	2·1	3·5	70·8	20·5	1·63
Helford River (B, C)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Dale Flats	*25*	0·100	0·2	0·1	0·1	0·3	0·2	18·5	79·3	1·38
Grève de S. Michel	*155*	0·085	0	0	0·1	0·1	0·3	6·1	93·0	0·35
Torbay[1]	*169*	0·140	0·3	0·7	0·6	0·6	0·3	45·1	51·8	0·61
Mean		0·198	0·9	0·4	1·0	11·4	7·0	45·8	32·8	0·71
COMMON										
Torr Abbey Sands	*139*	0·102	0·2	0·1	0	0·2	0·3	19·1	79·4	0·64
"	*141*	0·200	2·7	0·4	0·3	0·5	0·5	38·4	56·3	0·80
Paignton	*10*	0·187	0	0·6	1·6	5·9	3·5	79·5	8·7	0·20
Goodrington	*59*	0·287	2·0	3·7	3·5	5·0	3·0	67·3	15·2	0·34
"	*60*	0·169	0	0	0·1	3·4	7·4	82·6	6·4	0·17
Broadsands (C)	*2*	0·110	0	0	0·3	0·3	0·3	34·2	64·4	0·47
Broadsands (C)	*11*	0·199	1·3	0·7	0·5	1·0	0·8	77·8	17·7	0·29
Elbury Cove (C)	*106*	0·117	0·3	0	0	0·8	0·7	33·9	63·5	0·83
Par	*68*	0·096	0	0	0·1	0·4	0·5	20·0	77·6	1·40
Duporth	*62*	0·211	0·5	1·5	1·2	7·2	6·3	68·2	14·5	0·60
Flushing Cove (C)	*55*	0·169	0	0·1	0·4	11·0	9·3	49·5	28·5	1·28
Marazion	*52*	0·110	0	0·1	0·1	0·1	0·1	34·8	64·5	0·28
Dale Flats	*73*	0·139	0·1	0·1	0·1	0·3	0·1	66·1	32·1	1·11
Fairlie Sands	*18*	0·278	0	0	0·6	49·2	15·1	33·3	1·8	0·20
Torbay[1] (C)	*167*	0·089	0	0·1	0·1	0·2	0·3	10·8	87·7	0·77
Plymouth[1]	*22*	0·193	1·8	0·3	0·3	2·7	2·1	55·1	36·2	1·61
Mean		0·166	0·6	0·5	0·6	5·5	3·1	48·2	40·9	0·69

[1] Offshore records. (B), with *E. arcuatus*; (C), with *E. ensis*.

TABLE II. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS ARCUATUS* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.) >2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Salcombe, W.	*64*	0·739	14·4	9·6	3·1	8·8	11·4	47·7	4·5	0·58
"	*65*	1·453	33·2	22·7	5·9	6·7	4·0	19·4	7·9	0·28
Yealm River	*69*	2·144	60·8	16·0	8·0	2·7	0·4	2·6	5·7	3·77
"	*171*	0·772	15·2	3·5	5·8	47·0	9·8	11·4	5·8	1·67
Helford River	*164*	0·196	1·3	0·7	0·7	3·9	5·1	55·7	29·6	2·97
Helford River (A, C)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Flushing Cove	*56*	0·832	14·2	10·8	10·5	29·8	7·7	16·9	8·4	1·60
"	*57*	0·188	0·2	0·2	0·8	15·0	11·2	42·2	28·7	1·71
St Aubin's Bay	*15*	0·407	5·1	5·7	4·3	13·7	6·0	25·0	40·0	0·31
Térénès (C)	*149*	0·245	0·8	1·1	1·1	15·5	17·3	60·0	3·8	0·46
Plymouth[1] (C)	*31*	0·177	0·1	0·4	1·1	6·1	8·6	63·7	19·8	0·22
Mean		0·669	13·3	6·5	3·8	14·1	8·2	37·4	15·4	1·36
OCCASIONAL										
Salcombe, E. (A)	*6*	0·254	0·1	0·8	2·7	28·8	16·2	40·8	10·4	0·25
Yealm River	*70*	0·597	10·6	3·3	2·9	29·9	18·4	32·8	2·0	0·26
"	*71*	0·667	7·6	9·8	15·8	32·1	7·6	18·6	7·9	0·73
Drake's Is. (A, C)	*173*	0·433	0·7	2·2	8·0	81·8	4·4	1·8	0·7	0·45
Drake's Is.	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Foreman's Is.	*128*	0·866	16·2	9·3	8·3	36·9	6·5	11·1	10·7	1·21
Grouville Bay (C)	*16*	0·215	0	0·4	3·3	13·0	12·7	61·8	8·7	0·18
Goulven	*157*	0·208	0·2	0·4	2·2	16·0	7·0	56·7	17·6	0·05
Perharidy	*159*	0·726	7·3	7·8	19·5	64·0	0·6	0·6	0·1	0·05
Mean		0·471	4·7	3·9	7·4	37·8	9·4	28·3	8·0	0·57
COMMON										
Salcombe, E.	*3*	0·545	0·3	5·0	45·6	21·4	4·3	14·8	8·3	0·35
Salcombe, E. (C)	*12*	0·482	1·0	13·2	19·4	8·7	4·0	31·0	21·4	1·25
Yealm River	*1*	1·307	25·2	19·0	19·7	29·3	1·7	2·2	1·7	1·19
Tresco	*50*	1·242	10·6	42·5	32·0	10·6	2·1	1·9	0·3	0
Tresco-Bryher channel	*120*	0·961	11·7	21·0	21·1	27·7	6·3	10·7	1·4	0·13
"	*121*	1·267	25·7	16·0	15·9	30·9	5·0	5·5	0·8	0·19
"	*122*	0·978	10·6	23·3	20·6	38·0	3·2	3·8	0·4	0·19
Foreman's Is.	*126*	1·020	20·5	9·1	11·0	41·7	7·6	8·1	1·7	0·40
"	*127*	0·641	5·5	8·5	13·9	58·7	6·8	5·9	0·7	0·17
"	*129*	0·964	13·9	15·5	21·2	36·3	4·5	4·8	2·4	1·50
Tresco-Bryher channel	*130*	1·138	19·1	16·8	23·3	34·0	3·5	2·4	0·6	0·27
"	*131*	0·622	3·3	9·9	21·7	47·7	9·2	7·7	0·3	0·13
Newton Haven	*19*	0·195	1·1	0·6	1·2	3·3	1·5	64·7	27·3	0·25
Grouville Bay	*9*	0·314	0	0·3	6·3	48·4	17·1	25·2	2·7	0·12
Goulven	*158*	1·612	30·2	33·0	21·0	14·1	0·6	0·7	0·4	0·04
Mean		0·886	11·9	15·6	19·6	30·0	5·2	12·6	4·7	0·41

[1] Offshore records. (A), with *E. siliqua*; (C), with *E. ensis*.

TABLE III. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS ENSIS* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.) >2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Torr Abbey Sands	*140*	0·143	1·1	0·1	0·1	0·3	0·4	35·9	61·4	0·82
Elbury Cove	*105*	0·125	0	0	0	2·5	1·2	45·6	49·6	1·02
Elbury Cove (A)	*106*	0·117	0·3	0	0	0·8	0·7	33·9	63·5	0·83
Salcombe, W.	*163*	0·836	21·1	6·6	0·8	2·9	3·9	32·6	30·9	1·17
Yealm River	*72*	1·164	28·1	10·2	4·6	20·0	8·3	20·6	7·4	0·76
Drake's Is. (A)	*172*	0·620	7·9	15·9	7·2	5·1	3·3	21·1	37·5	1·95
Drake's Is.	*174*	0·225	2·3	1·6	1·1	4·9	4·3	34·9	49·1	1·87
Flushing Cove	*54*	0·142	0	0·3	1·2	7·9	3·3	29·2	55·7	2·45
Grouville Bay	*7*	0·168	0·3	0·1	0·1	2·0	3·3	82·7	11·4	0·12
Grouville Bay (B)	*16*	0·215	0	0·4	3·3	13·0	12·7	61·8	8·7	0·18
Térénès	*147*	0·267	1·3	3·7	3·1	6·1	3·5	64·8	16·7	0·82
Térénès (B)	*149*	0·245	0·8	1·1	1·1	15·5	17·3	60·0	3·8	0·46
Goulven	*156*	0·194	0	0·1	2·0	16·9	7·1	47·9	25·9	0·14
Plymouth[1]	*116*	0·185	0·1	0·1	0·6	9·2	17·4	57·1	13·8	1·80
Plymouth[1]	*113*	0·272	2·4	3·2	3·2	3·4	2·8	51·6	30·1	3·30
Plymouth[1]	*32*	0·158	0	0·2	0·7	2·9	4·8	69·6	19·8	2·12
Mean		0·317	4·1	2·7	1·8	7·1	5·9	46·8	30·3	1·24
OCCASIONAL										
Broadsands (A)	*2*	0·110	0	0	0·3	0·3	0·3	34·2	64·4	0·47
Broadsands (A)	*11*	0·199	1·3	0·7	0·5	1·0	0·8	77·8	17·7	0·29
Salcombe, E. (B)	*12*	0·482	1·0	13·2	19·4	8·7	4·0	31·0	21·4	1·25
Drake's Is. (A, B)	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Helford River	*24*	0·094	0	0·3	0·1	0·4	0·6	22·0	60·6	16·00
,,	*36*	0·177	0·7	0·1	0·2	2·5	3·9	73·1	18·6	0·83
,,	*40*	0·226	2·7	1·2	1·2	2·7	1·8	45·1	40·3	5·17
Helford River (A)	*41*	0·268	3·3	1·1	0·9	5·3	5·9	58·9	22·5	2·20
Helford River (A, B)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Flushing Cove (A)	*55*	0·169	0	0·1	0·4	11·0	9·3	49·5	28·5	1·28
Flushing Cove	*162*	0·147	0	0·4	0·6	6·0	4·7	41·9	45·3	1·26
Penpoull	*145*	0·443	7·4	4·5	2·8	3·4	1·8	62·9	16·1	1·14
Great West Bay, St. 12[1]	*27*	0·161	0·2	0·2	0·7	3·4	1·4	69·0	24·8	0·40
Great West Bay, St. 17[1]	*28*	0·136	0·1	0·3	0·9	5·6	2·2	33·8	53·6	3·45
Great West Bay, St. 16[1]	*29*	0·157	0·1	0·2	0·9	7·4	3·9	48·2	38·8	0·54
Torbay, St. 9[1]	*167*	0·089	0	0·1	0·1	0·2	0·3	10·8	87·7	0·77
Torbay, St. X[1]	*170*	0·080	0	0	0·1	0·2	0·1	1·4	97·3	0·91
Plymouth[1] (B)	*31*	0·177	0·1	0·4	1·1	6·1	8·6	63·7	19·8	0·22
St Austell Bay, St. 3[1]	*33*	0·145	0	0·2	1·0	5·5	5·3	46·4	30·9	10·74
St Austell Bay, St. 4[1]	*34*	0·098	0	0·1	0·6	2·0	1·6	19·6	60·9	15·29
St Austell Bay, St. 6[1]	*35*	0·109	0	0·4	1·3	4·2	1·2	7·0	80·2	5·71
Irish Sea[1]	*14*	0·203	0	0·7	1·6	12·2	8·8	65·0	10·6	1·22
Mean		0·189	0·8	1·2	1·8	6·0	3·9	43·5	39·5	3·29
COMMON										
St Aubin's Bay	*21*	0·180	0·5	1·8	1·8	8·2	5·1	20·3	61·9	0·42
Great West Bay, St. 19[1]	*30*	0·313	1·8	1·7	5·5	26·7	8·3	37·4	14·0	4·63
Aberporth Bay[1]	*160*	0·300	0·5	0·3	0·6	53·4	21·6	12·8	7·4	3·33
Mean		0·264	0·9	1·3	2·6	29·4	11·7	23·5	27·8	2·79

[1] Offshore records. (A), with *E. siliqua*; (B), with *E. arcuatus*.

canal has small salivary glands and reduced jaws, but well developed glands in the mid-oesophagus and no crystalline style. The stomach is relatively simple with two large ducts opening from the digestive gland. Two main types of cell occur in this gland, one digestive in function, the other believed to be excretory. In the nervous system the cerebral and pleural ganglia are united, the supra- and suboesophageal ganglia have migrated well forwards and a zygoneury and a left dialyneury both occur. The animals are successive hermaphrodites and are protandrous. In the male phase a grooved penis occurs connected by a seminal groove with the genital pore in the mantle cavity. Rudiments of female organs are already present at this stage and later form a capsule gland with an albumen gland and receptaculum seminis. No bursa copulatrix occurs in the female and the penis disappears.

In *Capulus ungaricus* the animal has become limpet-like with a round shell and no operculum. Oesophageal glands have been lost but otherwise the gut and other soft parts conform to the same pattern as those of *Trichotropis*. Of *Crepidula fornicata* and *Calyptraea sinensis* the same may be said. Fusion in the nervous system is more extensive and feeding is carried out entirely by ciliary means; there is no proboscis.

Capulus possesses an echinospira larval stage. Comparison of its anatomy with that of members of the Lamellariacea and Cypraeacea, which (*Simnia* apart) also possess such a larva, shows that it is nearer to the other members of the Calyptraeacea. Possession of the echinospira, therefore, suggests a general relationship of these three superfamilies rather than a closer relationship of *Capulus* to the Lamellariacea and Cypraeacea than to the Calyptraeacea.

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Printed in Great Britain

THE ECOLOGY OF BRITISH SPECIES OF *ENSIS*

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(Text-figs. 1–5)

CONTENTS

INTRODUCTION

In a recent paper describing the distinguishing characters of British species of *Ensis* (Holme, 1951), it was concluded that three valid species occur in British waters: *E. siliqua* (L.), *E. arcuatus* (Jeffreys) and *E. ensis* (L.). This paper is an attempt to analyse the ecological factors controlling the distribution of these species, particular attention being paid to the effect of soil grade.

In addition to those acknowledged in a previous paper, I would like to thank the following for their assistance: Dr A. G. Lowndes, Mr L. Birkett, Mr C. Edwards, and Mr O. D. Hunt. Specimens of *E. minor* were kindly supplied by Dr R. Tucker Abbott, U.S. National Museum.

I am indebted to the Director and Staff of the Station Biologique at Roscoff for facilities provided during a visit to the Station. Dr P. N. J. Chipperfield kindly placed at my disposal the research launch of I.C.I. Paints Division, Brixham, from which the dredgings in Torbay were made. I am grateful to

the Director of the National Institute of Oceanography for facilities provided on R.R.S. *Discovery II*, from which the bottom-sampling station in Aberporth Bay was worked.

The Habitat of *Ensis*

Ensis is found burrowing in sand at low-water mark of spring tides, and also occurs in shallow water offshore. On the beach, it burrows nearly vertically in the sand by means of a powerful foot, but it does not seem to possess a permanent burrow. The short siphons project just above the surface of the sand, when covered by water, and draw in water from just above the bottom. When disturbed, *Ensis* burrows rapidly into the sand, often emitting a jet of water into the air as it starts to burrow. Along a beach at low tide, *Ensis* may be located by the presence of keyhole-shaped depressions in the sand, which become more evident when the surface water has drained away, and also by the jets of water produced when they start to dig. It is possible to survey quite long stretches of beach in this way, only digging when a burrow is seen.

On a number of occasions the animals have been seen to come right out of the sand at low tide, and lie on the surface. This habit is also shared by certain other lamellibranchs living in the same habitat (e.g. *Spisula*, *Donax* and spiny *Cardium*).

On wave-exposed beaches, *Ensis siliqua* may be the only lamellibranch present, but in more sheltered areas the smaller *E. ensis* may be found, together with such species as *Venus striatula* (da Costa), *Donax vittatus* (da Costa), *Tellina fabula* Gmelin, *Gari fervensis* (Gmelin), *Mactra corallina cinerea* Montagu, and *Lutraria lutraria* (L.). The burrowing crab, *Corystes cassivelaunus* (Pennant), and the heart-urchin *Echinocardium cordatum* (Pennant) also occur in these localities.

Ensis arcuatus, which inhabits a coarser grade of soil, may be found with *Dosinia exoleta* (L.), *Tellina donacina* L., and the heart-urchins *Spatangus purpureus* O. F. Müller and *Echinocardium pennatifidum* Norman.

One or other of the species of *Ensis* occurs on practically every beach where there is sand of a sufficient depth and some slight protection from wave action. There may, however, often be none in small patches of sand between rocks: such sands are often highly reducing (see p. 158). *Ensis* does not occur in water of reduced salinity, although its absence from estuaries may sometimes be due to the lack of deposits of a suitable grade. A list of sandy beaches in which *Ensis* has not been recorded is given in Table VII (Appendix).

Methods

In order to eliminate so far as possible the effect of characteristics peculiar to individual beaches, collections were made over as wide an area as possible. On the shore, collections have been made on most of the sandy beaches in S. Devon, S. Cornwall, and the Scilly Isles (Fig. 1). I have also received

samples collected in various other parts of the British Isles. Additional samples have been obtained from certain beaches in Jersey and the north Finistère coast around Roscoff. A list of localities is given in Table VII (Appendix).

Grab or dredge collections are chiefly from Great West Bay (Holme, 1950), off Plymouth (some stations are in Holme, 1953), and in St Austell Bay off the Cornish coast (Fig. 5).

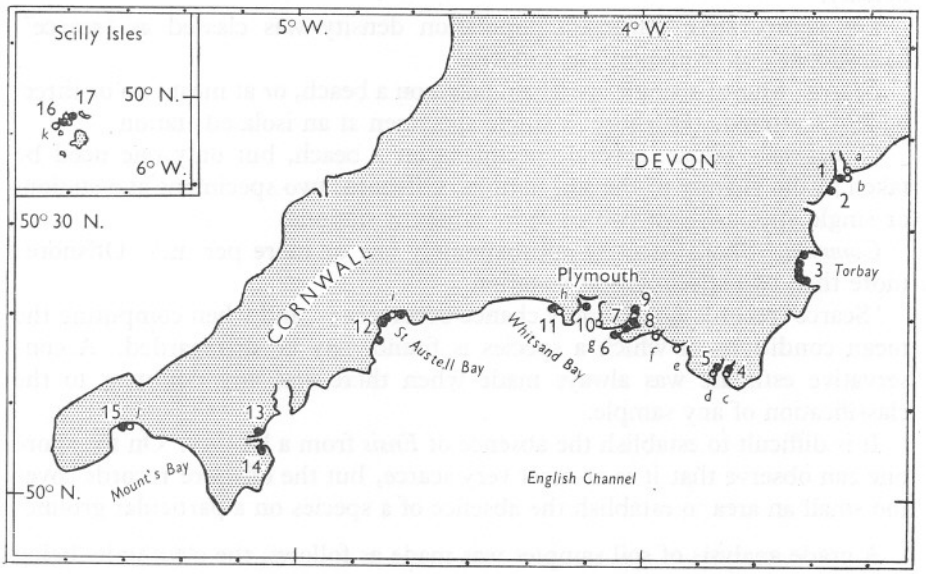

Fig. 1. Position of shore records (●) in Devon, Cornwall, and the Scilly Isles. Localities are numbered as follows: 1, Polesands; 2, Dawlish; 3, Torbay (see Fig. 5); 4, Salcombe, east; 5, Salcombe, west; 6, Cellars beach; 7, below Passage Wood; 8, Yealm sand bank; 9, Thorn Pt.; 10, Drake's Island; 11, Whitsand Bay; 12, St Austell Bay (see Fig. 5); 13, Helford; 14, Flushing Cove; 15, Marazion; 16, Tresco-Bryher channel; 17, Foreman's Island. Negative records (o): *a*, Maer Rocks, Exmouth; *b*, Polesands; *c*, south of Millbay, Salcombe; *d*, North Sands and South Sands, Salcombe; *e*, Borough Island; *f*, Mothecombe; *g*, Bovisand; *h*, Whitsand Bay; *i*, Charlestown (see Fig. 5); *j*, St Michael's Mount; *k*, Bryher.

At each locality a sample of the soil in which *Ensis* was living was taken for grade analysis. On the shore a sample from the top 5 cm. or so of soil was taken, all specimens taken within about a metre of the sample being considered to inhabit the same grade of soil. Samples were taken offshore either with the scoop-sampler (Holme, 1949*b*) or anchor-dredge (Forster, 1953). Where several hauls were made at a station, the soil sample was always taken from that in which *Ensis* was taken. There is little washing out of soil when the scoop-sampler is hauled up; but when using the dredge it was necessary to select a sample from an undisturbed portion of the dredge bag.

No accurate estimates of population density have been made. It is difficult to compare populations on different beaches, as tidal levels have to be taken into consideration. One is only sampling the fringe of the *Ensis* zone when collecting on the beach, and numbers are likely to change considerably over quite short vertical distances, so that the observed density may be much affected by the distance the tide recedes on a particular day. Offshore, the size of the sample, usually 0·5 m.^{2} or less, is too small for an assessment of density.

For comparative purposes, population density was classed as 'scarce', 'occasional', or 'common', as follows:

Scarce. Shore: a single specimen taken on a beach, or at most two or three, widely scattered. Offshore: a single specimen at an isolated station.

Occasional. Shore: several specimens on a beach, but only one need be taken in the vicinity of the soil sample. Offshore: two specimens at a station, or single specimens at two or more adjacent stations.

Common. Shore: density approximately one or more per m.^{2}. Offshore: more than two specimens at a station.

'Scarce' records may be only chance occurrences, and when computing the mean conditions in which a species is found may be disregarded. A conservative estimate was always made when there was any doubt as to the classification of any sample.

It is difficult to establish the absence of *Ensis* from a locality. On the shore one can observe that it is at most very scarce, but the offshore records cover too small an area to establish the absence of a species on a particular ground.

A grade analysis of soil samples was made as follows, the class units being based on the Wentworth system (2, 1, $\frac{1}{2}$, $\frac{1}{4}$ mm. etc.). The methods used and reliability of the results are described by Krumbein & Pettijohn (1938).

Samples were dried before storage, and for analysis were 'broken down' to a convenient size by dividing a flattened-out pile into quarters and taking opposite quarters. This operation was repeated until a sample of about 20 g. was obtained.

This was then treated with 20 vol. hydrogen peroxide on a hot plate for $\frac{1}{2}$ hr. to oxidize organic matter and break up aggregates, and then washed through a B.S.S. gauze sieve, with 0·124 mm. apertures.

The residue on the sieve was dried and screened through a set of B.S.S. sieves of the following apertures: 2·057, 1·003, 0·500, 0·251, 0·210, and 0·124 mm. (dimensions quoted by the manufacturers). The sieves were shaken mechanically by a machine which imparted a short but rapid to-and-fro motion, at the same time tilting them through a small angle to the horizontal by a slowly rotating eccentric. After 10 min. shaking a fairly accurate separation was obtained, and the fraction on each sieve was then weighed.

Material washed through the fine sieve after peroxide treatment was placed in a wide measuring cylinder having two marks, one 10 cm. above the other. Distilled water was added up to the top mark, and the material stirred into suspension. After allowing to settle for 1 min. 56 sec. (at 20° C.) the suspension was siphoned off down to the lower mark, the siphon having an upturned end so as not to disturb material which

had settled. Distilled water was added up to the top mark, and the operation repeated until the siphoned-off material was almost clear. By this means a separation of particles over and under $\frac{1}{32}$ mm. was obtained. The residue in the cylinder was dried and added to that passing the finest of the sieves in the shaker. To the siphoned-off suspension was added a little alum solution and a few drops of acetic acid, to flocculate the particles. After a day or two this was filtered through a weighed filter-paper, washed, dried, and the weight of solid determined.

A separation was thus made at the following approximate particle diameters: 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{8}$ and $\frac{1}{32}$ mm. Large particles, over about 10 mm., were taken out of the sample before analysis, and were not reckoned with when calculating the percentage grade composition of the soil. Besides inorganic particles, the analysis includes shell and other calcareous material.

When more than one sample with the same species was taken from a beach, it was sometimes necessary to reduce the records to a single one to avoid bias to the mean from all collecting grounds in favour of a well-worked beach. When two or more samples did not differ by as much as 10% in any one grade, all but one sample was eliminated. The sample taken last on any one day was usually retained, as this was generally at the lowest level on the beach, and therefore more representative of the typical habitat, which often extends below low-water mark.

The results of mechanical analysis are conveniently expressed as a cumulative curve (Fig. 3, p. 156), with grade size on a logarithmic scale. The cumulative curve for any soil is independent of the class units, provided these are sufficiently closely spaced, and is therefore a more satisfactory method of representation than a histogram, in which the shape of the figure is dependent on the class units employed.

A convenient term for comparing sands which may differ particularly in the percentage of coarser particles is the arithmetic mean (see Krumbein & Pettijohn, 1938, p. 240). The mean of the upper and lower limits of each class is multiplied by the percentage weight in that class. The total of the products is divided by 100 to obtain the arithmetic mean. The method of calculation is shown below:

Grade size (mm.)	Mean grade (mm.)	Percentage in sample	Product
(4)–2	3·0	14·24	42·72
2–1	1·5	10·84	16·26
1–$\frac{1}{2}$	0·75	10·51	7·88
$\frac{1}{2}$–$\frac{1}{4}$	0·375	29·81	11·18
$\frac{1}{4}$–$\frac{1}{5}$	0·225	7·72	1·74
$\frac{1}{5}$–$\frac{1}{8}$	0·1625	16·90	2·75
$\frac{1}{8}$–$\frac{1}{32}$	0·0781	8·38	0·65
$\frac{1}{32}$–0	0·0156	1·60	0·02
		100·00	83·20

Mean = 83·20/100 = 0·832 mm.

The value of the arithmetic mean is clearly more affected by changes in the percentages in the coarser grades than in the finer ones.

Geographical Distribution

Many previous identifications have been incorrect, owing to failure to distinguish *E. arcuatus* as a separate species, and the following notes are therefore based almost entirely on specimens which I have seen. (The records in Ford, 1923, are known to be correct, as I have been able to confirm identifications from specimens in Mr Ford's shell collection.) All three specimens are generally distributed on the Atlantic coast of Europe, and *E. siliqua* and *E. ensis*, at least, occur in the Mediterranean. A list of localities is given below, and a more detailed list of places where living specimens have been taken is given in Table VII (Appendix).

E. siliqua

England: S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Studland Bay, Dorset[1]. **Scotland**: Aberdeen[1]; Fairlie, Ayrshire. **Isle of Man**: Port Erin Bay. **Wales**: Criccieth, Caernarvonshire[1]; Dale, Pembrokeshire; Tenby, Pembrokeshire[1]. **Ireland**: Benderg Bay, Co. Down. **France**: Grève de S. Michel, Finistère. **Italy**: Naples (from specimen department).

E. arcuatus

England: Scilly Isles; S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Pevensey Bay, Sussex[1]; Newton Haven, Northumberland. **Scotland**: Millport, Firth of Clyde (from specimen department). **Isle of Man**: Derbyhaven. **Wales**: Benllech, Anglesey[1]. **Ireland**: Portaferry and Benderg Bay, Co. Down. **Channel Isles**: Bordeaux Harbour, Guernsey; Jersey. **France**: Finistère.

E. ensis

England: S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Pevensey Bay, Sussex[1]; North Sea (52° 45′ N., 02° 44′ E.); off Cumberland coast (approx. 54° 23′ N., 03° 35′ W.). **Isle of Man**: Port Erin Bay. **Wales**: Aberporth, Cardiganshire. **Channel Isles**: Jersey. **France**: Finistère.

The Mediterranean form of *E. siliqua* is apparently smaller than the Atlantic form (Bucquoy, Dautzenberg & Dollfus, 1887–98, as var. *minor*), but specimens from Naples agree with the Atlantic form in the form of the fourth aperture papillae described by Holme (1951). The same authors give a photograph of *E. ensis* from Roussillon, on the French Mediterranean coast (pl. 73, fig. 5, as *E. ensis* var. *minor*; fig. 4 may be a small *E. arcuatus*).

There is insufficient data to fix the northern and southern limits of the three species, but they are not recorded from Iceland by Madsen (1949).

Four[2] species or forms of *Ensis* have been recorded from N. America: *E. directus* Conrad, *E. minor* Dall, *E. californicus* Dall (all listed in Dall, 1900), and the recently described *E. minor megistus* Pilsbry & McGinty (1943).

[1] Empty shells.

[2] A fifth species has recently been described by Stillman Berry (1953).

E. directus resembles *E. arcuatus*, but the shell of the former is broader and more arcuate. In addition, Bloomer (1905) notes that the fourth aperture is bordered by several rows of papillae, whereas in *E. arcuatus* there is only a single row on each side. Pilsbry & McGinty have illustrations of both *E. minor* and *E. minor megistus*. The former closely resembles *E. ensis* in shell form, and may well be the same species. *E. minor megistus* is more slender and parallel-sided than either *E. minor* or *E. ensis*, and the anterior end is obliquely truncated, not rounded as in *E. ensis*. *E. minor megistus* does not correspond with any European species.

I have not been able to see specimens or figures of *E. californicus*.

Apart from the possibility that *E. minor* Dall is the same as *E. ensis*, I have no records of the British species from outside European waters (including the Mediterranean).

Environmental Factors

The distribution of any organism is controlled by a complex of factors, each of which may act at a different point in the life history of the individual. Some factors may be too subtle to be detected by the methods employed, but an analysis of the more readily measurable factors should provide clues to the presence or nature of these other factors.

Any one factor interacts both with the organism and with its environment, and it is often difficult to distinguish whether the action of a factor is direct or indirect. Soil grade, for example, may influence distribution directly (see below), but it also has an indirect effect on the slope and stability of the beach, so affecting tolerance of wave action (see p. 158).

Soil Grade

Analyses of soil grade for the three species are given in Tables I–III. In these tables the samples have been grouped according to population density as 'scarce , 'occasional' or 'common', as defined on p. 148. Fig. 2 shows the percentage in each grade in histogram form, and the distribution of arithmetic means is given in Fig. 4. The mean of 'occasional' and 'common' samples only is shown as a cumulative curve in Fig. 3.

E. siliqua occurs in sands of fairly fine grade, in which particles between 0·21 and 0·0313 mm. preponderate. This species is not confined to sands of any particular grade composition within these limits, but seems to avoid those in which particles over 0·5 mm. form more than about 5% of the total. The percentage of silt (< 0·0313 mm.) is always small, and is usually less than 1%. *E. siliqua* is thus characteristic of ordinary 'clean' beach sands.

E. ensis occurs in soils of a grade similar to that in which *E. siliqua* is found. The histograms showing percentage weights in the different grades are very similar (Fig. 2), as is the distribution of arithmetic means (Fig. 4). Only eight of the *E. ensis* samples also contain *E. siliqua*, so the coincidence of the

TABLE I. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS SILIQUA* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.) >2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Polesands	*47*	0·236	0·4	1·0	1·3	15·2	14·9	64·8	2·3	0·05
Saltern Cove	*53*	0·245	0	0·2	2·1	33·1	11·9	40·6	11·9	0·22
Salcombe, E.	*63*	0·646	3·1	17·4	25·5	10·2	5·3	22·7	15·0	0·76
Drake's Is. (C)	*172*	0·620	7·9	15·9	7·2	5·1	3·3	21·1	37·5	1·95
Drake's Is. (B, C)	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Drake's Is.	*176*	0·103	0·1	0·1	0·3	1·7	1·3	15·4	79·7	1·36
Whitsand Bay	*117*	0·276	0	0·1	1·0	44·2	22·2	31·0	1·5	0·07
Porthpean	*61*	0·701	11·1	10·6	5·8	21·5	12·1	34·7	4·0	0·31
Helford River (C)	*41*	0·268	3·3	1·1	0·9	5·3	5·9	58·9	22·5	2·20
Flushing Cove	*58*	0·110	0	0·1	0·5	6·0	1·8	10·2	78·1	3·35
Mean		0·348	2·6	4·8	4·9	18·0	9·0	33·0	26·7	1·22
OCCASIONAL										
Dawlish	*74*	0·304	5·0	0·6	0·3	1·2	1·3	75·4	15·9	0·33
Salcombe, E. (B)	*6*	0·254	0·1	0·8	2·7	28·8	16·2	40·8	10·4	0·25
Par	*66*	0·287	0·2	0·1	3·2	44·9	14·3	31·8	5·2	0·38
Pentewan	*136*	0·222	0	0·3	1·3	18·9	17·9	57·1	4·4	0·16
Helford River	*38*	0·183	1·1	0·2	0·2	2·1	3·5	70·8	20·5	1·63
Helford River (B, C)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Dale Flats	*25*	0·100	0·2	0·1	0·1	0·3	0·2	18·5	79·3	1·38
Grève de S. Michel	*155*	0·085	0	0	0·1	0·1	0·3	6·1	93·0	0·35
Torbay[1]	*169*	0·140	0·3	0·7	0·6	0·6	0·3	45·1	51·8	0·61
Mean		0·198	0·9	0·4	1·0	11·4	7·0	45·8	32·8	0·71
COMMON										
Torr Abbey Sands	*139*	0·102	0·2	0·1	0	0·2	0·3	19·1	79·4	0·64
"	*141*	0·200	2·7	0·4	0·3	0·5	0·5	38·4	56·3	0·80
Paignton	*10*	0·187	0	0·6	1·6	5·9	3·5	79·5	8·7	0·20
Goodrington	*59*	0·287	2·0	3·7	3·5	5·0	3·0	67·3	15·2	0·34
"	*60*	0·169	0	0	0·1	3·4	7·4	82·6	6·4	0·17
Broadsands (C)	*2*	0·110	0	0	0·3	0·3	0·3	34·2	64·4	0·47
Broadsands (C)	*11*	0·199	1·3	0·7	0·5	1·0	0·8	77·8	17·7	0·29
Elbury Cove (C)	*106*	0·117	0·3	0	0	0·8	0·7	33·9	63·5	0·83
Par	*68*	0·096	0	0	0·1	0·4	0·5	20·0	77·6	1·40
Duporth	*62*	0·211	0·5	1·5	1·2	7·2	6·3	68·2	14·5	0·60
Flushing Cove (C)	*55*	0·169	0	0·1	0·4	11·0	9·3	49·5	28·5	1·28
Marazion	*52*	0·110	0	0·1	0·1	0·1	0·1	34·8	64·5	0·28
Dale Flats	*73*	0·139	0·1	0·1	0·1	0·3	0·1	66·1	32·1	1·11
Fairlie Sands	*18*	0·278	0	0	0·6	49·2	15·1	33·3	1·8	0·20
Torbay[1] (C)	*167*	0·089	0	0·1	0·1	0·2	0·3	10·8	87·7	0·77
Plymouth[1]	*22*	0·193	1·8	0·3	0·3	2·7	2·1	55·1	36·2	1·61
Mean		0·166	0·6	0·5	0·6	5·5	3·1	48·2	40·9	0·69

[1] Offshore records. (B), with *E. arcuatus*; (C), with *E. ensis*.

TABLE II. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS ARCUATUS* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.) >2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Salcombe, W.	*64*	0·739	14·4	9·6	3·1	8·8	11·4	47·7	4·5	0·58
,,	*65*	1·453	33·2	22·7	5·9	6·7	4·0	19·4	7·9	0·28
Yealm River	*69*	2·144	60·8	16·0	8·0	2·7	0·4	2·6	5·7	3·77
,,	*171*	0·772	15·2	3·5	5·8	47·0	9·8	11·4	5·8	1·67
Helford River	*164*	0·196	1·3	0·7	0·7	3·9	5·1	55·7	29·6	2·97
Helford River (A, C)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Flushing Cove	*56*	0·832	14·2	10·8	10·5	29·8	7·7	16·9	8·4	1·60
,,	*57*	0·188	0·2	0·2	0·8	15·0	11·2	42·2	28·7	1·71
St Aubin's Bay	*15*	0·407	5·1	5·7	4·3	13·7	6·0	25·0	40·0	0·31
Térénès (C)	*149*	0·245	0·8	1·1	1·1	15·5	17·3	60·0	3·8	0·46
Plymouth[1] (C)	*31*	0·177	0·1	0·4	1·1	6·1	8·6	63·7	19·8	0·22
Mean		0·669	13·3	6·5	3·8	14·1	8·2	37·4	15·4	1·36
OCCASIONAL										
Salcombe, E. (A)	*6*	0·254	0·1	0·8	2·7	28·8	16·2	40·8	10·4	0·25
Yealm River	*70*	0·597	10·6	3·3	2·9	29·9	18·4	32·8	2·0	0·26
,,	*71*	0·667	7·6	9·8	15·8	32·1	7·6	18·6	7·9	0·73
Drake's Is. (A, C)	*173*	0·433	0·7	2·2	8·0	81·8	4·4	1·8	0·7	0·45
Drake's Is.	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Foreman's Is.	*128*	0·866	16·2	9·3	8·3	36·9	6·5	11·1	10·7	1·21
Grouville Bay (C)	*16*	0·215	0	0·4	3·3	13·0	12·7	61·8	8·7	0·18
Goulven	*157*	0·208	0·2	0·4	2·2	16·0	7·0	56·7	17·6	0·05
Perharidy	*159*	0·726	7·3	7·8	19·5	64·0	0·6	0·6	0·1	0·05
Mean		0·471	4·7	3·9	7·4	37·8	9·4	28·3	8·0	0·57
COMMON										
Salcombe, E.	*3*	0·545	0·3	5·0	45·6	21·4	4·3	14·8	8·3	0·35
Salcombe, E. (C)	*12*	0·482	1·0	13·2	19·4	8·7	4·0	31·0	21·4	1·25
Yealm River	*1*	1·307	25·2	19·0	19·7	29·3	1·7	2·2	1·7	1·19
Tresco	*50*	1·242	10·6	42·5	32·0	10·6	2·1	1·9	0·3	0
Tresco-Bryher channel	*120*	0·961	11·7	21·0	21·1	27·7	6·3	10·7	1·4	0·13
,,	*121*	1·267	25·7	16·0	15·9	30·9	5·0	5·5	0·8	0·19
,,	*122*	0·978	10·6	23·3	20·6	38·0	3·2	3·8	0·4	0·19
Foreman's Is.	*126*	1·020	20·5	9·1	11·0	41·7	7·6	8·1	1·7	0·40
,,	*127*	0·641	5·5	8·5	13·9	58·7	6·8	5·9	0·7	0·17
,,	*129*	0·964	13·9	15·5	21·2	36·3	4·5	4·8	2·4	1·50
Tresco-Bryher channel	*130*	1·138	19·1	16·8	23·3	34·0	3·5	2·4	0·6	0·27
,,	*131*	0·622	3·3	9·9	21·7	47·7	9·2	7·7	0·3	0·13
Newton Haven	*19*	0·195	1·1	0·6	1·2	3·3	1·5	64·7	27·3	0·25
Grouville Bay	*9*	0·314	0	0·3	6·3	48·4	17·1	25·2	2·7	0·12
Goulven	*158*	1·612	30·2	33·0	21·0	14·1	0·6	0·7	0·4	0·04
Mean		0·886	11·9	15·6	19·6	30·0	5·2	12·6	4·7	0·41

[1] Offshore records. (A), with *E. siliqua*; (C), with *E. ensis*.

TABLE III. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS ENSIS* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.) >2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Torr Abbey Sands	*140*	0·143	1·1	0·1	0·1	0·3	0·4	35·9	61·4	0·82
Elbury Cove	*105*	0·125	0	0	0	2·5	1·2	45·6	49·6	1·02
Elbury Cove (A)	*106*	0·117	0·3	0	0	0·8	0·7	33·9	63·5	0·83
Salcombe, W.	*163*	0·836	21·1	6·6	0·8	2·9	3·9	32·6	30·9	1·17
Yealm River	*72*	1·164	28·1	10·2	4·6	20·0	8·3	20·6	7·4	0·76
Drake's Is. (A)	*172*	0·620	7·9	15·9	7·2	5·1	3·3	21·1	37·5	1·95
Drake's Is.	*174*	0·225	2·3	1·6	1·1	4·9	4·3	34·9	49·1	1·87
Flushing Cove	*54*	0·142	0	0·3	1·2	7·9	3·3	29·2	55·7	2·45
Grouville Bay	*7*	0·168	0·3	0·1	0·1	2·0	3·3	82·7	11·4	0·12
Grouville Bay (B)	*16*	0·215	0	0·4	3·3	13·0	12·7	61·8	8·7	0·18
Térénès	*147*	0·267	1·3	3·7	3·1	6·1	3·5	64·8	16·7	0·82
Térénès (B)	*149*	0·245	0·8	1·1	1·1	15·5	17·3	60·0	3·8	0·46
Goulven	*156*	0·194	0	0·1	2·0	16·9	7·1	47·9	25·9	0·14
Plymouth[1]	*116*	0·185	0·1	0·1	0·6	9·2	17·4	57·1	13·8	1·80
Plymouth[1]	*113*	0·272	2·4	3·2	3·2	3·4	2·8	51·6	30·1	3·30
Plymouth[1]	*32*	0·158	0	0·2	0·7	2·9	4·8	69·6	19·8	2·12
Mean		0·317	4·1	2·7	1·8	7·1	5·9	46·8	30·3	1·24
OCCASIONAL										
Broadsands (A)	*2*	0·110	0	0	0·3	0·3	0·3	34·2	64·4	0·47
Broadsands (A)	*11*	0·199	1·3	0·7	0·5	1·0	0·8	77·8	17·7	0·29
Salcombe, E. (B)	*12*	0·482	1·0	13·2	19·4	8·7	4·0	31·0	21·4	1·25
Drake's Is. (A, B)	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Helford River	*24*	0·094	0	0·3	0·1	0·4	0·6	22·0	60·6	16·00
,,	*36*	0·177	0·7	0·1	0·2	2·5	3·9	73·1	18·6	0·83
,,	*40*	0·226	2·7	1·2	1·2	2·7	1·8	45·1	40·3	5·17
Helford River (A)	*41*	0·268	3·3	1·1	0·9	5·3	5·9	58·9	22·5	2·20
Helford River (A, B)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Flushing Cove (A)	*55*	0·169	0	0·1	0·4	11·0	9·3	49·5	28·5	1·28
Flushing Cove	*162*	0·147	0	0·4	0·6	6·0	4·7	41·9	45·3	1·26
Penpoull	*145*	0·443	7·4	4·5	2·8	3·4	1·8	62·9	16·1	1·14
Great West Bay, St. 12[1]	*27*	0·161	0·2	0·2	0·7	3·4	1·4	69·0	24·8	0·40
Great West Bay, St. 17[1]	*28*	0·136	0·1	0·3	0·9	5·6	2·2	33·8	53·6	3·45
Great West Bay, St. 16[1]	*29*	0·157	0·1	0·2	0·9	7·4	3·9	48·2	38·8	0·54
Torbay, St. 9[1]	*167*	0·089	0	0·1	0·1	0·2	0·3	10·8	87·7	0·77
Torbay, St. X[1]	*170*	0·080	0	0	0·1	0·2	0·1	1·4	97·3	0·91
Plymouth[1] (B)	*31*	0·177	0·1	0·4	1·1	6·1	8·6	63·7	19·8	0·22
St Austell Bay, St. 3[1]	*33*	0·145	0	0·2	1·0	5·5	5·3	46·4	30·9	10·74
St Austell Bay, St. 4[1]	*34*	0·098	0	0·1	0·6	2·0	1·6	19·6	60·9	15·29
St Austell Bay, St. 6[1]	*35*	0·109	0	0·4	1·3	4·2	1·2	7·0	80·2	5·71
Irish Sea[1]	*14*	0·203	0	0·7	1·6	12·2	8·8	65·0	10·6	1·22
Mean		0·189	0·8	1·2	1·8	6·0	3·9	43·5	39·5	3·29
COMMON										
St Aubin's Bay	*21*	0·180	0·5	1·8	1·8	8·2	5·1	20·3	61·9	0·42
Great West Bay, St. 19[1]	*30*	0·313	1·8	1·7	5·5	26·7	8·3	37·4	14·0	4·63
Aberporth Bay[1]	*160*	0·300	0·5	0·3	0·6	53·4	21·6	12·8	7·4	3·33
Mean		0·264	0·9	1·3	2·6	29·4	11·7	23·5	27·8	2·79

[1] Offshore records. (A), with *E. siliqua*; (B), with *E. arcuatus*.

histograms is not due to both being made up of the same set of samples. *E. ensis* is able to tolerate a slightly higher percentage of coarse material over 0·5 mm., but typically occurs where there is 3% or less. *E. ensis* is found in

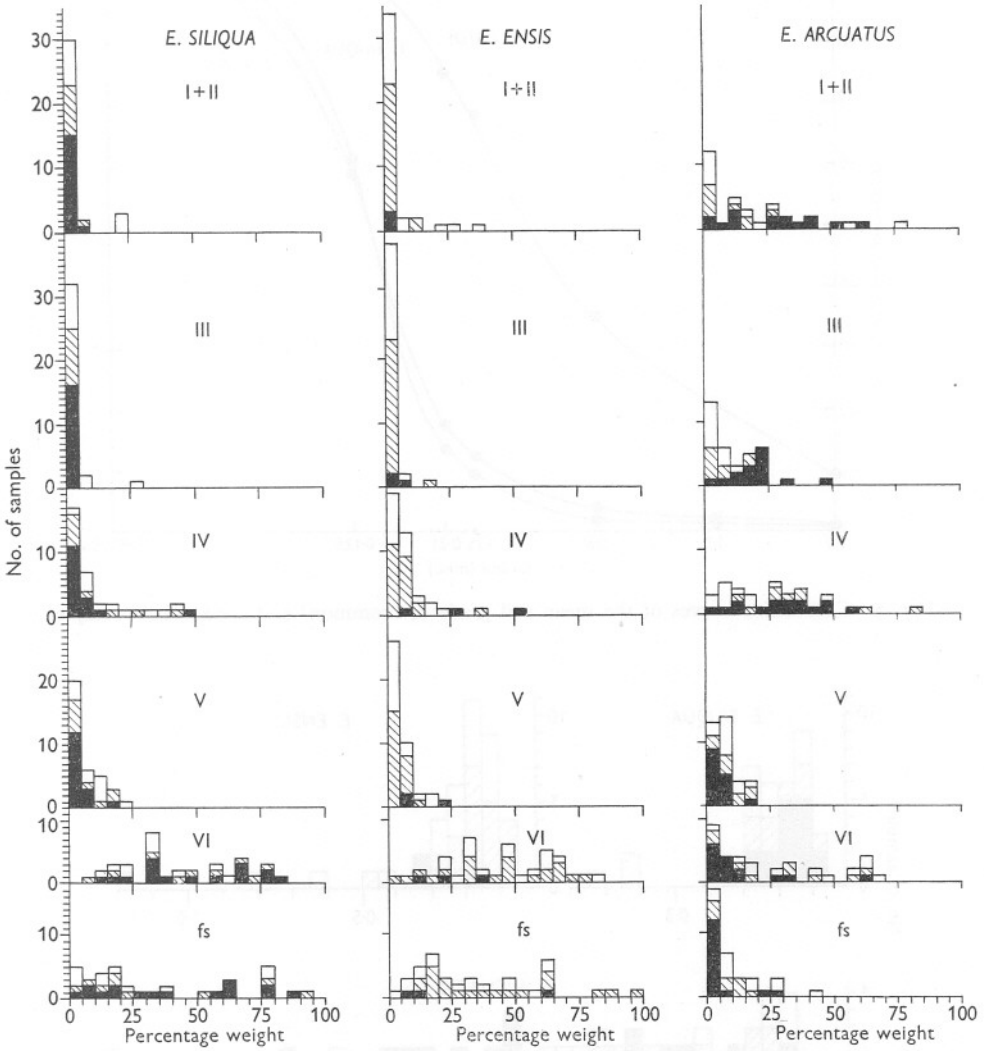

Fig. 2. Diagram summarizing the soil analyses given in Tables I–III. The percentage weights in each grade have been grouped in 5% units, and the number of samples in each 5% group are shown in histogram form. The silt grade has been omitted. Samples are represented thus: ■, common; ▨, occasional; □, scarce.

soils with a silt percentage ranging from 0·1 to 16%, the mean being about 3%. One might expect the mean silt percentage for this species to be rather higher than for *E. siliqua*, since most of the samples are from sheltered

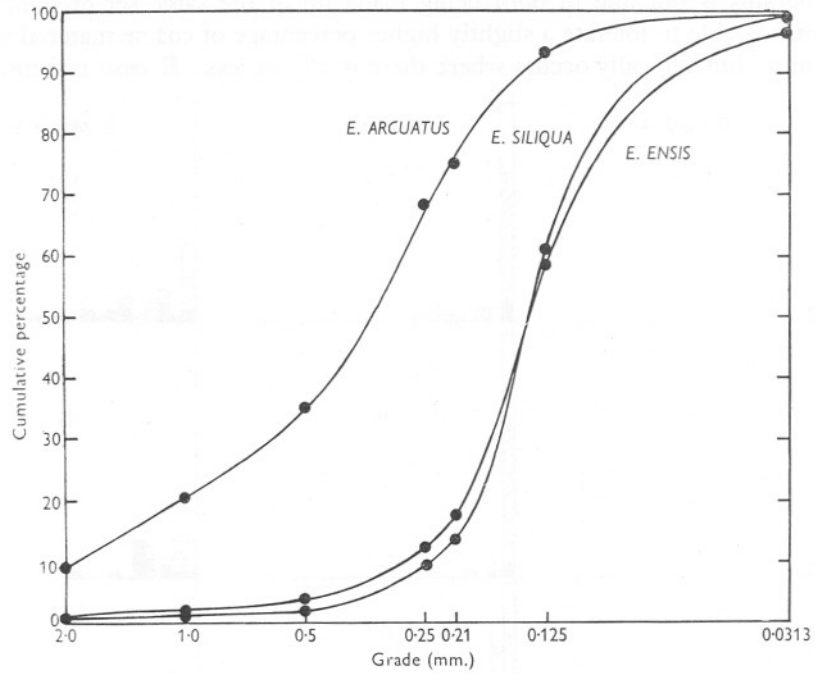

Fig. 3. Cumulative curves of the mean soil grade of 'common' and 'occasional' samples.

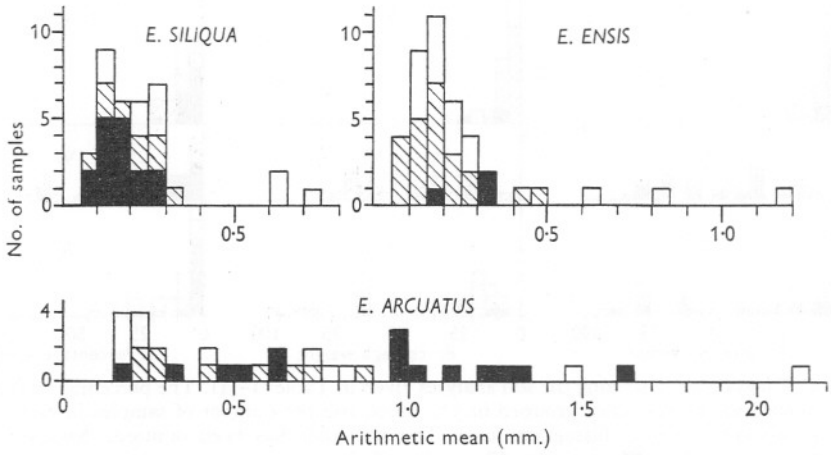

Fig. 4. The arithmetic mean of soils inhabited by the three species. The mean is grouped in units of 0·05 mm. Samples are represented thus: ■, common; ▨, occasional; □, scarce.

localities or from offshore, whereas the silt content is usually higher than on an open beach, but the occurrence of *E. ensis* in three samples containing over 10% silt indicates that it does have a greater silt tolerance.

E. arcuatus inhabits soils of a coarser grade than the other two species. The arithmetic mean of the soils ranges from less than 0·2 mm. to over 1·5 mm. In fine-grade soils its distribution overlaps that of *E. siliqua* and *E. ensis*, and it occurs with either or both of these species on certain beaches. Sands inhabited by *E. arcuatus* typically contain about 35% over 0·5 mm., and naturally show a correspondingly lower percentage in the finer grades. It is therefore not possible to say whether the distribution of this species is correlated with the presence of coarse or the relative scarcity of fine particles.

The density of *Ensis* populations is usually greater in sands approaching the 'mean' grade of the samples for that species. This is clear from an inspection of Figs. 2 and 4. In particular, population densities of *E. siliqua* and *E. ensis* are less in soils coarser than the mean. The densities on different beaches cannot readily be related to the grade of deposit, as so many other factors may be concerned. In St Austell Bay, however, there are a number of open beaches within a few miles of each other (Fig. 5), on which the populations of *E. siliqua* are very different. These differences may be correlated with the varying grade of beach deposit, but the effect could be an indirect one due to the greater disturbance by waves on beaches of coarse sand (see p. 158).

Beach	Arith. mean (mm.)	Population density
Porthpean	0·701	Scarce
Par (nr. Callyvador rocks)	0·287	Occasional
Pentewan	0·222	Occasional
Duporth	0·211	Common
Par (E. end beach)	0·096	Common

Nature of the Soil Particles

The sands in which *Ensis* occurs are usually predominantly of quartz grains, although the percentage of calcareous material is often high.

Sandy beaches in Devon and Cornwall are usually discontinuous, being separated by rocky headlands, so that little movement of material along the coast can occur. The sand grains on any beach are probably formed largely from rocks in the vicinity, together with material washed in from offshore. The size, shape, and surface texture of the quartz grains will depend on the rocks from which they are derived (Hatch & Rastall, 1952, pp. 86–90), although some subsequent degree of rounding may be achieved on exposed beaches.

Between Paignton and Exmouth the cliffs are mainly of New Red Sandstone, which produces fine-grade quartz grains with some degree of rounding. Much of the coastline between Torbay and Mount's Bay is formed of Devonian slates and grits: these produce more angular grains, similar to the Polzeath

sand figured by Wilson (1948, plate XVII). Grains derived from the chain of granite bosses extending through Devon and Cornwall are probably found in most of these sands. Where there are china clay workings, kaolin and mica may be carried down to the sea in large quantities, contributing both to littoral and offshore deposits. This is particularly noticeable in St Austell Bay. The granite of the Scilly Isles produces a coarse-grained quartz sand, in which *E. arcuatus* is abundant.

In Jersey and Brittany the local granite and metamorphic rocks produce quartz grains of a much smaller size than in Scilly. Pruvot (1897) has described the geology and deposits of the N. Brittany coast, and has shown that many of the beaches have a high calcium carbonate content.

There is no evidence that any species of *Ensis* is restricted to sand of a particular mineralogical character, degree of roundness, or surface texture. Wilson (1953) suggests that an organic coating on certain sands affects the settlement of *Ophelia* larvae. Most sands probably possess an organic coating of some sort, but this matter has not been investigated. Certain sands from the Scilly Isles have a conspicuous green coating on the grains, which may be of algal or fungal nature.

Reducing Conditions in the Sand

Ensis typically occurs in sands which are not black below the surface, indicative of reducing conditions. Where seaweed or other organic matter gets buried in and incorporated in the sand, resulting in a black layer containing ferrous sulphide, *Ensis* is absent. The absence of *Ensis* from beaches at Mothecombe and Bovisand, near Plymouth, is probably due to such conditions. *Ensis* can tolerate sands which are slightly reducing, in which there is a grey layer below the surface, however. Such conditions occur on beaches of firm fine sand in which the organic content is not high, but there is little circulation of water. *E. siliqua* has been found in 'grey' sands at Torr Abbey, Elbury, S. Michel, etc.

Wave Exposure

On exposed beaches where the sand is continually churned by waves, *Ensis* is absent. Such beaches are typically barren. *E. siliqua* is only found where there is at least slight shelter from wave action. Tolerance of wave action seems to be dependent on the stability of beach deposits. King (1951) has shown that on an exposed beach the depth of disturbance during storms is unlikely to exceed 20 cm., but if wholesale transport of material occurs the sand may be disturbed to a much greater depth. The depth of disturbance in a coarse sand is greater than in a fine one, on account of the steeper slope of beaches of coarse material.

Although *E. siliqua* is capable of rapid burrowing, it is sometimes washed inshore in large numbers after storms, as are the other species. In very

exposed areas it is only able to live below low-water mark, where wave disturbance is less severe (e.g. in Bigbury Bay). Some of those washed on to an exposed beach after a storm may burrow into the sand and establish a temporary foothold until disturbed by the next storm. This may account for occasional specimens found on the beach at Whitsand, also on the Polesands, where wave action is not extreme, but the bank is too shifting for *Ensis* to persist. On the other hand, *E. siliqua* is found in large numbers on the exposed beach at Marazion, in Mount's Bay. Its occurrence may be due to the sand there being fine and gently sloping, so that the depth of disturbance is minimized. The arithmetic mean of a sample from Marazion was 0·110 mm., as against 0·276 mm. in Whitsand Bay.

E. arcuatus seldom occurs on open beaches in south-west England, and this may be due to the relatively greater depth of disturbance for a given wave height on beaches of the coarse sand which it inhabits. It is confined to sheltered harbours and estuaries where accumulations of coarse material occur. It is also found in relatively sheltered beaches between the islands in Scilly.

Although occurring in sands of a similar grade to those occupied by *E. siliqua*, *E. ensis* is absent from the more exposed beaches on which the former occurs. In such exposed areas, it may occur only below low-tide mark (e.g. St Austell Bay, Fig. 5). In Jersey and Finistère, however, it occurs on exposed beaches (see pp. 162–3).

It is difficult to obtain an objective estimate of the severity of wave action on a beach. On nearly all beaches on which *Ensis* occurs, wave action may at times be severe, and a comparison of beaches is perhaps best made in relation to the normal conditions which prevail, rather than to the extreme conditions when a storm is blowing from an unusual quarter. On the basis of the normal wave exposure, a tentative classification has been made as follows:

(1) Beaches fully exposed to prevailing wind and swell (from south-west).

(2) Beaches with some slight shelter from prevailing wind and swell.

(3) Beaches sheltered from prevailing wind and swell, but exposed to waves from another direction.

(4) Beaches in bays which are sheltered from prevailing wind and swell.

(5) Sheltered beaches in harbours and estuaries.

Such a classification cannot take into account individual differences due to submarine topography, etc., but in the absence of detailed observations is the nearest approximation which can be achieved.

The occurrence of species of *Ensis* in relation to wave-exposure is summarized in Table IV. *E. siliqua* is found in all five categories, but *E. ensis* and *E. arcuatus* are more or less confined to groups 4 and 5. Notable exceptions are the occurrence of these last two species on exposed beaches on the south side of the Channel.

The occurrence of *E. siliqua* and *E. ensis* in St Austell Bay and Torbay is shown in Fig. 5. St Austell Bay is sheltered from the west, but is open to the south and south-east. *E. siliqua*, only, occurs on the beaches there, exposure being too great for *E. ensis*, which occurs offshore. (*E. siliqua* has not been recorded offshore, where it no doubt occurs in shallow water.)

TABLE IV. OCCURRENCE OF *ENSIS* IN RELATION TO WAVE EXPOSURE

(Names in italics are of beaches on the south side of the Channel. +, 'common' or 'occasional' records; (+), 'scarce' records. Samples from certain areas (e.g. Scilly Isles) are lumped together. *, probably washed in from offshore.)

Exposure	Beach	*siliqua*	*arcuatus*	*ensis*
1	Whitsand	(+)*	–	–
	Marazion	+	–	–
2	*St Aubin's Bay*	–	(+)	+
	Goulven	–	+	(+)
3	Polesands	(+)*	–	–
	Dawlish	+	–	–
	Paignton	+	–	–
	Goodrington	+	–	–
	Saltern	(+)	–	–
	Par	+	–	–
	Duporth	+	–	–
	Porthpean	(+)	–	–
	Pentewan	+	–	–
	S. Michel	+	–	
	Perharidy	–	+	–
	Grouville	–	+	(+)
4	Torr Abbey	+	–	(+)
	Broadsands	+	–	+
	Elbury	+	–	(+)
	Cellars Beach	–	+	(+)
	Scilly Is.	–	+	–
	Dale	+	–	–
	Térénès	–	(+)	(+)
5	Salcombe	+	+	+
	Yealm (excl. Cellars Beach)	–	+	–
	Drake's Is.	(+)	+	+
	Helford	+	(+)	+
	Flushing	+	(+)	+
	Penpoull	–	–	+

Torbay is more sheltered, being open only to the east. *E. siliqua* occurs on all sandy beaches, and in shallow water offshore. *E. ensis* is absent from beaches in the middle of the bay, being found only at Torr Abbey Sands, in the north-west corner, and Broadsands and Elbury in the south-west corner. These beaches are probably a little more sheltered than those in the middle of the bay.

Currents

On open beaches the effects of currents are negligible in comparison with the water movements produced by breaking waves. No correlation has been found between the occurrence of *Ensis* and the strength of currents, although

these have an indirect effect by their action on the distribution of particles, particularly in sheltered waters.

Depth Distribution

On the shore all species occur from about MLWST downwards, often increasing in number toward extreme low-water mark.

E. siliqua extends seawards to a depth of *c.* 20 m. on suitable substrata. It seems to be most common at extreme low water and just below. Several specimens were taken in Bigbury Bay in 20 m., and it has also been taken in the anchor-dredge in Torbay in depths of 1·6–6·9 m., where it is common.

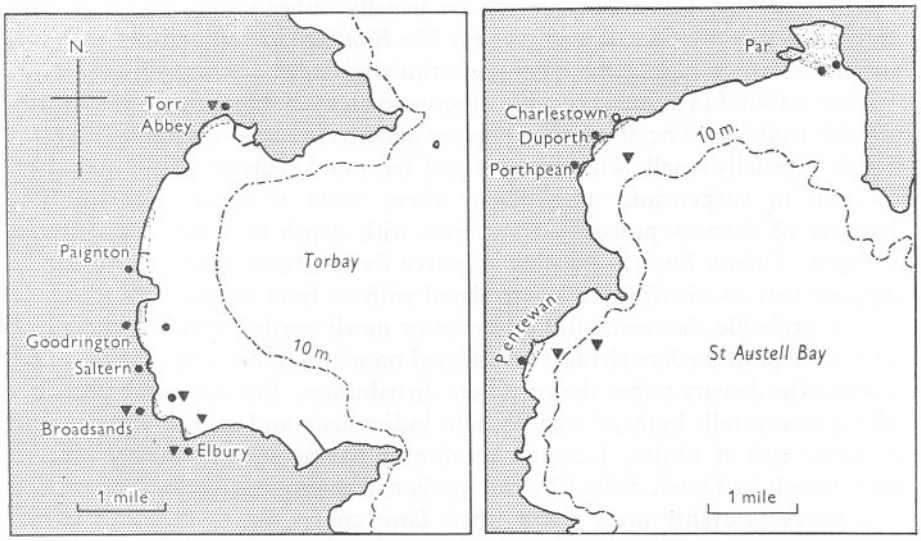

Fig. 5. Charts of Torbay and St Austell Bay, showing the distribution of *E. siliqua* (●) and *E. ensis* (▼). Beach samples are indicated on the land masses, offshore samples on the sea, the latter in their approximate positions. A negative record for Charlestown is also shown. Sandy beaches are stippled. The 10 m. depth contour is shown.

Other specimens have been taken in shallow water in Whitsand Bay and Plymouth Sound.

E. ensis occurs in small numbers at LWST in south-west England, but is much more common in depths of *c.* 10 m. (see Table VII, Appendix, and Ford, 1923). Single specimens have been taken in depths of 58·5 m. and 60 m. off Plymouth (Holme, 1953).

E. arcuatus occurs at LWST and has been recorded in 14·5 m. in Whitsand Bay. It is sometimes taken when dredging in the Eddystone shell gravel in *c.* 42 m. Ford (1923) records it from a number of stations in Plymouth Sound.

Moore (1936) has shown that *Echinocardium cordatum*, which lives in a similar habitat to *Ensis siliqua*, settles below low-water mark, and may

subsequently migrate into the intertidal region. There is no evidence of a similar migration in *Ensis*. Although small specimens of *Ensis* were not often dug on the shore, they might easily have been missed since sieving methods were not employed. On those days when *Ensis* came out of the sand at low tide, small specimens of a size seldom taken when digging were sometimes seen. These would normally be missed because their burrows are inconspicuous, and they would rarely be taken during random digging. The only movements of populations so far recorded occur when *Ensis* are washed in during storms (p. 158).

Biotic Factors

The inhabitants of a sandy beach are usually rather sparse, and it is difficult to see that filter-feeding organisms like *Ensis* are in competition with one another for a food supply that is being continually renewed by water movements.

Ensis is found in habitats in which there are great differences in the quantity of food available. The amount of organic detritus in suspension over an open beach is usually small, while in enclosed bays and estuaries large quantities may be in suspension, particularly where there is much *Zostera*. The quantity of detritus probably also varies with depth of water and distance offshore. Unless *Ensis* is a highly selective feeder, there seems no reason to suppose that its distribution is correlated with its food supply.

It is probable that some toll of larvae or newly settled spat is inflicted by filter or deposit feeders already established on a beach, but this is more likely to affect the density rather than absolute distribution. Similarly, fish probably take a heavy toll, both of small whole individuals and of the siphons and posterior end of adults. I cannot recall ever having seen an empty shell of *Ensis* which had been drilled by the predatory gastropod *Natica*.

A heavy mortality must result when *Ensis* comes out of the sand at low tide (see p. 146). While some probably survive the exposure and burrow in again when the tide returns, many must be eaten by gulls.

Distribution on the Southern Side of the English Channel

The distribution and abundance of *Ensis* on beaches in Finistère and Jersey is rather different from that in south-west England. Although the grade of soil occupied by each of the three species is much the same as in England (Tables I–III), there are differences in tolerance of wave-exposure (Table IV).

In Jersey the tidal range is large, being 32·5 ft. (9·91 m.) at spring tides. The beaches are gently sloping in most places, so that the distance between high and low water may be as much as a kilometre or more. St Aubin's Bay and Grouville Bay are relatively exposed, but on such gently sloping beaches, mostly of firm fine sand, the depth of wave disturbance cannot be great (cf. King, 1951). In the middle of St Aubin's Bay (near Beach Rock), wave action is so severe as to exclude burrowing lamellibranchs, although small

clumps of eel-grass (*Zostera*), indicating fairly stable conditions were found. Close to St Aubin's Fort, on the west side of the bay, where there is more shelter, *E. ensis* was found in some abundance, together with several *E. arcuatus*. This is the only beach on which *E. ensis* has been taken in any numbers. *Solen marginatus* Montagu also occurs, but at a slightly higher level on the beach.

In Grouville Bay, which is open to the east and only sheltered in this direction by isolated reefs, *E. arcuatus* and *E. ensis* were quite common near S. Etac, as was *Solen marginatus*.

These beaches differ from those in south-west England in the presence of *Ensis ensis* and *E. arcuatus* under more exposed conditions, in the absence or rarity of *E. siliqua* (none was found during 3 days' collecting), and in the abundance of *Solen marginatus*, which is restricted to sheltered conditions in muddy sand of estuaries, etc., in England.

E. arcuatus and *E. ensis* are fairly common on beaches in N. Finistère, but *E. siliqua* is not, being only found on one of the beaches investigated, S. Michel. Here *E. siliqua* was present in small numbers on the fairly exposed beach, together with many specimens of the pod-razor, *Pharus legumen major* Bucquoy, Dautzenberg & Dollfus, and *Mactra corallina cinerea*, in great abundance. *Ensis ensis* was not taken on this beach. Both *E. arcuatus* and *E. ensis* were found on the relatively sheltered beach at Térénès. At Goulven, a wide sandy beach some 5 km. across, the sand at the eastern, exposed, end is almost barren (one *Corystes cassivelaunus* was the only burrowing animal found). Towards the middle of the bay there are patches of *Zostera*, under conditions similar to those in which it was found in St Aubin's Bay, but no burrowing lamellibranchs were taken. In the middle of the bay there is slightly more shelter from the headland on the west side of the bay, but the sand is thrown up into ridges at right angles to the direction of wave incidence, evidence of considerable wave disturbance. On these ridges were found *Callista chione* (L.), *Donax variegatus* (Gmelin), *Spisula* sp., and *Ensis ensis* and *E. arcuatus* in small numbers. *Solen marginatus* is rare or absent on exposed beaches in Finistère, but is found in sheltered areas, as at Penpoull, in a habitat similar to that in which it occurs in S.W. England.

E. siliqua is also scarce farther south: in a survey of sandy beaches near Quiberon (S. Brittany), Prenant (1932) makes no mention of the occurrence of *E. siliqua*, although species living in a similar habitat, such as *E. ensis* and *Donax vittatus* (da Costa) are recorded. *Ensis siliqua* could hardly have been mistakenly identified as *E. ensis*.

Sea temperatures on either side of the Channel are given in Tables V and VI. The mean annual temperature at Plymouth and Jersey is almost the same, but the range of monthly means in Jersey is greater. At Roscoff the mean annual temperature is perhaps a little higher than at Plymouth, but the range of monthly means is much the same. Although the slightly higher summer

temperatures on the southern side of the Channel may be important for the breeding of a species at the northern end of its range, such differences can hardly explain the differences in ecology of *Ensis* which ranges both north and south of the area. Another possible cause of the differences in ecology is discussed on p. 168.

TABLE V. MEAN MONTHLY AND ANNUAL SEA SURFACE TEMPERATURES FOR 1903–27 (° C.) AT THE NEAREST POSITIONS TO PLYMOUTH, JERSEY, AND ROSCOFF FOR WHICH SUCH DATA ARE AVAILABLE

(Data from Lumby, 1935.)

	50° 15′ N., 04° 15′ W. (Plymouth)	49° 08′ N., 02° 22′ W. (Jersey)	48° 52′ N., 04° 38′ W. (Roscoff)
Jan.	10·13	9·08	10·60
Feb.	8·70	8·06	9·83
Mar.	8·87	8·42	9·98
Apr.	9·43	9·29	9·74
May	10·96	11·45	11·10
June	12·88	13·75	12·43
July	15·16	15·59	14·72
Aug.	15·65	16·74	15·15
Sept.	15·02	16·27	14·22
Oct.	14·45	15·03	13·97
Nov.	12·76	12·86	12·90
Dec.	10·71	11·06	11·16
Mean annual	12·06	12·30	12·15

TABLE VI. MEAN MONTHLY AND ANNUAL SEA TEMPERATURES (° C.) AT PLYMOUTH AND JERSEY FOR THE YEARS 1947 TO 1951 INCLUSIVE

(Samples taken from the shore, daily. Data for Plymouth kindly supplied by G. H. Ivory and Partners, Plymouth; that for Jersey taken from the *Société Jersaise, Bulletin Annuel*, for 1948 to 1952.)

	Plymouth	Jersey
Jan.	9·0	6·9
Feb.	7·9	6·3
Mar.	8·3	7·3
Apr.	9·8	8·7
May	11·7	11·6
June	14·2	14·2
July	15·6	16·3
Aug.	16·6	17·6
Sept.	16·2	16·8
Oct.	14·7	14·6
Nov.	12·3	11·4
Dec.	10·2	8·9
Mean annual	12·2	11·7

NOTE ON THE LARVA OF *ENSIS*

All three species have a pelagic larva. Attempts at artificial fertilizations have not been entirely successful; adults collected during the spring of 1950 were never fully ripe, although Lebour (1938) states that *E. siliqua* breeds in early

spring. A few larvae of each species were obtained, but only *E. ensis* was reared to metamorphosis. Shelled larvae, as described by Lebour, were obtained for all three species; these possess a large central cilium similar to that described by Lebour (1938, p. 139) for *Hiatella gallicana*, although Lebour states that no such cilium is present in *Ensis siliqua*.

Only a very few specimens of *E. ensis* were reared to metamorphosis, which took about 1 month at 13–15° C. The larvae were fed on Flagellate I, *Isochrysis galbana*. At metamorphosis these larvae had a shell length of *c.* 290μ. A late larva was seen swimming by its velum and occasionally extending its foot: at this stage it will presumably settle under suitable conditions.

Discussion

The factors believed to be of importance in the distribution of species of *Ensis* may be summarized as follows. *E. siliqua* and *E. ensis* are restricted to sands of a fairly fine grade in which the percentages both of silt and of coarse particles are fairly low. *E. ensis* shows a rather greater tolerance of silt and of coarse particles than *E. siliqua*. *E. arcuatus* inhabits sands of a coarser grade, but has a wide tolerance of different grades, so that its distribution overlaps that of the preceding species to some extent.

Sands black below the surface are avoided by all species.

E. siliqua can withstand a moderate degree of wave-exposure, but is absent from fully exposed beaches. *E. arcuatus* and *E. ensis* are restricted to more sheltered beaches. The stability of the soil is an important factor in determining the occurrence of all species on fairly exposed beaches.

On the shore, *Ensis* occurs only below mean low-water mark of spring tides. Offshore, all three species are common in shallow water. *E. siliqua* occurs down to only *c.* 20 m., but the other species occur in small numbers in deeper water on the Continental Shelf. Where wave action is so severe as to exclude a species from a particular beach, it may occur below low-tide marks where disturbance is less extreme.

South of the English Channel *E. siliqua* is much less common, while the tolerance of *E. arcuatus* and *E. ensis* to wave action appears to increase, so that they are found on moderately exposed beaches.

There is no evidence that the mineralogical nature of the sand grains, their shape or surface texture, currents, food supply or other biotic factors influence the absolute distribution of the species, although certain biotic factors may influence population density.

Ecological factors may limit the distribution of a species, either through a direct lethal effect, or by their influence on the choice of a particular environment. In the latter instance the adult may be living well within the lethal limits imposed by the environment, due to the effect of some form of 'habitat-selection', and as a result of which not all habitats in which the individual

could survive become populated (cf. Elton, 1935, pp. 40–1). In sedentary marine organisms there is some evidence that the distribution of the adult is partly related to a choice by the larva of a suitable substratum in which to metamorphose.

Postponement of metamorphosis until a suitable substratum is reached has been recorded for a number of species (summarized by Wilson, 1952), including at least one mollusc, *Teredo*. Although larvae of several species have been shown to respond to the nature or particle size of the substratum, it is possible that others are induced to settle in the vicinity of adults or newly settled spat of the same species (cf. Knight-Jones, 1951). If the larva reacts to some feature of the environment other than the presence of other individuals of the same species, suitably devised settling experiments should explain some features of distribution. No such experiments have so far been made owing to difficulties in rearing the larvae. If, however, the larva reacts to the presence of other individuals of the same species, or if there is no highly specific settling reaction, the distribution of adults must be explained in terms of conditions favourable or otherwise to the individual after settlement.

The preference of species of *Ensis* for soils of a particular grade may well be due to a larval settlement reaction. The three species are so similar in structure and habits that it seems likely that any one species could burrow into and live in any of the full range of soils inhabited by all species. Silt, alone, may be a limiting factor to survival of the adult. The high silt percentages tolerated by *Ensis ensis* may be lethal to the other species. Pratt (1953) has shown that the lamellibranch *Venus mercenaria* grows more rapidly in sand than in a muddy sand, and this may be explained by the observations of Loosanoff & Tommers (1948), who found that even small quantities of suspended silt decrease the filtration rate of oysters (*Ostrea virginica*), which would adversely affect their food intake.

Reducing conditions in the soil, which are probably lethal to the adult, might affect distribution either at the settling stage or through subsequent mortality of newly settled individuals.

It is hard to see that *Ensis* larvae could distinguish the normal conditions of wave-exposure on a beach. Abortive colonization of more exposed beaches probably occurs, perhaps during calm weather, and when normal conditions of wave-action return the spat are washed out of the sand.

Ensis is probably restricted to low spring-tide level on the shore because it is not adapted to withstand the fluctuations of temperature, salinity, etc., that occur higher up the beach, but no explanation can be offered of the restricted depth range of *E. siliqua* offshore. Certain other species, e.g. *Donax vittatus* and *Tellina fabula*, have a similar depth range to *Ensis siliqua*.

Previous work on the ecology of benthic animals provides little information on the precise effects of particular limiting factors. The importance of soil

grade has long been recognized (e.g. Allen, 1899; Ford, 1923; Davis, 1925; Prenant, 1932; Powell, 1937), but most workers have directed their attention to a classification of species or 'communities' in relation to soil grade rather than to the study of the occurrence of individual species in different localities, as has been attempted here. The *range* of substrata inhabited by different species is very variable, but one may expect members of the infauna to be more restricted in their range than members of the epifauna. Powell (1937) considers that deposit feeders are more restricted in their choice of bottom than herbivores and suspension feeders, while carnivores appear to show no particular preference. Holme (1949*a*) has shown that the silt content of the soil has an important effect on the distribution of certain species on the shore.

Properties of sands other than particle size may also affect distribution. Wilson (1953) has shown the importance of 'attractive' or 'repellent' substances, possibly of organic origin, on the surface of sand grains for the settlement of the larvae of the polychaete *Ophelia bicornis*. Ekman (1947) has correlated the distribution of certain invertebrates with the firmness of the soil, as measured by a cone penetrometer. Preliminary experiments by the writer with a small cone penetrometer attached to the end of a slender rod have not been entirely satisfactory. It is essential that measurements be made in the undisturbed substratum, and not, as was done by Ekman, in a dish in which the sample has been allowed to settle for 24 hr. Under such conditions the original packing is not regained.

On the effects of wave action on burrowing organisms, there are little but general observations. It is generally accepted that wave action excludes many species from exposed beaches, and the importance of this factor is emphasized by Southward (1953).

Davis (1923) has shown that water movements in the North Sea may influence the distribution of settling larvae of *Spisula*. Settlement of spat in limited areas will occur only where the adults are more or less confined to isolated patches. In *Ensis*, where the adults of all species are widely distributed in coastal areas, there should usually be sufficient numbers of larvae in any one place for all suitable substrata to be occupied. A possible exception may be the Scilly Isles, where only *E. arcuatus* has been found, although there are undoubtedly patches of sand of a suitable grade for the other species. A few miles from the shore the bottom slopes away to some 80 m. depth, so it is unlikely that *E. ensis* and *E. siliqua* occur in any numbers in the vicinity of the islands. The Scilly Isles are 23 miles from the Cornish coast, so that the density of larvae of the latter two species in the Scilly Isles plankton is probably small. Consequently small patches of soil of a suitable grade may be only occasionally colonized by these species. Similarly, the relative scarcity of coarse-grade deposits in Great West Bay will result in a scarcity of breeding stock of *E. arcuatus*, so that suitable deposits in the area may not always be colonized.

Detailed observations have been confined to an area bordering the western half of the English Channel. The apparent differences in distribution on either side of the Channel makes one chary of applying conclusions to other areas, however. Temperature differences seem to be insufficient to account for the changes in distribution (pp. 163–4). There is probably little interchange between populations on either side of the Channel, so it is possible that races with different habitat-requirements occur. The western end of the English Channel is known to be an important boundary to the distribution of certain species (Ekman, 1953, pp. 81–2), and this boundary may be as much due to the geographical isolation of shallow-water species on each side as to temperature differences. Rees (1950) has shown that larvae of *Octopus vulgaris*, which have a pelagic life of at least a month, can spread to the south coast of England from breeding centres in the Channel Islands region, and one can presume that a similar interchange of *Ensis* populations does occur from time to time. Such interchange is probably on a restricted scale, and it is possible that different races could survive in spite of it.

Summary

An attempt is made to analyse the ecological factors affecting the distribution of the three European species of *Ensis*: *E. siliqua* (L.), *E. ensis* (L.) and *E. arcuatus* (Jeffreys).

All three species appear to be confined to European waters, but the exact limits of their range have not been established.

None of these species appears to occur outside Europe, but the North American *E. minor* Dall may be the same as *E. ensis*.

Soil grade is shown to be an important factor limiting distribution, although no species is narrowly confined to soil of a particular grade composition. Population densities are in general higher in soils approaching the 'average' grade inhabited by each species.

There is no evidence that the shape or mineralogical composition of the sand grains affects distribution.

All species avoid black, reducing, sands.

All species require some shelter from wave action, but *E. siliqua* can withstand much greater wave exposure than the other two. Tolerance of wave-action seems to depend on the stability of the beach deposits.

The three species are found at LWST on the beach, and in shallow water offshore. *E. siliqua* has a restricted depth range of *c.* 20 m., but the other species may be found in small numbers in deeper water.

Distribution in Jersey and Finistère differs from that in south-west England in greater tolerance of wave exposure by *E. arcuatus* and *E. ensis*.

Each species has a pelagic larva. *E. ensis* larvae were reared to metamorphosis in about a month.

The mode of action of limiting factors is discussed, and the results are considered in the light of previous work on the ecology of benthic invertebrates.

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APPENDIX

TABLE VII. LIST OF LOCALITIES

The name of the locality is followed by the serial number of the soil sample, enabling cross-reference to Tables I–III to be made. The occurrence and density of the species is shown next, in brackets: A, *Ensis siliqua*; B, *E. arcuatus*; C, *E. ensis*. This is followed by a National Grid Reference to the station, or its latitude and longitude. The depth of water at offshore stations is also given.

Shore Collections

DEVON

Polesands, Exmouth. S.W. corner. 47 (A, scarce). 20/998792.

Dawlish. Mid-way between Dawlish and Langstone Pt. 74 (A, occasional). 20/971773.

Torr Abbey Sands. 139 (A, common), 140 (C, scarce), 141 (A, common). 20/912635.

Paignton beach, S. end. 10 (A, common). 20/896605.

Goodrington Sands. N. end of beach. 60 (A, common). 20/895598.

Middle of S. half of beach. 59 (A, common). 20/895593.

Saltern Cove. 53 (A, scarce). 20/896585.

Broadsands. N. end of beach. 11 (A, common; C, occasional). 20/897578.

Middle of beach. 2 (A, common; C, occasional). 20/897576.

Elbury Cove. 105 (C, scarce), 106 (A, common; C, scarce). 20/903570.

Salcombe, E. S. side of Millbay. 6 (A, occasional; B, occasional). 20/740382.

N. side of Millbay. 3 (B, common), 12 (B, common; C, occasional), 63 (A, scarce). 20/740383.

Salcombe, W. 200 yd. S. of Marine Hotel. 64 (B, scarce). 20/738385.
Woodville Rocks. 65 (B, scarce), 163 (C, scarce). 20/736384.
Yealm River. Cellars beach. 70 (B, occasional), 71 (B, occasional), 72 (C, scarce). 20/531476.
Below Passage Wood. 69 (B, scarce). 20/537476.
Yealm sand bank. 1 (B, common). 20/539481.
Thorn Pt. 171 (B, scarce). 20/542490.
Drake's Island. E. end of N. side. 172 (A, scarce; C, scarce). 20/470529.
E. side. 173 (B, occasional). 20/471528.
E. of pier. 174 (C, scarce), 176 (A, scarce). 20/469529.
W. of pier. 175 (A, scarce; B, occasional; C, occasional). 20/468529.

CORNWALL

Whitsand Bay, Sharrow Pt. 117 (A, scarce). 20/393521.
Par Sands. E. end of beach. 68 (A, common). 20/087524.
Nr. Callyvador rocks. 66 (A, occasional). 20/083524.
Duporth. 62 (A, common). 20/036511.
Porthpean. 61 (A, scarce). 20/033507.
Pentewan. 136 (A, occasional). 20/019464.
Helford River, Gate Beach. 24 (C, occasional). 10/760268; 36 (C, occasional), 38 (A, occasional), 40 (C, occasional), 41 (A, scarce; C, occasional), 164 (B, scarce), 165 (A, occasional; B, scarce; C, occasional). 10/762268.
Flushing Cove (Gillan harbour). 56 (B, scarce). 10/787252. 54 (C, scarce), 55 (A, common; C, occasional), 57 (B, scarce), 58 (A, scarce), 162 (C, occasional). 10/784252.
Marazion. 52 (A, common). 10/509309.

SCILLY ISLES

Tresco, E. of Rushy Pt. 50 (B, common). 00/903151.
Tresco-Bryher channel. 120 (B, common), 121 (B, common). 00/886145. 122 (B, common), 00/886144. 130 (B, common). 00/884149. 131 (B, common). 00/884152.
Foreman's Island. 126 (B, common). 00/901158. 127 (B, common). 00/901160. 128 (B, occasional). 00/900161. 129 (B, common). 00/898162.

AYR

Fairlie Sands. 18 (A, common). 26/196541.

NORTHUMBERLAND

Newton Haven. 19 (B, common). 46/247241.

PEMBROKE

Dale Flats. 25 (A, occasional), 12/813058. 73 (A, common). 12/814060.

CHANNEL ISLANDS

Grouville Bay. 7 (C, scarce), 9 (B, common), 16 (B, occasional; C, scarce). 49° 10′ 25″ N., 02° 00′ 45″ W.
St Aubin's Bay. 15 (B, scarce), 21 (C, common). 49° 11′ 00″ N., 02° 09′ 35″ W.

FINISTÈRE

Penpoull. 145 (C, occasional). 48° 41′ 42″ N., 03° 57′ 00″ W.
Térénès. 147 (C, scarce), 149 (B, scarce; C, scarce). 48° 40′ 45″ N., 03° 51′ 12″ W.
Grève de S. Michel. 155 (A, occasional). 48° 41′ 15″ N., 03° 36′ 00″ W.
Grève de Goulven. 156 (C, scarce), 157 (B, occasional), 158 (B, common). 48° 39′ 40″ N., 04° 15′ 00″ W.
Perharidy. 159 (B, occasional). 48° 43′ 55″ N., 04° 00′ 06″ W.

TABLE VII (*cont.*)

Grab and Dredge Samples

Great West Bay. Station 12. 27 (C, occasional). 50° 35′ 12″ N., 03° 26′ 25″ W. 5·5 m.
Station 16. 29 (C, occasional). 50° 34′ 40″ N., 03° 26′ 48″ W. 10·5 m.
Station 17. 28 (C, occasional). 50° 34′ 25″ N., 03° 27′ 05″ W. 10·5 m.
Station 19. 30 (C, common). 50° 33′ 10″ N., 03° 27′ 00″ W. 16·5 m.
Torbay. Station 9. 167 (A, common; C, occasional). 50° 24′ 30″ N., 03° 32′ 54″ W. 3·2 m.
Station 18. 169 (A, occasional). 50° 25′ 24″ N., 03° 33′ 12″ W. 6·1 m.
Station X. 170 (C, occasional). 50° 24′ 12″ N., 03° 32′ 36″ W. *c.* 5·0 m.
Plymouth. 22 (A, common). 50° 15′ 45″ N., 03° 53′ 00″ W. 20 m.
116 (C, scarce). 50° 10′ 00″ N., 04° 10′ 00″ W. 60 m.
113 (C, scarce). 50° 15′ 00″ N., 04° 12′ 30″ W. 49 m.
32 (C, scarce). 50° 11′ 00″ N., 04° 05′ 00″ W. 58·5 m.
31 (B, scarce; C, occasional). 50° 19′ 42″ N., 04° 14′ 45″ W. 14·5 m.
St Austell Bay. 33 (C, occasional). 50° 17′ 15″ N., 04° 46′ 18″ W. 9 m.
34 (C, occasional). 50° 17′ 24″ N., 04° 45′ 42″ W. 13 m.
35 (C, occasional). 50° 19′ 30″ N., 04° 45′ 06″ W. 6 m.
Aberporth Bay. 160 (C, common). 52° 08′ 48″ N., 04° 31′ 48″ W. 13 m.
Irish Sea. 14 (C, occasional). *Approx.* 54° 23′ N., 03° 35′ W.

'Negative' Records

Records of sandy beaches on which no *Ensis* was found. Where the beach was examined on a very low spring tide, the name is in italics. The probable reasons for absence of *Ensis* are given thus: R, black, reducing sands; S, shifting, unstable sands; E, wave action extreme; L, lowered salinity owing to fresh water running over the beach at low tide. *, beaches where *Ensis* may have been washed up from deeper water, most of the beach being barren.

DEVON
Exmouth, nr. Maer Rocks. R. (30/010798.)
Polesands.* S. (30/002794.)
Salcombe, S. of Millbay. S. (20/737379.)
North Sands. L? (20/731381.)
South Sands. L? (20/729377.)
Borough Island and Challaborough Bay. E. (20/655439, 648443, 648448.)
Mothecombe. R., L? (20/612471.)
Bovisand. R. (20/492506.)
Saunton Sands (N. Devon). E. (21/435345–438378.)

CORNWALL
Whitsand Bay.* E. (*ca.* 20/407510.)
Charlestown. L? (20/040514.)
St Michael's Mount. R. (10/515303.)

SCILLY ISLES
Bryher, two beaches at S.W. end of island. E. (00/873141, 876140.)

JERSEY
St Aubin's Bay (middle). E. (49° 11′ 18″ N., 02° 08′ 42″ W.)

FINISTÈRE
Grève de Goulven (E. end). E. (48° 39′ 48″ N., 04° 14′ 00″ W.)
South of Isle de Sieck. E? (48° 42′ 00″ N., 04° 04′ 30″ W.)

J. Mar. biol. Ass. U.K. (1954) 33, 173–175
Printed in Great Britain

EFFECT OF EXTERNAL MILIEU ON LUMINESCENCE IN *CHAETOPTERUS*

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In a previous paper some effects of anisosmotic and unbalanced salt solutions on the luminescent responses of *Chaetopterus variopedatus* have been described (Nicol, 1952). Similar experiments have been carried out on polynoid elytra, and the results are described elsewhere in this Journal, together with a brief review of the relevant scientific literature (Nicol, 1954*b*). The present experiments were undertaken to investigate the effects of single salts in physiological concentrations, balanced by the addition of some inert substance, and to explain the possible mechanism of stimulation by hypo-osmotic solutions.

The experiments were simple. Whole specimens of *Chaetopterus*, or isolated anterior regions, were placed in the solutions to be tested. Salts (NaCl, KCl, $CaCl_2$, $MgCl_2$), sucrose and choline chloride were made up in solutions osmotically equivalent to normal sea water. Alkalinity of salt solutions was raised to pH 8·2.

Results were as follows:

Choline Cl	No luminescent response
Sucrose	Faint light appeared slowly
NaCl 81 + choline Cl 19	Faint brief light
KCl 2 + choline Cl 98	Faint brief light
$CaCl_2$ 3 + choline Cl 97	Little faint light, especially when container agitated
NaCl 81 + sucrose 19	Luminescent response
KCl 2 + sucrose 98	Luminescent response
$CaCl_2$ 3 + sucrose 97	Luminescence fairly bright
$MgCl_2$ 15 + sucrose 85	No light
NaCl 81 + fresh water 19	Luminescent response
KCl 2 + fresh water 98	Faint, brief light
$CaCl_2$ 3 + fresh water 97	Luminescent response
$MgCl_2$ 15 + fresh water 85	Luminescent response

Specimens were anaesthetized for half an hour in 0·2% chloretone or 0·5% cocaine (made up in sea water). They were then transferred to sucrose containing the same quantity of anaesthetic. A feeble luminescent response resulted.

These results confirm those obtained in earlier experiments, namely that solutions of the single salts NaCl and KCl are excitatory and evoke

luminescence; $CaCl_2$ raises irritability, resulting in faint light, especially if the preparation is subjected to gentle mechanical stimulation. In addition, the results show that these three salts produce their effects not only when acting in excess, but also when, balanced with choline chloride, they occur in normal physiological amounts.

Contrary to the effect of choline chloride, which appears to be physiologically inert so far as the luminescent response is concerned, sucrose evokes a faint luminescent response which is not blocked by anaesthesia with chloretone or cocaine, but which does not appear in the presence of $MgCl_2$.

As previously noted, hypo-osmotic sea water (25%) evokes luminescence, and luminescence appears in solutions of each of the four ions, Na, K, Ca and Mg, in physiological concentration plus fresh water. It is noteworthy that neither Ca nor Mg, in physiological quantity, block hypo-osmotic excitatory stimulation.

In an investigation of the viability of various marine animals in diluted sea water, Pearse (1928) found that *Chaetopterus* would tolerate 50% sea water for long periods. A previous study has shown that luminescence does not appear until the sea water is diluted 50% or more (Nicol, 1952). This is the physiological limit of the species, and the harmful effects of dilutions greater than 50% are not offset by $CaCl_2$ and $MgCl_2$ in physiological quantities.

In any system consisting of excitable tissue dependent upon a properly balanced ionic environment for stability, any radical alteration in the concentrations or proportions of the external ions will produce great changes in excitability, and depolarization of external boundaries. Changing the external milieu could affect either the nervous system or the luminescent cells. Evidence has recently been adduced to show that the latter possess a contractile mechanism for discharging the luminescent secretion. The excitatory effect of Na and K on excitable tissues is well known, and these two ions probably act on both the photogenic cells and nervous system of *Chaetopterus*. The responses which they induce are by no means as bright or as long-lasting as those evoked by strong electrical stimulation, probably owing to initial localized depolarization, followed by loss of excitability as this process becomes complete. The increased irritability produced by Ca has been reported for other invertebrate groups, and may be linked with Mg-lack (Robertson, 1941; Nicol, 1954*a*, *b*).

As in the vertebrate preparation, choline chloride appears to be inert; any possible blocking action such as it exerts on the neuro-muscular junction has not been ascertained. Sucrose, on the other hand, has a weak stimulatory effect in isosmotic concentration. Sucrose, therefore, is osmotically but not ionically inert. Its stimulatory effect on the luminescent response is not abolished by prolonged nervous anaesthesia, and it probably acts directly on the photogenic cells apart from any effect on the nervous system. The ionic unbalance produced by the introduction of isosmotic sucrose produces some

degree of depolarization owing to ionic shifts, and results in excitation and luminescence. The same effect is probably responsible for the luminescence appearing in diluted sea water or fresh water.

In a recent paper Bonhomme (1953) gives a histological picture of luminescent secretion in *Polycirrus* rather similar to that in *Chaetopterus*. The course of secretion is presented as opening of the cell, hydrolysis of secretory granules, and expulsion of photogenic material, possibly as the result of swelling or changed osmotic relations. The possibility that cytolysis or raised intracellular pressure resulting from imbibition of water may be responsible for the luminescence of *Chaetopterus* in dilute media has been considered, and seems most unlikely in view of the faint and transitory light produced, compared with the brighter response which can be evoked by electrical stimulation, and with the amount of photogenic material present. Dilute sea water and fresh water probably exert a transitory depolarizing effect on the excitable tissues, which rapidly lose irritability. A similar explanation is probably applicable to the many instances of luminescence in marine animals evoked by application of fresh water.

Summary

The effects of hypo-osmotic solutions, and of isosmotic solutions of choline chloride, sucrose, NaCl, KCl, $CaCl_2$ and $MgCl_2$ on the luminescent response of *Chaetopterus variopedatus* are reported. Choline chloride, as an inert substance, can be used in conjunction with each of the other salts to test the effects of the latter in physiological quantities. Sodium and potassium excite in physiological concentrations; calcium raises irritability. Sucrose produces a weak luminescent response, which is not blocked by nervous anaesthetics (cocaine, choloretone). Changes in the ionic environment induce localized depolarization, followed by complete depolarization and loss of irritability of both nervous and photogenic tissue. This explanation will probably account for most instances of luminescence evoked by ionically abnormal media.

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J. Mar. biol. Ass. U.K. (1954) 33, 177–186
Printed in Great Britain

FATIGUE OF THE LUMINESCENT RESPONSE OF *CHAETOPTERUS*

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(Text-figs. 1–6)

When stimulated in some suitable manner *Chaetopterus* secretes a luminescent material into the surrounding sea water. The luminescent secretion is discharged from glands which are widely dispersed over the surface of the animal; the most conspicuous are two glandular patches on the dorsal surface of segment XII. Secretory material is forced out of the cells by some contractile process; discharge is not merely the result of secretion pressure. Under repetitive stimulation the intensity of the luminescent response decreases owing to the intervention of fatigue. Fatigue has been interpreted as a gradual exhaustion of luminescent material in the glandular cells (Nicol, 1952*b*, *c*). The present investigation seeks to analyse the onset and progress of fatigue in greater detail.

The cost of part of the equipment used in this research was met by a grant-in-aid of scientific research from the Royal Society. I acknowledge with gratitude technical assistance from Mr A. E. Stoate and Mr F. J. Warren.

Material and Methods

Fresh specimens of *C. variopedatus* were employed. These were stimulated by brief condenser shocks from an electronic or mechanically operated circuit. The luminescent response was recorded by means of photomultiplier tube (RCA 931), direct coupled amplifier, and oscilloscope. Deflexions of the spot on the oscilloscope screen were photographed with moving paper.

Results

Diminution of Response under Repetitive Stimulation

Specimens were stimulated electrically for different periods and at different frequencies. When subjected to a series of shocks, with long intervals between stimuli, the response takes the form of separate flashes. Fig. 1 shows a series of records obtained by administering an electrical shock every 10 min. Each shock evokes a luminescent response, which quickly rises to a peak, and gradually decays over the course of 5 min. The intensity of consecutive flashes is plotted in Fig. 2. The first few responses are of about equal magnitude, but thereafter intensity rapidly decreases. The decay curve approaches a quarter

ellipse in form. At this very slow rate of stimulation, 1 per 10 min., it is reasonable to assume that no fatigue of the contractile mechanism is occurring, and that diminution of the response is due to intervention of other factors.

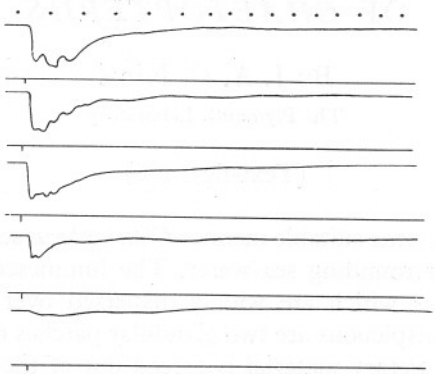

Fig. 1. Fatigue of the luminescent glands of *Chaetopterus* (glands on segment XII). Five consecutive records from the same animal, responses separated by an interval of 10 min. Stimulus on each occasion was a single condenser shock, shown as a pip on the lower line. Luminescent response appears in these records as a deflexion downwards of the middle trace. Time scale above, 1 per 30 sec. Paper speed halved after $2\frac{1}{2}$ min.

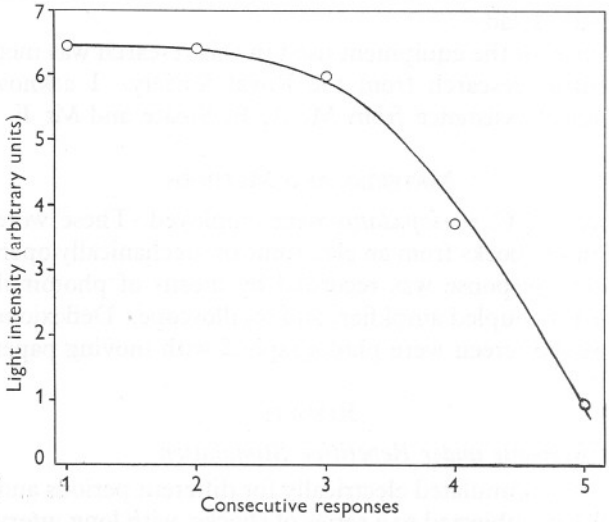

Fig. 2. Curve showing decrease of intensity of consecutive luminescent responses. Each response evoked by a single stimulus.

A burst of stimuli at slow rates (2–6 per min.) evokes recognizably discrete responses which summate, especially at higher frequencies (Fig. 3). When stimulation is maintained at these frequencies for some time, the response (light intensity) gradually falls off. With rest periods of 5 min. between

successive bursts of stimuli, each successive period of stimulation evokes a luminescent response which is progressively less than that of its predecessor (Figs. 3B, 4A). A point of some importance for the argument which will be

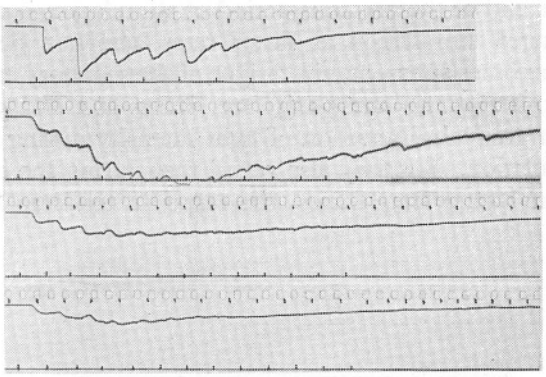

Fig. 3. A, luminescent responses evoked by a burst of stimuli (8 stimuli at 2 per min.) for 4 min. Time scale, 1 per 30 sec. B, responses to repetitive stimulation. Three consecutive periods of stimulation at 6 per min. for 2 min. Intervals of 5 min. between periods of stimulation. Time scale, 1 per 10 sec.

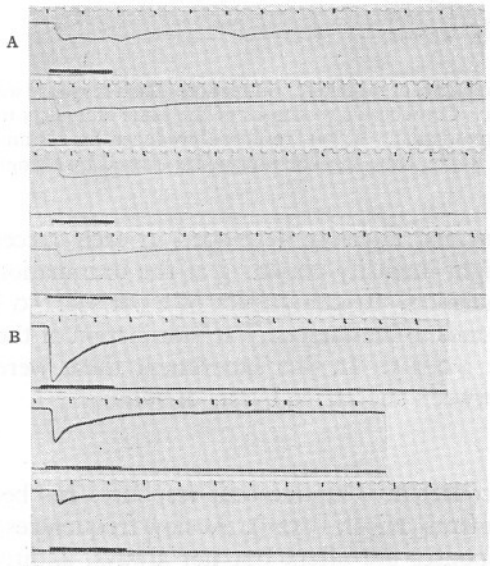

Fig. 4. A, consecutive responses to repetitive stimulation. Each response was evoked by a 50 sec. burst at 1 per sec. Interval of 5 min. between successive periods of stimulation. Duration of stimulation is shown in heavy black on lower line of each record. Time scale, 2 per min. B, responses to repetitive stimulation. Experimental conditions and details of recording similar to above, except that a frequency of 2 per sec. was used.

developed is that the maximal intensity developed in each response (after the first) is about equal to that developed at the termination of the previous period of stimulation and, in any event, is no greater. At a stimulation frequency of 1 per sec., the ratio of maximal light intensity at the beginning of a response to intensity at the end of the previous period of stimulation is unity.

With prolonged stimulation at faster rates, above 1 per sec., another phenomenon becomes apparent, viz. fatigue of the secretory mechanism. This is brought out in Figs. 4B and 5A (50 sec. bursts at 2 and 5 per sec.). The records show a gradual diminution of light intensity during each successive period of stimulation. But they also reveal that during the course of a prolonged burst at higher frequencies, the response tends to fall off rapidly.

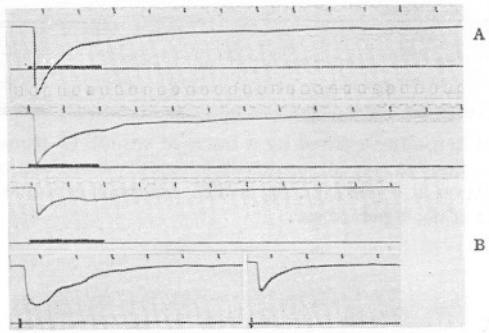

Fig. 5. A, responses to bursts of repetitive stimulation. Each response was evoked by a 50 sec. burst at 5 per sec. Consecutive responses of the same animal; 5 min. interval between each period of stimulation. B, left, response produced by 2 stimuli (interval between shocks 0·5 sec.). Right, same specimen, response evoked by a single shock. Time scale, 2 per min.

Moreover, the maximal intensity developed in each successive response is greater than the light intensity obtaining at the termination of the previous period of fast stimulation. At a frequency of 2 per sec. (50 sec. burst), ratios of initial luminescence to luminescence at termination of the previous period of stimulation are 2·0–2·5. In this experiment there were rest periods of 5 min. or more between successive bursts of stimuli.

Summation

Summation of consecutive luminescent responses has been demonstrated in previous experiments (Nicol, 1952*c*). At slow frequencies, from 2 to 12 per min., separate responses are often, but not always, additive, and light intensity may rise during progress of stimulation (Fig. 3). Fusion of discrete responses occurs above 30 per min. An attempt made previously to demonstrate facilitation in the luminescent response was unsuccessful, and fresh experiments were devised in which the nerve cord was stimulated by strong

shocks, instead of direct stimulation of the luminescent glands. Comparison was made of 1 versus 2 condenser shocks; in the latter event the two stimuli were separated by an interval of 0·5 sec. As previously noted, the response following two stimuli is sometimes equal to, or less than, that resulting from a single stimulus (Nicol, 1952*c*). But with the present technique some records were obtained (two out of five) in which the response produced by a pair of stimuli was significantly greater than that evoked by a single stimulus (Fig. 5B). Magnitude of response (maximal intensity, total light) produced by two stimuli was not more than twice that produced by a single shock. This appears to preclude facilitation and to demonstrate that increment of response, under repetitive stimulation, is due to summation.

Recovery from Fatigue

Earlier attempts to demonstrate recovery of the luminescent response after fatigue were unsuccessful. A few observations suggest that a rest of several hours following stimulation permits recovery of the luminescent response (Nicol, 1952*b*). Recovery of luminescence has been investigated in the following manner. Whole specimens were stimulated with a prolonged burst of shocks at slow frequencies, and records were obtained of the response. Electrodes were placed either directly on the glandular cells, or over the ventral nerve cord of segment XII. With electrodes in the latter position stimulation invariably caused autotomy of the anterior region as well, and these preparations were used only to investigate recovery over short periods not exceeding 24 hr. After stimulation the specimens were returned to sea water. Those animals to be examined in 24 hr. or later were placed in empty *Chaetopterus* tubes. Whole animals soon demonstrated normal activity by fabricating new sections on cut ends of the tubes. Thirty-three animals were investigated.

It was found that specimens varied considerably in time taken to achieve complete recovery. After periods of $4\frac{1}{2}$, 24 and 48 hr., animals displayed 10–100% of the luminescent response (original response rated at 100%) (Fig. 6). After 72 hr. all specimens which were examined gave a luminescent response equal to or greater than that originally recorded. Complete recovery of the luminescent response, following exhaustive stimulation, therefore, can take place within 5 hr.

Histological Observations

The histology of the luminescent glands of *Chaetopterus* is reviewed in an earlier paper (Nicol, 1952*a*). On the dorsal surface of segment XII the glands consist of dense aggregations of elongated cells packed with secretory granules The secretory contents form a bag-shaped mass invested by a cytoplasmic sheath, the cell wall. The small nucleus lies at the base of the cell, from which

fibres run to the basement membrane. The cell walls are strongly argentophilic, suggesting a dense groundwork, but no structural differentiation is apparent. Interspersed between the glandular cells are numerous ciliated cells. These cells have been carefully examined in preparations stained with iron haematoxylin or treated with silver protargol. It is found that the ciliated cells are very slender elongated elements lying between the luminescent cells. Distally the ciliated cells flare out into trumpet-shaped expansions which partially cover the external region of the luminescent cells. They bear long cilia on their external surfaces; the cilia arise in the usual way from basal granules, from which a cone of fibres can be followed a short distance into the cell towards the nucleus.

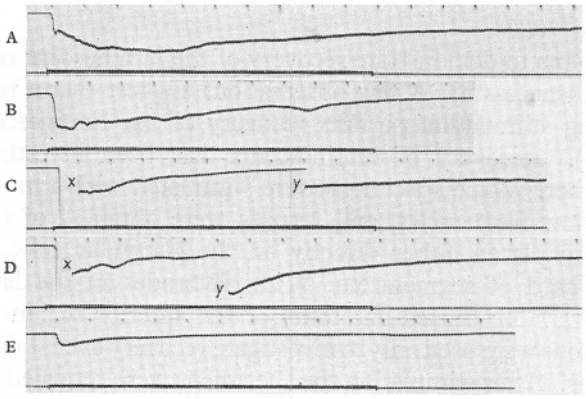

Fig. 6. Fatigue of luminescent response and recovery from fatigue. Stimulation in each record, 5 min. burst at 30 per min. Time scale, 1 per 30 sec. A, first response; B, second response from the same specimen after an interval of 4½ hr., C, first response from another specimen; D, second response from the same after an interval of 24 hr.; E, another response, 3 min. after D. At *x*, amplification was reduced to one-fifth and increased again at *y*.

Ciliated cells in the intestine of *Lumbricus* are said to possess contractile intracellular fibrils which assist in the extrusion of the secretory material from contiguous glandular cells by exerting compression on the latter (Millott, 1948). The appearance of these cells is not dissimilar to that seen in the luminescent glands of *Chaetopterus*, except that I have not been able to satisfy myself that any system of strongly developed intracellular fibrils extends through the entire length of the ciliated cell. The appearance of the luminescent cells when secreting strongly suggests that the glandular contents are being forcibly expelled, presumably by contraction of the cytoplasmic sheath of the glandular cell, which may contain contractile protein. Secreting cells differ in appearance from quiescent cells in that the glandular contents of the former are pushed towards the free surface of the cell while the base of the cell is compressed and

tapering. The ciliated cells serve to drive the discharged luminescent material towards a ciliated tract on the median dorsal surface, in which it is carried anteriorly, and dispersed away from the animal.

Discussion and Conclusions

The data which have been secured in this investigation can be summarized as follows. A single stimulus evokes a bright response, and repetitive stimuli result in summation of luminescent responses. There is no evidence for facilitation in the neuro-effector system. Repetitive stimulation at slow rates (below 1 per sec.) brings about gradual exhaustion of the luminescent response, as evidenced by the effect of further stimulation after a short period of rest. Complete recovery of luminescent ability is achieved in 4–5 hr. in some specimens, longer in others. Under repetitive stimulation at fast rates, above 1 per sec., the response quickly decays during the course of stimulation, but recovers after a brief interval of 5 min. so that the maximal intensity developed during the second period of stimulation is greater than that measured towards the end of the previous period of stimulation. Histological examination of the luminescent glands reveals that much secretion is still present in the glandular cells, even after exhaustive stimulation. The following hypothetical interpretation is offered to account for these facts.

Prolonged stimulation at high frequencies (above 1 per sec.) probably brings about gradual fatigue of the contractile mechanism responsible for extruding the luminescent material. Consequently, the cell is no longer able to maintain its contraction, and extrusion of luminescent material declines. A rest period of 5 min. is sufficient to allow recovery of contractile efficiency, and to permit another maximal response. The correspondence to muscle fatigue is obvious. Curves of tetanic contraction recorded from the longitudinal muscle of the sabellid *Branchiomma*, for example, show a similar sequence of events, i.e. rapid decline of tension and contraction-height during the course of stimulation, and recovery of contractile ability after a period of rest, so that the initial tension developed by the rested muscle is greater than the tension developed during the terminal period of stimulation in the previous contraction (Nicol, 1951). This similarity, of course, sheds no light on the physico-chemical events responsible for fatigue, but it emphasizes that the same sort of contractile mechanism is operating in the discharge of luminescent material as in muscular contraction.

In the absence of any other apparent mechanism it is suggested that contraction is accomplished by the cytoplasmic sheath of the glandular cells. These may be endowed with contractile proteins capable of exerting pressure and producing movement.

The progressive diminution of light intensity which occurs in successive discrete responses is due to factors other than contraction-fatigue. Part of this decrease must result from progressive exhaustion of luminescent material

available for discharge. At first sight this would seem to provide an adequate explanation for the gradual decline in brightness of successive responses. There are several reasons for considering this explanation inadequate. First, if glands are electrically stimulated to exhaustion until the luminescent response beomes very weak, and are then mechanically disrupted, a great deal more light is produced. Secondly, if glands electrically stimulated to exhaustion are examined histologically, it becomes evident that a large amount of secretory material is still present inside the cells. It is evident, therefore, that some other factor is operating which is not merely fatigue of the contractile mechanism, causing decreased efficiency of contraction during prolonged activity, and resulting in conservation of luminescent material intracellularly.

It is noteworthy that the curve showing progressive diminution of light intensity in successive isolated responses has the form roughly of a quarter ellipse. That is, the first few responses (2 or 3) maintain a high intensity, after which maximal light intensities fall off at an increasing rate. Similar curves showing decrease of intensity in consecutive luminescent responses of polynoids take the form of rectangular hyperbolas. That is, the intensities of luminescent responses after the first one fall off at a decreasing rate along an asymptotic curve. In these latter animals the controlling factor is probably progressive exhaustion of intracellular luminescent material, or some other substance controlling oxidation of the latter.

Any suggestions concerning the factor responsible for this apparent fatigue of luminescent ability which develops progressively in isolated consecutive responses of *Chaetopterus* must be speculative, with the indirect information at hand. Attention should be directed first of all to the saccular organization of the glandular cells. Once the cell starts to secrete a distal pore opens on the cell surface, and secretory granules are forced out. A fresh unstimulated glandular cell will be distended and its wall stretched. When stimulated it will compress the cell contents and the pressure will find relief on extrusion of some of the cellular contents. A small proportion of the intracellular secretory material is lost with each contraction. A second stimulus and contraction will find the cell less distended than on the first occasion. From this viewpoint the glandular cell can be regarded as a semi-fluid hydraulic system in which pressure exerted on an enclosed column leads to movement of the latter and compensatory pressure-release once the cell opens to the outside. An analogy is at hand in the fluid-filled cavities of soft-bodied animals such as annelids. But in these forms the fluid-volume remains constant, and provides a hydrostatic system against which the muscles can operate. The contents of the glandular cell, however, are gradually dissipated during successive responses. On the assumption that the contractile mechanism of the cell behaves like muscle under stretch, it may be expected to produce maximal compression when stretched or expanded, and the force which it develops will fall off as it

progressively diminishes in pace with reduction of cellular contents and cell-size. The curve in Fig. 2 showing progressive diminution in light intensity of successive responses is very similar to a curve for striated muscle when tension developed is plotted against decreasing length along the *x*-axis.

It is impossible from these data, and probably impossible from experiments designed in this way, to obtain a true picture of the course of recovery and restitution of secretory material in the luminescent cells. Even after an exhaustive stimulation of the luminescent glands the cells show no visible decrease of secretory material, and the problem cannot be resolved by histological means. The luminescent response is regenerated to full intensity in some 4–5 hr., even in isolated heads severed from the rest of the body. This possibly could be due to restitution of glandular material. It could also result from temporary intake of sea water, restoring cell volume.

This hypothesis and these analogies are presented for argument. As a tentative hypothesis it has this to commend it. It provides an explanation of the progressive decrease in intensity of successive responses. It accounts for progressive exhaustion of the secreting (contractile) mechanism in the face of high residual levels of intracellular luminescent material. And it evokes no new principles not already described for contractile tissues.

Summary

Further experiments have been carried out on the luminescent glands of *Chaetopterus* in an attempt to provide a functional explanation of the mechanism of glandular secretion. Attention has been confined to the luminescent glands in segment XII, which were excited by electrical stimulation. Responses have been followed and recorded by photomultiplier tube and oscilloscope.

A bright response is evoked by a single stimulus, and repetitive stimulation at rates slow enough to allow separate responses to be distinguishable produces summation. Paired stimuli provide no evidence for facilitation. The intensity of the response produced by two stimuli is not more than twice that produced by one.

Evidence is adduced for the participation of three factors in fatigue of the luminescent response. At rapid rates of stimulation the contractile mechanism responsible for extruding the secretory material becomes fatigued. At very slow rates of stimulation, below that producing fatigue of contractility, light-intensity falls off in consecutive responses. This is only in small part due to gradual exhaustion of available luminescent material, since the glandular cells still contain large amounts of secretion even after stimulation to apparent functional exhaustion. The hypothesis is advanced that the contractile mechanism exerts maximal contraction when fully stretched in a cell heavily loaded with secretory material, and its efficiency decreases as it shrinks with reduction of cell volume.

Complete recovery of luminescent ability, to intensities initially recorded, was attained by some specimens in 5 hr.; all specimens examined showed complete recovery in 72 hr.

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J. Mar. biol. Ass. U.K. (1954) 33, 187–224
Printed in Great Britain

THE CREVICE FAUNAS OF THE UPPER INTERTIDAL ZONE AT WEMBURY

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(Text-figs. 1–7)

The main picture of the zonation of life between tide marks on British shores has been added to by a good deal of recent work, such as that of Colman (1932) and Evans (1947) at Plymouth, and the regional studies by Stephenson & Stephenson (1949). Of the more restricted special habitats within the tidal area there have been fewer accounts, though Colman (1940) has made a detailed survey of the faunas inhabiting intertidal seaweeds, which was later followed by Wieser (1952). Most recently there has appeared a paper by Glynne-Williams & Hobart (1952), working at Anglesey, which for the first time analysed clearly the composition and food relations of the restricted fauna living in crevices in this habitat. This part of the tidal zone forms an interesting meeting place of two faunal elements, those intertidal animals of terrestrial origin and those which are truly marine. In the summer of 1950 and 1951 the present writer had made a similar study in the Plymouth area, at Wembury and some other localities. The work was begun as part of an ecological study of the two marine pulmonates *Leucophytia bidentata* and *Otina otis*, and was later extended to take account of the other animals hidden in crevices and those living on the exposed rock surface throughout the upper half of the tidal zone.

The work of Glynne-Williams & Hobart lends a further interest to the Devon results as a comparative study, and—while in many respects both this and the Anglesey work must serve as preliminary studies only—the following account may be of value (i) in making a comparison with the larger and well-developed crevices in another type of foliaceous rock, (ii) in giving some quantitative data on the vertical and horizontal distribution of animals in crevices, (iii) in showing the differences in detail between the essentially similar crevice faunas in the two localities, and (iv) in making a comparison between conditions applying to ecological succession in crevices with conditions of life on the open rock surface.

The seaweed-inhabiting faunas are not further discussed here, with the single exception of the lichen *Pygmaea pumila* (= *Lichina pygmaea*). The observations of Glynne-Williams & Hobart on the origin, and the food and moisture content of the deposits present in crevices are also taken as broadly

applicable to Devon conditions, and here again the present writer has no new contribution to make.

This work was carried out at the Plymouth Laboratory of the Marine Biological Association, where I enjoyed the use of the University of London Table. I am grateful to Mr F. S. Russell, F.R.S., and the Plymouth Staff for much assistance, and in particular I have to thank Mr G. M. Spooner for the identification of many of the crustacea and Mr F. G. C. Ryder for help with the levelling of the area. Mr T. E. Hughes of Birkbeck College, London, was kind enough to identify the mites, and my special thanks are due to Prof. A. Graham for his continued kindness and encouragement while I was a research student at Birkbeck College during 1951 and 1952.

The Locality and Classification of the Habitats

The principal area studied was the rocky shore at Wembury, near Plymouth, where on either side of a small sand-beach two reefs run seaward in a south-westerly direction, Church Reef as discussed and surveyed by Colman (1932) and a similar reef immediately to the west. Both reefs are composed of Dartmouth Slates, and form near the landward edge a series of strongly projecting ledges, obliquely overthrust chiefly towards the north or north-west. The geological formation of slates is perhaps the most ideal for a study of crevice-dwelling animals, the successive slabs weathering apart to form narrow, oblique fissures often as much as 12 in. deep. The survey was confined to rocks above mean sea-level which is at 8·2 ft. above Chart Datum (C.D.) zero for Plymouth. Re-levelling of the portion of the area studied in detail was carried out, and the tidal levels have been taken to be those determined for Wembury by Colman (1932). As in that paper and in the work of Orton (1929), a height of 2 ft. has been allowed for the raising of effective water-level by the action of wave surge and splash. Following Colman (1932) heights above C.D. have been expressed throughout in feet.

A typical portion of the ground selected for sampling is shown in the profile diagram (Fig. 1) drawn from an outcrop of rock near the landward edge of the west reef. Here there is a vertical range up to 22 ft. above C.D. On the topmost outcrops of rock, above 20 ft. C.D., which were never covered by the tide or regularly subjected to wave splash, the surface is brightly daubed with the yellow terrestrial lichen *Xanthoria*. Down to *c.* 16 ft. C.D., a little way above MHWS the tarry black lichen, *Verrucaria maura*, encrusts most of the rock, being sprinkled at the lower limits with tufts of the bushy lichen *Pygmaea pumila*. From here down to *c.* 10 ft. C.D. there is a continuous zone of the barnacle *Chthamalus stellatus*, intermixed especially at the lower half with more tufts of *Pygmaea*. Below *c.* 10 ft. C.D. species of *Fucus* form a thick covering, in most places continuous, on the flatter rock surfaces, and there is

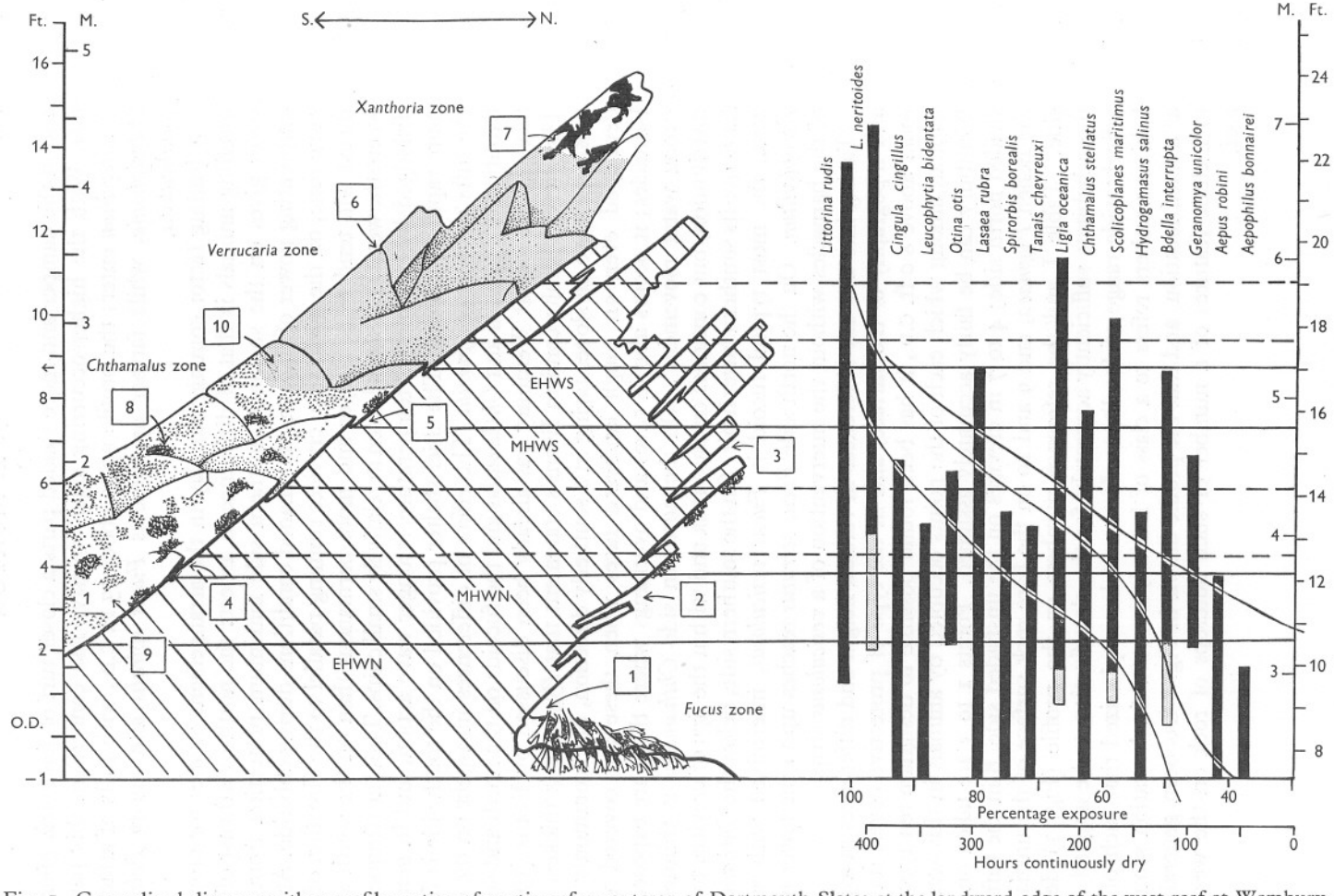


Fig. 1. Generalized diagram with a profile section of portion of an outcrop of Dartmouth Slates at the landward edge of the west reef at Wembury, showing typical positions for locations 1–10 referred to in Table I (p. 191). Tidal levels have been superimposed on the profile, the broken lines representing splash levels 2 ft. above the respective tidal heights. On the right are shown the vertical ranges of some of the commoner crevice-dwelling and other animals. A stippled portion of a column represents an extension of range on the exposed southward side. On the right are shown (below) the two curves for percentage of exposure at various levels, the 'splash curve' being the higher, and (above) the curve for the maximum period of continuous exposure (see Colman, 1932).

also in the damper and more shaded parts a closer turf of *Laurencia pinnatifida*. With the higher-occurring algae we have less concern in this paper; *Ascophyllum* enters the profile up to about 13 ft. C.D., with tufts of attached *Polysiphonia*, while farther up there is *Fucus spiralis* and finally *Pelvetia canaliculata*.

A leading factor coming into play in the arrangement of sites for crevice-dwelling animals is that of difference in aspect: the north overthrust of the slates gives on the sheltered northern side maximum protection from the evaporating power of the sun, and shaded conditions during ebb-tide. At the same time, on the north side, crevices running parallel to the direction of the thrust will tend to be deeper and more permanent than on the south side, where the weathered slates loosen in relatively small chips. These are frequently dislodged by extremes of temperature, stronger wave action, and in general more rapid weathering. The chief shelter provided on the south side is from the tufts of *Pygmaea pumila*. The effect of differences in aspect on the distribution of *Pygmaea* and *Chthamalus* and the fucoids on the north and south faces of the reef at Wembury has already been discussed by Evans (1947). The present results confirm Evans's statement that *Pygmaea* is thickest and most extensive on slopes facing in a southerly direction, but 'commonly well developed even on rough landward faces when these are covered with barnacles; it occurs also in caves and overhangs where these are exposed to strong water movement'. On the northern side *Chthamalus* is thinner in distribution, but continues on bare rock and within the lips of crevices to not far above its southern lower limit. On the southern side, below the *Chthamalus* zone, the most typical fucoid is *Fucus vesiculosus*, intermixed with some *Ascophyllum*. On the north side *Fucus serratus* reaches up to the lower limit of the barnacles without the intervention of a *vesiculosus* strip.

Taking into account differences in vertical height and differences in aspect and topography of the surface, and in the type of protective cover, on the rocks above 8·0 ft. C.D., it has been found possible to designate ten types of habitat, among which each of the points occupied by animals in this zone at Wembury can be fairly accurately assigned. Points **1** to **3** are taken on the shaded north side; **4** to **7** in crevices on the unshaded side, **8** in the tufts of the lichen *Pygmaea*, and **9** and **10** on the bare rock surface of the unshaded side. Table I and the reference numbers on the profile (Fig. 1) define these habitats sufficiently accurately for the classification to be applied over a fairly wide range of localities. It should be emphasized that each of the habitat numbers refers to a class of habitat, not to a single station sampled at a fixed location, and that the profile drawn in Fig. 1 has been generalized to include features of a number of such outcrops of rock on the western reef.

TABLE I. CLASSIFICATION OF HABITATS IN UPPER TIDAL ZONE AT WEMBURY

Type number	Description	Tidal levels (C.D.)
1	Crevices below upper limit of *Fucus vesiculosus* and *Laurencia pinnatifida*, shaded by damp algae	*c.* 10 ft. and below
2	Deep crevices on shaded (north) aspect in lower half of *Chthamalus* zone	EHWN to MHWN, *c.* 10·5–12·5 ft.
3	Deep crevices on shaded (north) aspect, near upper limit of *Chthamalus-Pygmaea* zone	Around splash zone of MHWN, *c.* 14·5 ft.
4	Shallow crevices on exposed (south) aspect, in lower half of *Chthamalus-Pygmaea* zone	EHWN to MHWN, *c.* 10·5–12·5 ft.
5	Shallow crevices on exposed (south) aspect, near upper limit of *Chthamalus-Pygmaea* zone	Around splash zone of MHWN, *c.* 14·5 ft.
6	Shallow crevices on exposed (south) aspect, in zone of black lichen, *Verrucaria maura*	Around splash line of MHWS, *c.* 17–19 ft.
7	Shallow crevices never reached by tide, exposed or sheltered aspect, in zone of yellow lichen, *Xanthoria parietina*	*c.* 20–22 ft.
8	In tufts of *Pygmaea pumila* on unshaded rock surface, in *Chthamalus-Pygmaea* zone (cf. Colman, 1940)	*c.* 11–14 ft.
9	Bare rock surface on unshaded (south) side, in lower half of *Chthamalus-Pygmaea* zone	EHWN to MHWN, *c.* 10·5–12·5 ft.
10	Bare rock surface on unshaded (south) side, in upper half of *Chthamalus-Pygmaea* zone	*c.* 13–16 ft.

METHOD—SAMPLING PROCEDURE

In attempting to give a quantitative estimate of the faunas present in each of the locations selected, several difficulties arise which are not experienced on the more uniform environment of a soft-bottomed shore, where the classical methods of notation are best brought into use. First, the environment is never entirely uniform or perfectly comparable in detail from one station to another. There is every gradation of such variable factors as differences of shade, differences of crevice substratum and differences in shelter from wave attack. On moving from one outcrop of rocks to another, often not even a rough replica of the previous environment is obtainable. Secondly, there is the extreme patchiness of many of the species themselves, even in environments that appeared to be approximately uniform. Thus the excursions made by the mite *Bdella* between crevices 4 and the bare rock surface vary extremely from hour to hour. *Anurida maritima*, to take another example, is sometimes present in several hundreds in one crevice and is likely to be quite wanting in another close by. With *Otina* and other animals aggregating together, perhaps a dozen may be found in a favoured part of a crevice and in a sample of another part altogether missed. Further, such very abundant dominants as *Lasaea rubra* vary within wide limits in apparently replicate areas—this is exemplified by Colman's three counts of *Lasaea* from 50 g. of *Pygmaea*. None the less, it would be misleading not to recognize in general a fairly constant relation—with large enough series of samples—between crevices of comparable locations. This tends to hold good over Wembury and other Plymouth localities

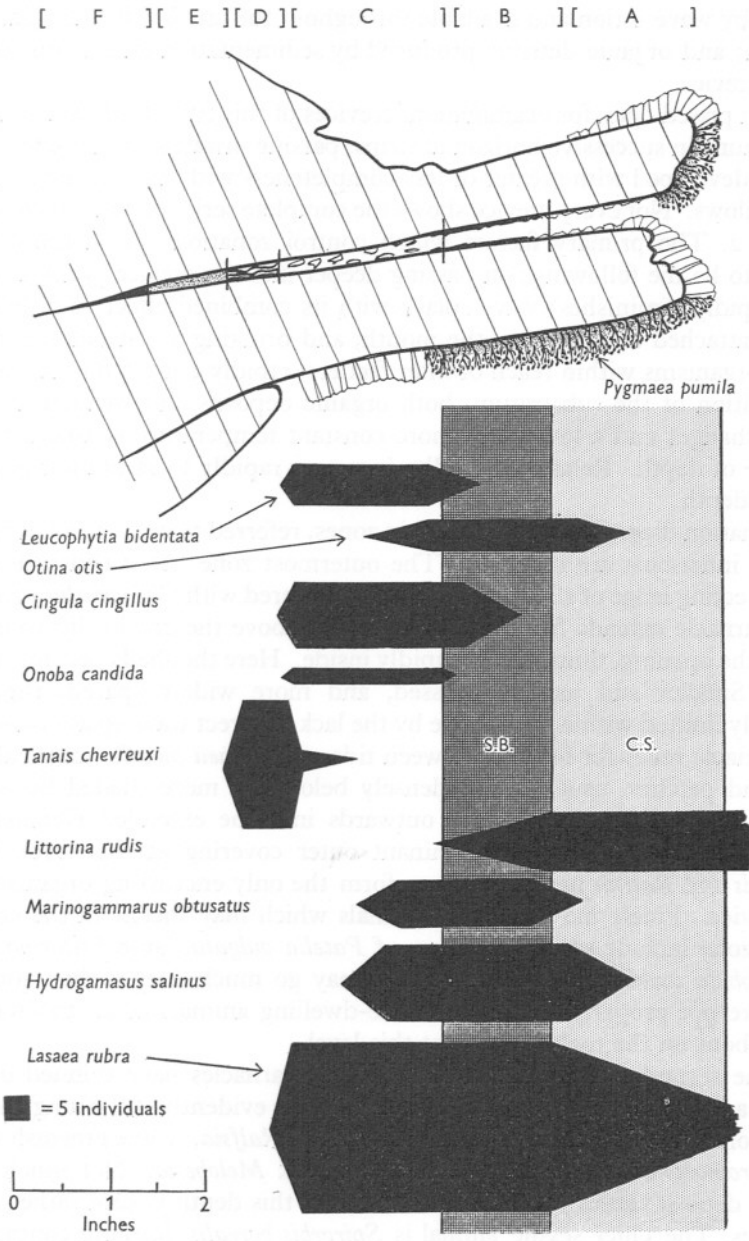
as well, and to vary in roughly the same way with gradations in ecological conditions. There is one reservation: a single series of samples from any one chosen station may not give a complete picture, and variations within 30–40% are to be regarded as the normal range. On the profile drawn in Fig. 1, each location was sampled in at least three or four typical crevices, and the final distribution diagrams (Fig. 6) are also generalized from the results of two seasons' representative sampling over the whole area of the reef. It was thus found less useful to quote actual numbers of animals than to adopt the scale of densities as given in the figures. The area sampled in each case was a portion of the crevice of 15 cm.^{2}. On opening a crevice this was marked out in as quick time as possible, usually by laying down a sheet of stiff card and making marks at each corner. The upper and lower slate of the crevice were then sampled, giving a total area of rock surface of 2×15 cm.^{2}. The total population was sucked up into a $5 \times 1\frac{1}{2}$ in. collecting tube, with an entomology-bottle glass intake, and the contents examined in the laboratory. Swift-moving animals such as insects and crustaceans were secured first, and mites and insects drawn on to a layer of water to immobilize them from crawling out of the tube. Sessile organisms were then counted at leisure and tufts of *Pygmaea* were brought home and the animals collected in an enamel pan after remaining overnight in $1\frac{1}{2}$% formalin.

The Habitats and Their Faunas

Location 2

Shaded aspect, deep crevices between EHWN and MHWN, lower limit to middle of *Chthamalus* zone

A profile diagram with distribution figures for horizontal succession of animals in this type of crevice is given in Fig. 2. A description of location 2 will be given first, as this is the richest habitat with the most favourable combination of conditions for the development of the greatest number of species. It shows in a complete form a sequence represented elsewhere only partially or in fragments. Crevices here are seldom entirely dry between tides. With a northern aspect they receive maximum shade and the crevices slope down deeply, sometimes to a depth of 9–12 in. between the slates. They are rather sheltered from wave action, and the material of the substratum, both organic and derived from weathering, will tend to stay here longest and, as shown by Glynne-Williams & Hobart, there may be a rough grading of sediments on passing more deeply into the narrowest part of the crevice. Of ecological factors, the most significant is the absence of prolonged exposure (see Fig. 1) for more than a single intertidal period. The temperature within the crevice is both low and uniform throughout tidal exposure, and relative humidity remains always at—or just below—saturation point. The food supply comes apparently from two sources, suspended particles brought to the mouth of the



2. Diagrammatic section of a typical intertidal crevice in Dartmouth Slate at location 2. The letters refer to the successive zones within the crevice discussed in the text. Distribution diagrams are given below for the occurrence of nine of the more abundant species of animal within the crevice. The blacked area represents the actual number of animals taken in typical samples, apportioned between the zones, the scale being given by the small black square which is equivalent to five individuals. The stippled areas indicate the approximate limits of the two encrusting organisms, *Chthamalus stellatus* (P.) and *Spirorbis borealis* (*S.B.*).

crevice by wave action and available throughout the crevice during tidal submersion; and organic detritus produced by sedimentation on the substratum of the crevice.

When prised open for examination, crevices of this type disclose a zonation of the fauna in successive horizontal strips, passing inwards from the opening. This is developed with greater or less completeness within each crevice as the depth allows. Not every crevice shows the complete series of zones recognized in Fig. 2. The primary factors which control zonation within the crevice appear to be the following: on passing deeper into the crevice penetration of light rapidly diminishes; wave splash with its combined effect of dislodging loosely attached animals near the mouth, and bringing a constant supply of micro-organisms within reach of filter feeders, rapidly ceases; the nature and composition of the substratum, both organic deposits and weathering products, change; and a lower and more constant temperature is attained with increase of depth. Relative humidity increases rapidly to saturation point at greater depth.

A zonation diagram shows ideally six zones, referred to as A–F, all of which but the innermost are inhabited. The outermost zone (A) is constituted by the projecting ledge of slate which is closely covered with *Chthamalus stellatus*. This barnacle extends from its thickest layer above the crevice lip round to within the opening, thinning out rapidly inside. Here the shells become rather paler. Smaller and more depressed, and more widely spaced, they are evidently limited within the crevice by the lack of direct wave splash on which the barnacle relies for feeding between tides. *Pygmaea pumila* occurs also in tufts and patches, usually most densely below the more shaded lip of the crevice. The whole zone grades outwards into the extended *Chthamalus-Pygmaea* zone which is the dominant outer covering at this level. With *Spirorbis* and *Ralfsia* in zone B these form the only encrusting organisms in the crevice. Freely moving larger animals which may shelter in the crevice at this zone include small specimens of *Patella vulgata*, large *Littorina rudis* and *Gibbula umbilicalis*. The two latter may go much deeper than zone A. They are not properly speaking crevice-dwelling animals at all and wander freely about on the rocks outside at this level.

At the second zone of the crevice (B) the barnacles have thinned out to final disappearance, but the penetration of light is evidently still strong enough to support an encrusting film of the brown alga *Ralfsia*, a fine greenish scum of *Enteromorpha* and often an encrusting pink *Melobesia*. Not much permanent deposit settles here and the crevice at this depth is kept rather clean by waves. The chief sessile animal is *Spirorbis borealis*, forming continuous sheets or patches, where the animals are still able to filter out nutriment from particles washed in by waves. This level in the crevice is the favourite site for *Otina otis*, which always, unlike *Leucophytia*, avoids regions of thickest accumulation of detritus. It prefers to browse upon a fairly clean surface, picking up

organic fragments, and especially diatoms lodged by the wave-splash in slight irregularities of the surface. From the splash, *Spirorbis* filters out nutriment of much the same kind as *Otina* secures with its radula. *Otina* by no means confines itself to this zone. It is able to venture outwards, even while the tide is out, to the projecting ledge of barnacles. Here it crawls about actively, kept moist by splash, and often ventures among the barnacles on the under side of the lip of the crevice.

A deeper zone (C), beyond the *Spirorbis* fringe, occurs wherever a space of about $\frac{1}{2}$ in. is still left between the roof and floor of the crevice. Small stones, pieces of shell, pebbles and coarser sand are able to lodge here. There collects also a deposit of fine clay-like weathering products, and a rich organic debris lodges in the interstices between the larger particles. The fauna is richer here. While *Spirorbis* is entirely lost, the chief species become *Cingula cingillus*, *Lasaea rubra*, *Leucophytia bidentata*, much reduced numbers of *Otina otis*, and many small individuals of *Littorina rudis*, which, however, is a good deal more common towards the mouth of the crevice. All the animals mentioned feed in various ways on the rich organic deposit. They include several species with purely marine affinities, such as *Lasaea rubra*, *Cingula cingillus* and *Littorina rudis*. A striking feature of the location, however, is the migration of a terrestrial element back between tide marks, into the specialized crevice locality. In smaller numbers, but usually in each crevice with one to a dozen specimens, are represented the myriapod, *Scolicoplanes maritimus*; the pseudoscorpion, *Neobisium maritimum*; a collembolan, *Anurida maritima*; a machilid, *Petrobius maritimus*; and the coleopteran, *Aëpus robini*; while the pulmonates *Leucophytia* and *Otina* are themselves supratidal rather than marine in affinities. In addition, the small, reddish brown marine mite, *Hydrogamasus salinus*, is present, often in immense numbers, forming one of the chief items of the species list. The air interlocked in small spaces at this level of the crevice is evidently sufficient to enable most of these animals to remain air-breathing between tides and to live compatibly side by side with marine members of the fauna. Some species, such as the mite and the collembolan, evidently retain air by a setose or hairy covering and are difficult to wet; but others, such as *Leucophytia*, are probably truly amphibious and breathe dissolved air from the sea water during high tides. Other members of the fauna in considerable numbers in zone (C) includes the amphipods *Hyale nilssoni*, *Marinogammarus obtusatus* (also found at the crevice mouth in the barnacle fringe), and frequently a specimen of the little teleost, *Blennius galeritus*, which regularly lodges in air-filled crevices between the tides. Among the isopods, *Sphaeroma serratum*, and *Naesa bidentata* are probably most important. Of the molluscs, another rissoid, *Onoba candida*, is regularly present, though in much smaller numbers than *Cingula*, as well as *Mytilus edulis*, pale brown in colour, up to 5 mm. in length and attached by byssus threads to the rock. The worms include several eunicids and terebellids, and also *Eulalia*

viridis, which is typical both here and, perhaps even more, in the *Spirorbis* and *Chthamalus* zones. The high tidal nemertean, *Lineus longissimus*, is very frequent; there are often five or six in each crevice.

In a narrow, but fairly constant zone at a deeper level (D) the accumulation of sand and detritus becomes thick enough for the peracaridan *Tanais chevreuxi* to form its system of galleries. Each occupies the whole width of a narrow zone of compacted sand, about 1 in. across at most, and the burrows form a closely branching system in general parallel with the direction of the crevice. The chief animals accompanying *Tanais*, crawling back into deeper recesses between lodgements of muddy sand, are the isopod *Sphaeroma serratum*, reduced numbers of *Lasaea*, the shells now pale and transparent, and *Cingula* and *Leucophytia*.

Deeper still in the crevice, in zone (E), the clay deposit, chiefly the reddish product of the weathering of slate, becomes even more compact and finer in grade, and is penetrated by few animals with the exception of the deposit-feeding worms, *Amphitrite gracilis* and *Cirratulus cirratus* whose food-collecting tentacles extend outwards to the more open zones, C and D, in places where the *Tanais* zone is interrupted or unrepresented.

Zone E may extend backwards 3 or 4 in. into the crevice, after which, at the level F, the sheer faces of the slate laminae remain in contact or separated only by a film of red or yellow clay formed by weathering action. Here no space remains for penetration by living organisms.

Location 3

Shaded aspect, deep crevices at and around splash line of MHWN, near the upper limit of the *Chthamalus-Pygmaea* zone

The fauna here is a good deal less rich than that just described, and there are a number of differences in the impact of the environment. The chief ecological difference is evidently that of greater temperature fluctuation (see Fig. 3). There is a higher average temperature with longer exposure between tides and much greater insolation of rock surfaces. Consequently, relative humidity is lower because of both longer evaporation and lack of water-retaining deposits. The crevices tend to be less deep, and this is probably in some measure due to the different nature of the weathering: the slates tend to be sheared off or delaminated more by subaerial erosion than by the deeper action of water. Type 3 crevices may remain dry for a total period of up to 200 hr. within a tidal cycle. This ensures that deposit feeders will find conditions unsuitable. Many of the animals of the lower fauna have dropped out. Perhaps this has as much to do with unfavourable conditions of food supply as with temperature or humidity.

Leucophytia, which was typical of deposit feeders at 2, is lost entirely. *Otina* is still able to do well here and nestles among the barnacles or with *Lasaea*. It evidently reaches here the higher limit of its intertidal range. Like the

limpet, *Otina* is able to crawl about actively and forage for wave-lodged diatoms or filamentous algae which are often found in its gut; it is able to feed by means of its radula from a rather clean-swept surface. Many tufts of *Pygmaea* still occur at the barnacle fringe at the mouth of the crevice. They shelter vast numbers of *Lasaea rubra*, which, as long as *Pygmaea* persists, is as abundant

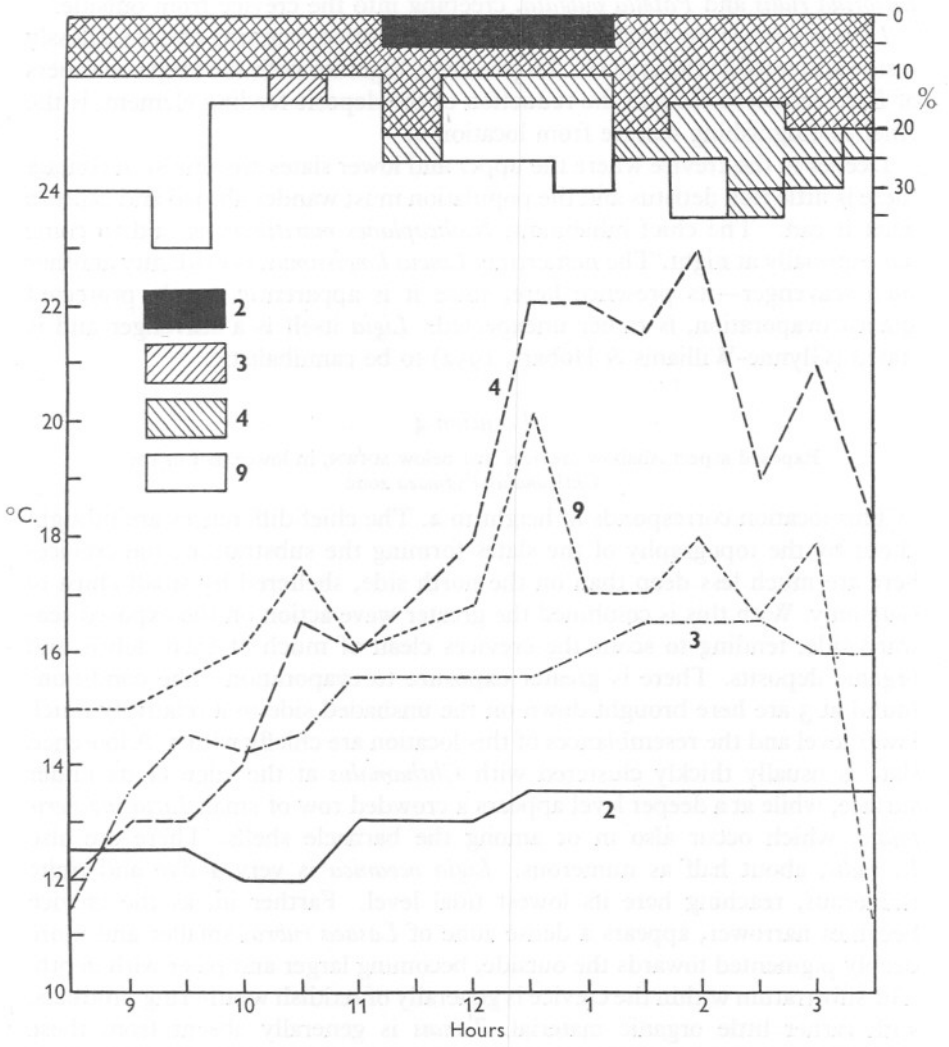


Fig. 3. Graphs showing half-hourly temperature readings over a complete intertidal period on a portion of the western reef at Wembury, on 18 June 1951; and (above) diagrams showing the percentage relative humidity by which the points selected fell short of saturation at the same intervals. Position (i), referred to in the text, was just above the bare rock surface at location **9**, and the remaining three stations are at typical positions of locations **2**, **3** and **4**.

as below. *Lasaea* is a suspension feeder and must at higher levels have a stringent tidal periodicity imposed on its feeding and other activities. *Littorina neritoides* is here almost as abundant as *Lasaea*. It prefers to nestle in barnacle shells and also presses in dense clusters as far into the crevice as it is possible for smaller individuals to go. There are still at this level many *Littorina rudis* and *Patella vulgata*, creeping into the crevice from outside.

Ligia oceanica now reaches its densest distribution, running about restlessly inside and outside the crevice. Its presence, together with the larger numbers of *Littorina neritoides* and the reduction of the deposit-feeding element, is the chief distinguishing feature from location **2**.

Deeper in the crevice where the upper and lower slates are almost in contact there is little rich detritus and the population must wander abroad and capture what it can. The chief inhabitant, *Scolicoplanes maritimus*, is said to come out especially at night. The nemertine, *Lineus longissimus*, is evidently another such scavenger—its presence here, since it is apparently poorly protected against evaporation, is rather unexpected. *Ligia* itself is a scavenger and is stated (Glynne-Williams & Hobart, 1952) to be cannibalistic.

Location 4

Exposed aspect, shallow crevices just below MHWN, in lower half of the *Chthamalus-Pygmaea* zone

This location corresponds in height to **2**. The chief differences are brought about by the topography of the slates forming the substratum; the crevices here are much less deep than on the north side, sheltered by small chips of slate only. With this is combined the greater wave action on the exposed seaward side, tending to scour the crevices clean of much of their debris and organic deposits. There is greater exposure to evaporation. The conditions found at **3** are here brought down on the unshaded side to a relatively much lower level and the resemblances of this location are chiefly with **3**. A loosened slate is usually thickly clustered with *Chthamalus* at the edge of its under surface, while at a deeper level appears a crowded row of small *Littorina neritoides*, which occur also in or among the barnacle shells. There are also *L. rudis*, about half as numerous. *Ligia oceanica* is very active and quite numerous, reaching here its lowest tidal level. Farther in, as the crevice becomes narrower, appears a dense zone of *Lasaea rubra*, smaller and more deeply pigmented towards the outside, becoming larger and paler with depth. The substratum within the crevice is generally of reddish weathering products, with rather little organic material. *Tanais* is generally absent from these crevices which are scarcely deep enough, nor with sufficient supply of building material, to enable it to construct its burrows. Occasionally a small patch of mud may support a dozen or so *Tanais* and here conditions are often sedimented enough for *Cingula* which—with or without *Tanais*—may continue quite abundant. *Leucophytia* is present in these patches, but reduced both in

size and number; on the unsheltered side this is about the upper limit of its extent. *Otina*, on the other hand, being less tied to thick deposits and doing better on cleaner ground, seems to tolerate these crevices quite well, often sheltering in empty barnacle shells. The local humidity at such points must closely approach saturation, although in general it may fall a good deal lower at **4** (Fig. 3). The carnivores or scavengers, *Machilis* and *Aëpus*, are represented here, but more poorly than at **2**. *Anurida maritima* often swarms. Of the mites *Hydrogamasus* does not often turn up here, but the large reddish *Bdella* and *Cyrthydrolaelaps* are usually abundant, creeping out upon the heated rock surface over the barnacles.

Location **5**

Exposed aspect, shallow crevices at splash line of MHWN, near upper limit of *Chthamalus-Pygmaea* zone, same level as **3**

This zone is most readily comparable with **3**, but with the unshaded conditions of south exposure, the environment is a good deal more harsh. In many ways the fauna approaches that of **6**. The crevice is mainly filled with clean, pinkish brown weathering products along the joint plane of the slates, and little life exists in such places. At the outer part of the crevice, however, a fringe of *Pygmaea* helps to accumulate a small amount of organic deposits and provides shade for a narrow zone of *Lasaea rubra* and *Littorina neritoides* occurring within the mouth of the crevice. These are the two dominant animals. *Lasaea* is near the top of its range at this point and *Littorina neritoides* near its optimum. Both yielded over 200 individuals in the area sampled. *L. rudis* accompanies *L. neritoides* but is of small size and much less abundant. *Ligia oceanica* wanders about actively and specimens of the *Geranomya* larva were found which had probably strayed from the *Pygmaea* zone. *Scolicoplanes maritimus* was at times found here, but conditions seemed too dry for *Anurida maritima*. There are no *Tanais* and no worms. Deposit feeders are at a great disadvantage and the chief nutriment available consists of suspended particles borne by the waves.

Locations **6** *and* **7**

6, exposed aspect, shallow crevices in the zone of black lichen, *Verrucaria maura*, above the barnacle zone, at 17–19 ft. C.D. **7**, exposed or sheltered aspect, cracks in the rock never reached by the tide, in the zone of yellow lichen, *Xanthoria*, 19–21 ft. C.D.

These were the highest points sampled, and both were usually taken on the unshaded side of the profile. Conditions tend to be increasingly harsh and the fauna greatly impoverished. At **6**, although there are no barnacles exposed on the surface, *Chthamalus* may exist in one or two sparse clusters in the crevices. *Pelvetia canaliculata* may still be growing at the surface, and its thallus and holdfasts provide shelter for a few animals (Colman, 1940). In the crevices *Littorina neritoides* is now by far the dominant organism, in

practically terrestrial conditions reached only by the splash of high-water springs. *L. rudis* is still present but much more scarce. *Ligia oceanica* is active and plentiful, but none of the mites appear to persist. *Lasaea rubra* just reaches crevice **6** though it is outstripped in numbers by *Littorina neritoides*; its presence here is a remarkable achievement for a lamellibranch relying on suspension-feeding.

Finally, at **7**, small *L. neritoides* almost alone persists in an environment that is perfectly terrestrial. Occasionally small specimens of *L. rudis* make an appearance. There is little detritus or wave-lodged material; temperature extremes and lack of shade are very great; insolation is prolonged and high, and humidity very low. *Ligia* has practically disappeared and we find in its place small numbers of a slower terrestrial oniscid.

Location **8**

In tufts of the lichen *Pygmaea pumila*, on the unshaded rock surface in the zone of *Chthamalus-Pygmaea*, at 11–14 ft. C.D. (cf. Colman, 1940).

Tufts of this lichen have remarkable water-retaining power and provide almost the only cover on the surface of bare rock for an extension of the faunas normally sheltering in crevices. The faunas of *Pygmaea* tufts were discussed by Colman (1940). The chief differences to be noted from crevice **4** are the smaller number of actual crevice-dwelling species to be found among *Pygmaea*, and the extraordinary abundance of two species, *Lasaea rubra* and the isopod *Campecopea hirsuta*. Colman remarks on the teeming masses of *Lasaea*, the highest number recorded by him being 12,140 in a sample of 100g. of damp lichen. A comparable order of density for 15 cm.^{2} of *Pygmaea*-covered surface was obtained during this survey, a typical figure being 898. *Campecopea hirsuta* is present here in numbers far greater than at **2** and **4** where it was also seen. Colman's figures of 2204 and 3772 per 100g. of lichen are of similar order to the sample of 494 for one square recorded in this survey. Of the acarines found here, not named by Colman, the red mite *Bdella* appears to be the most numerous. Of other migrants from the crevices, *Littorina neritoides*, *L. rudis*, *Anurida maritima* and *Petrobium maritimum* figure prominently. A new arrival is the tipulid dipteran *Geranomya unicolor*, of which large numbers of larvae were always found. This insect gave a remarkable demonstration of osmotic regulation in its resistance to a 5% solution of formalin in which it remained alive in immersed tufts of lichen overnight.

Locations **9** *and* **10**

9, bare rock surface on unshaded side, between EHMN and MHWN near the lower level of the *Chthamalus-Pygmaea* zone. **10**, bare rock surface on unshaded side above MHWN near the upper limit of the *Chthamalus-Pygmaea* zone.

These locations are among the least favourable of all, and very few of the animals present in crevices at **4** and **5** are able to venture out here. The fauna

is denuded of practically all species except tiny specimens of *Littorina neritoides* and *L. rudis*, which nestle in empty barnacle shells and are evidently able to crawl about at night and in conditions of greater shade. At **9** *L. rudis* is far predominant; at **10** it is reduced in numbers and *L. neritoides* has well overtaken it, being present in samples of more than 400 to a 15 cm.^{2} patch. *Lasaea rubra* has totally disappeared on the open surface. Evidently the evaporation rate is too great even with the tight closing of the shell during the withdrawal of the tide. Of the scavengers, *Ligia* probably comes out by night, as may also *Scolicoplanes*, but in the daytime *Bdella* alone remains swarming over the open rock surface in ceaseless activity.

Location 1

Crevices at upper limit of *Fucus serratus–Laurencia pinnatifida* zone, 10 ft. C.D., and below, shaded by damp thallose algae.

The fauna of crevices of this type presents a strong contrast with all of the locations higher up. The barnacles of the outer lip of the crevice are lacking, tending to be replaced by portions of the carpet of *Laurencia*, which creeps in slightly around the mouth of the crevice though never quite removed from the light. There follows inside the entrance to the crevice a rather narrow zone, which is often silted up, or—in cleaner conditions—supports a growth of encrusting red algae and bryozoa. *Ralfsia* never occurs here and there is usually too much silt for *Spirorbis*, which drops out almost entirely from the crevice fauna in most cases, but is still able to settle in abundance at this level on clean blades of *Fucus*. In this part of the crevice and farther in, on the organic deposits, *Lasaea rubra* still flourishes. Specimens are of large size and pale colour (Fig. 4). *Leucophytia bidentata* does well too, sometimes on very thick yellow-brown silt. *Cingula* is decidedly less common and *Otina* quite lacking. *Sphaeroma* is much less seen, the chief isopods being *Naesa bidentata* (♀=*Dynamene* spp.). There appear to be few *Tanais* in any crevices of this type. Entrants into the fauna which are never seen higher up are a small *Amphiura*, a pilumnid crab, *Anomia ephippium* of small size, and the very distinctive half-crab *Porcellana platycheles*. Tiny *Mytilus edulis* also appear, being sometimes also seen higher up at **2**. Most of the insects seem to be able to extend down to this zone where the crevice is open and clean enough to allow them, but the accumulation of moist silt is probably their limiting factor. *Aëpus* is on the whole less abundant than at **2**, but a new arrival is the heteropteran *Aëpophilus bonnairei* which is never present higher up and is distinctly a low-tidal form. With it occurs the bright green-bodied juvenile stage of the ectoparasitic isopod *Gnathia oxyuraea*.

The deeper part of the crevice is often permanently filled with water-logged, but fairly stiff, sand and detritus. The most typical new entrant here is the burrowing lamellibranch *Hiatella arctica*, which forms horizontal galleries in the mud communicating by its red siphons with the outer part of

the crevice. It is never found farther up, and with *Porcellana*, *Gnathia* and *Aëpophilus* forms a group of species diagnostic of this type of habitat. Among the worms present in the substratum *Perinereis marioni*, and *Eulalia viridis* are as common as above, accompanied by *Amphitrite gracilis* and *Cirratulus cirratus*, and—especially where there is much clay—*Phascolosoma minutum*. Groups of sabellariid tubes are common on the cleaner parts of the rock.

The Environment

The moisture content and the proportion of organic detritus present in the substratum of the crevice have been quantitatively assessed and discussed by Glynne-Williams & Hobart (1952). During the present work the two factors of relative humidity and temperature within selected crevices have been measured for a typical summer day. The temperature was recorded at each of four selected points on the outcrop of the west reef illustrated in Fig. 1, at half-hourly intervals during a complete period of intertidal exposure on 18 June 1951. This was selected as an average warm day on which conditions of temperature and evaporation approached their higher extreme. The meteorological conditions for Plymouth for the day included 'Sunshine 11·6 hr., Max. Temperature (in shade) 62°F, Sea Temperature 58·5°F, Barometer 30·08'.

The first series of temperature readings was taken at position (i), in air, 1 cm. from the exposed rock surface, on the unshaded south aspect corresponding to location 9. A second series of readings was recorded from within a crevice of type 4 on the unshaded side. On the shaded north side, observations of temperature were made at the bottom of a crevice of type 2, and higher up at the bottom of a crevice of type 3. Temperatures were recorded by Cambridge Instrument Company thermocouples, with a narrow flexible thermometer antenna which could be thrust into the full depth of a crevice, with care to avoid contact with the rock surface and without dislodgement of the slate or opening up of the surroundings. The results are presented in the lower half of Fig. 3. The internal temperature of crevice 2 remained low and uniform, rising by 0·5°C at 11.00 hr. to continue through the afternoon at its highest value of 13·5°C. In crevice 3 (where the intertidal exposure was longer) the temperature had risen at 09.30 hr. to 14·5°C, continuing, though less steadily, at an average of 2° higher than in crevice 2. On the exposed rock face at position (i), the temperature had risen to 15° within 30 min. after the tide had ebbed from that point, and, with a recession during the morning due to a light shower of rain, continued to rise throughout the morning to reach its peak of 20°C at 12.30 hr. In the crevice of type 4 the temperature, beginning at the same level as in crevices 2 and 3, rose rapidly during the early part of the morning till it exceeded at 11.00 hr. that of the exposed rock surface (i). This was apparently to be explained by the rapid heating up of the unshaded rock mass surrounding the crevice and the absence of the cooling

effect of breezes and air currents that came into play throughout the day upon the exposed surface. The temperature in this crevice reached a peak of 24°C at 14.00hr. and continued during the afternoon higher than, but with as great fluctuations as, that of (i).

Half-hourly measurements of relative humidity were made simultaneously with temperature readings at the same points by exposing small squares of cobalt thiocyanate paper, which was kindly supplied by Dr M. E. Solomon of the Pest Infestation Laboratory, D.S.I.R., Slough. Standardizing was carried out by exposure in desiccators in atmospheres of known relative humidity over solutions of potassium hydroxide of increasing specific gravity after the method of Buxton & Mellanby (1934). The colour standards so obtained were then compared with the reading of Edney paper hygrometers, and were regarded as having an accuracy of within 5%. Squares of humidity paper of $\frac{1}{2}$ cm. were introduced into crevices within the end of glass rods inserted into the crevices with care to avoid moisture condensed upon the rocky surface, and the papers, removed after 30 min. exposure, were placed in tubes of liquid paraffin for comparison in the laboratory.

The readings of relative humidity at (i) (location 9) and locations 2, 3 and 4 are presented on the upper portion of Fig. 3. Crevice 2 maintained much the highest relative humidity throughout the intertidal period, falling to 95% between the hours of 11.30 and 13.00, and remaining the rest of the time at saturation. The lower extreme is represented by the exposed rock surface at point (i) where the relative humidity fluctuated throughout the day, frequently falling to 65%. Intermediate values were obtained for crevices of type 3, which did not exceed 90% during the intertidal period and fell to 80% at 11.30hr. and later during the afternoon. The type 4 crevice maintained a relative humidity similar to 3 during the early part of the day, later falling rapidly with rise in air temperature.

It would be an over-simplification to emphasize too strongly the apparently close correspondence between the peaks of temperature and the points of greatest saturation deficit. Obviously the variations of relative humidity form an ecological factor with very complex underlying causes. The water content of enclosed air may vary locally under the direct influence of the temperature of the atmosphere and of the substratum, together with variations in the amount of shade and exposure, and drainage of the crevice substratum (these two factors being dependent on the detailed topography of the ground); the percentage of intertidal exposure; the amount of wave-splash and the incidence of local breezes.

On the profile diagram (Fig. 1) have been superimposed curves for two sets of factors established by Colman (1932) with application to intertidal zonation at Wembury. The two lower curves are generalized compound curves showing the average percentage of exposure, for actual tidal level and for the splash level 2 ft. higher, during a complete tidal cycle. Such a curve is 'built up of

(1) the semi-diurnal tide curve and (2) the curves, having a wave-length of a fortnight, formed by the height of high and low waters as they vary between one set of springs and the next'. The upper curve represents 'the length of time during which any given level may be continuously dry during one fortnight (Vernal Equinox)'.

The last curve is applicable to an area lying above EHWNT, below which no place is continuously exposed during the whole of any tidal period. Referring to the upper limits of the ranges recorded in Fig. 1, it will be noticed that *Spirorbis borealis* reaches the splash zone of EHWNT; this species is one that appears to need daily, even at the lowest high neap tides, some amount of wave-splash and access to food. *Chthamalus stellatus* extends well above this

TABLE II. PERCENTAGES OF EXPOSURE, AND MAXIMUM NUMBER OF HOURS OF EXPOSURE AT UPPER LIMIT, FOR SOME CREVICE-DWELLING ANIMALS

Species	Average % of daily exposure (splash figures in brackets)		Maximum number of hours exposed (upper limit)
	Upper limit	Lower limit	
Ligia oceanica	100 (100)	57 (48)	Never submerged
Chthamalus stellatus	98 (90)	52 (45)	325
Tanais cavolinii	85 (66)	*	165
Hydrogamasus salinus	86 (70)	*	185
Spirorbis borealis	86 (70)	*	180
Lasaea rubra	100 (95)	*	370
Cingula cingillus	93 (83)	*	250
Littorina neritoides	100 (100)	82 (64)	Never submerged
L. rudis	100 (100)	60 (50)	Never submerged
Leucophytia bidentata	93 (65)	*	165
Otina otis	93 (78)	60 (49)	230

* Lower limit not observed in present survey.

level to MHWST. During neap tides it may remain out of the high-water splash zone at its upper limit, as may also *Lasaea rubra*. For the greater portion of a fortnightly tidal cycle, *Ligia oceanica* and *Littorina neritoides* appear to be quite independent of wetting by splash. For some of the animals whose vertical distributions are recorded in Fig. 1, we may extract Table II, expressing the average percentages of daily exposure at their upper and lower limits, and also the total number of hours during which these species—at the upper limit of their range—may remain continuously exposed during a fortnightly tidal cycle.

DISTRIBUTION OF THE FAUNA

A species list for the habitats of the upper intertidal zone at Wembury is given in Table III. It has not been attempted to give actual numbers of single counts at particular crevices sampled, but rather to record conditions of density, from dominance to occasional presence. In the distribution diagrams (Fig. 6), quantitative data have been expressed by the scale of density shown on the figure. Each estimate is again not the result of any single sample, but

gives a generalized picture from the results of two seasons' records from a large number of stations. Twenty-two species of animals are dealt with, the distributions being grouped according to taxonomic relationships, to give a comparative picture of the ecology of fairly closely related types of animals. In many cases, it will be realized that a uniform distribution of a species over the area of 10 cm.^{2} sampled is not implied. Thus, with *Lasaea*, individuals will frequently be crowded together in a narrow row a centimetre wide, and the remaining drier parts of the sampled square may show few or no specimens. An even distribution is much more a feature of crevices at 1 and 2. At 3, 4 and 5, the greater part of a crevice may be too dry to be inhabited, and this, rather than lower density at points of actual occurrence, accounts for the diminishing numbers of several of the species at these locations.

Some observations in more detail on the ecology of several groups of species of special importance in upper tidal crevices may be presented here. Glynne-Williams & Hobart (1952) have already recorded much useful data, especially about the terrestrial element in the fauna, in relation to feeding habits and position in the crevice.

Isopoda

The principal isopods are five in number, *Ligia oceanica*, an unnamed terrestrial oniscid of the wood-louse type, *Campecopea hirsuta*, *Sphaeroma serratum* and *Naesa bidentata*. Of these we may neglect the oniscid species which is of chief interest in showing the close approach to terrestrial conditions at 7. *Ligia* is typical of the upper half of the profile, most abundant at 3, next at 4, wandering about actively outside the crevice, especially at night. Its upper limit is probably governed by the dryness and shallowness of the crevice and the lack of adequate food supplies. It is thus almost absent at 7 and is in no sense truly terrestrial. It likewise appears to avoid *Pygmaea* (Colman, 1940) and is markedly reduced in numbers at 2 and quite absent at 1. Nicholls (1931 *a*, *b*) gives some account of its ecology and feeding, and this aspect has also been studied by Glynne-Williams & Hobart (1952). *Ligia* is replaced at 2, and also accompanied at 3, by the much more sluggish isopod *Sphaeroma serratum*. This species is far less tolerant of drying-out or temperature fluctuations. It is limited above at the MHWS line, and at its lower range just reaches 1. *Naesa bidentata* is found together with *Sphaeroma* in 2, but both these isopods are much more typical of deeply silted crevices and they extend downwards to reach their greatest abundance, in the area studied, at 1. They are found very little if at all on the unshaded south side. Numerically, by far the most important isopod is the small *Campecopea hirsuta*. As found by Colman (1940) in his sample from *Pygmaea*, this species is present in teeming numbers at 8, and there are always a few individuals represented at 2 and at 4 where they are likely to be overlooked on account of small size.

TABLE III. SPECIES LIST FROM INTERTIDAL CREVICES AND RELATED HABITATS

	Location type no.									
	1	2	3	4	5	6	7	8	9	10
Lineus longissimus (Gunnerus)	—	O	O	O	O	—	—	—	—	—
Small pink nemertine (indet.)	—	O	O	O	—	—	—	—	—	—
Perinereis marioni (Aud. and Edw.)	O	R	O	O	—	—	—	—	—	—
Unidentified eunicids	O	O	—	—	—	—	—	—	—	—
Eulalia viridis (O. F. Müller)	R	R	R	R	—	—	—	—	—	—
Harmothoë imbricata L.	R	—	—	—	—	—	—	—	—	—
Cirratulus cirratus (O. F. Müller)	R	O	—	O	—	—	—	—	—	—
Amphitrite gracilis Grube	R	O	—	O	—	—	—	—	—	—
Spirorbis borealis (Daudin)	O	D	—	O	—	—	—	—	—	—
Phascolosoma minutum Keferstein	O	—	—	—	—	—	—	—	—	—
Chthamalus stellatus (Poli)	—	D	D	D	O	—	—	—	—	—
Tanais chevreuxi Milne-Edwards	O	D	—	O	—	—	—	—	—	—
Sphaeroma serratum (Fabr.)	O	A	A	A	—	—	—	—	—	—
Naesa bidentata (Adams)	A	R	O	O	—	—	—	—	—	—
Campecopea hirsuta (Mont.)	—	R	—	O	—	—	—	D	—	—
Ligia oceanica L.	—	O	A	A	A	R	R	O	O	O
Unidentified terrestrial oniscid	—	—	—	—	—	—	O	—	—	—
Gnathia oxyuraea (Lillj.)	R	—	—	—	—	—	—	—	—	—
Hyale nilssoni (Rathke)	*O	—	—	—	—	—	—	—	—	—
Marinogammarus obtusatus (Dahl)	—	A	A	O	O	—	—	—	—	—
Porcellana platycheles (Pennant)	R	—	—	—	—	—	—	—	—	—
Juvenile pilumnid crab	R	—	—	—	—	—	—	—	—	—
Anurida maritima (Guérin)	O	A	A	A	O	O	—	O	—	—
Petrobius maritimus Leach	O	R	R	R	—	—	—	—	—	—
Aëpophilus bonnairei (Sig.)	R	—	—	—	—	—	—	—	—	—
Micralymma marinum (Stroem.)	—	O	—	O	—	—	—	—	—	—
Aëpus robini (Laboulb.)	R	A	—	O	—	—	—	—	—	—
Unidentified caddis	—	—	—	—	—	—	—	O	—	—
Geranomya unicolor	—	—	—	O	—	—	—	D	—	—
Scolicoplanes maritimus (Leach)	—	R	R	R	R	O	—	—	—	—
Neobisium maritimum	—	O	—	—	—	—	—	—	—	—
Cyrthydrolaelaps hirsutus Berlesé.	O	O	—	—	—	—	—	—	—	—
Hydrogamasus salinus Laboulbene	A	D	O	O	—	—	—	—	—	—
Bdella interrupta Evans	—	O	O	A	O	—	—	—	A	—
Protereupetes sp.	—	O	—	A	—	—	—	—	A	—
Anomia ephippium L.	O	—	—	—	—	—	—	—	—	—
Mytilus edulis L. (juv.)	R	O	—	—	—	—	—	—	—	—
Lasaea rubra (Montagu)	D	D	D	D	D	O	—	D	—	—
Hiatella arctica (L.)	D	—	—	—	—	—	—	—	—	—
Tapes pullastra	O	—	—	—	—	—	—	—	—	—
Acanthochitona crinitus (Pennant)	O	—	—	—	—	—	—	—	—	—
Littorina neritoides (L.)	—	—	D	A	D	D	A	R	R	D
L. rudis (Maton)	—	A	A	A	A	A	O	R	D	A
L. littoralis (L.)	O	O	—	—	—	—	—	—	—	—
Gibbula umbilicalis (da Costa)	—	R	R	—	—	—	—	—	—	—
Cingula cingillus (Montagu)	A	D	D	R	—	—	—	—	—	—
C. cingillus var. *rupestris*	*D	D	O	O	—	—	—	—	—	—
C. semistriata (Montagu)	R	O	—	—	—	—	—	—	—	—
Onoba candida (Brown)	R	O	—	—	—	—	—	—	—	—
Onchidella celtica (Cuvier)	*O	R	—	—	—	—	—	—	—	—
Otina otis (Turton)	—	A	R	R	—	—	—	—	—	—
Leucophytia bidentata (Montagu)	A	A	—	O	—	—	—	—	—	—
Blennius galeritus L.	O	O	—	—	—	—	—	—	—	—

D, dominant; A, abundant; R, regularly present; O, occasionally present; —, absent.
* Occurs at Whitsands but not seen at Wembury.
Nomenclature as in Plymouth Marine Fauna (Marine Biological Association, 1931).

Tanais chevreuxi

In choice of site, both with respect to tidal height and to depth in the crevice, this is one of the most restricted animals encountered in the present study. Its requirements appear to be a sandy mud, richly silted by weathering products and organic detritus, with an admixture of sufficient coarse sand to enable its galleries to be given a compact structure. As described above (p. 196) it presses deeply into crevices to a point where there is room for one layer only of animals between the two slates. Its appearance is an indicator of fairly mature conditions of succession in locations of type **2**. The only other place where it is at all abundant is sometimes at **4**. It never appears at **3**, where its absence is one of the criteria of this type of crevice, and it apparently becomes cut out at **1** where the conditions of silting up and waterlogging of the crevice are too extreme. Dennell (1937) has described the feeding habits and ecology of *Apseudes talpa*, and this is the species of tanaidacean found by Glynne-Williams & Hobart at Anglesey. It would be interesting to know something more about the distribution and ecological requirements of *Tanais chevreuxi*, and to compare its occurrence at Wembury with that of the allied species, *T. cavolinii*, which would seem not to occur in crevices.

Mites

These may be taken together as a group, though they are by no means without clear-cut ecological distinctions, as between the species mentioned at Wembury. On the shaded side, in greatest concentration at **2** and extending abundantly down to **1**, the chief mite is *Hydrogamasus salinus*, small in size and polished tan-brown. It is accompanied by a species of *Protereupetes*. On the south exposed side, most at home at **4** and **5** and wandering on to bare rock at **9**, though found also on the shaded side, are two larger and more spectacular mites, the bright red *Bdella interrupta* and *Cyrthydrolaelaps hirsutus*. These are able to extend well up the shore into terrestrial conditions. They are air-breathing, evidently able to retain atmospheric air imprisoned between their fine setae. They appear to be widely foraging scavengers and also some of the chief carnivores of this area of the reef. The present writer has no direct observations on their feeding, but reference should be made to Glynne-Williams & Hobart (1952), and to previous observations quoted by them.

Lasaea rubra

This little lamellibranch is the most numerous animal in the upper shore. Its astonishing abundance at location **8**, in tufts of *Pygmaea*, has already been remarked upon by Colman. It is no less dominant at **1**, **2**, **3** and **4**, where it crowds closely into the tighter parts of the crevices almost as thickly as it is possible to aggregate. As found by Colman, numerical differences between successive samples of such large numbers may be very great, and on the

distribution diagrams (Fig. 6) its fluctuations do not show up, as wherever it occurs it may be expected to exceed 200–300 in the 2×15 cm.^{2} area sampled. It seldom if ever occurs uniformly over the whole of such a patch, except at **2** and **1**, where larger-sized individuals may be more evenly spaced out. At **3** and **4** its most typical occurrence is in a mass of smaller individuals pressed close together in a narrow line at the shallowest and innermost portion of the crevice. Figures for three of a number of samples taken were, for example, at a crevice of type **2**, 865, at **8**, 898, at **5**, 455, and most samples at **1** and **4** totalled well over 400. The distribution of *Lasaea* is terminated abruptly by its complete disappearance above the level of **6**; and unlike the Littorinas it is more or less coincident in its upper level with the barnacle zone, but even here it needs more shelter—which is evidenced by the presence of not a single specimen on the open surface at **9** and **10**. It does best where it can take shelter by pressing into the smallest cracks or into empty *Chthamalus* shells, but it goes down well below the *Chthamalus* level, as may be seen from the present results at **1**, and from Glynne-Williams & Hobart. Colman (1940), in his account of the fauna of the holdfasts of the larger brown algae (*Ascophyllum*, the fucoids and *Laminaria digitata*), finds no abundance of *Lasaea*, as in *Pygmaea* tufts. Though this bivalve is plentiful in crevices of type **1**, below the *Chthamalus* zone, its maximum numbers always appear to occur in the neighbourhood of *Chthamalus* and *Pygmaea*.

There is a rather marked differentiation between *Lasaea* from two different groups of habitats. In the well sheltered conditions at **1** and **2** and the deeper parts of crevices at **3**, the average size is much larger, length of shell up to 3 mm. or more and the colour pale. At **4**, **5** and **6**, and especially in tufts of *Pygmaea* at **8**, the mean length is smaller. There is a larger element of tiny individuals below 1 mm. in length and the predominant group is of $1\frac{1}{2}$–2 mm. (Fig. 4). The shells are all much darker brown or reddish purple in colour.

Lasaea rubra anchors itself in a confined space by a tiny byssus, and it appears to show the same thigmotactic behaviour as *Otina*. It is probably claustrophilic for the same reason as *Otina*, both tending to remain aggregated in chinks and small cavities with the greatest local humidity. It appears to be entirely a suspension feeder; even in the richest organic deposits at **2** and at **1**, it appears to have no particular modifications for sucking-up detritus in the manner of a deposit feeder. At **6** and at **5** it may undergo a total dry period for at times as long as 300 hr. continuously. Here the amount of nutritive deposits must be exceedingly small, and *Lasaea* must have become highly efficient at capturing the suspended particles splashed up to it by wave action in its relatively short period of contact with the water. Popham (1940) has given a detailed account of the pallial cavity of this bivalve. In addition, many questions still present themselves with respect to the physiology and cycles of activity in *Lasaea*. At its upper limits this lamellibranch must show

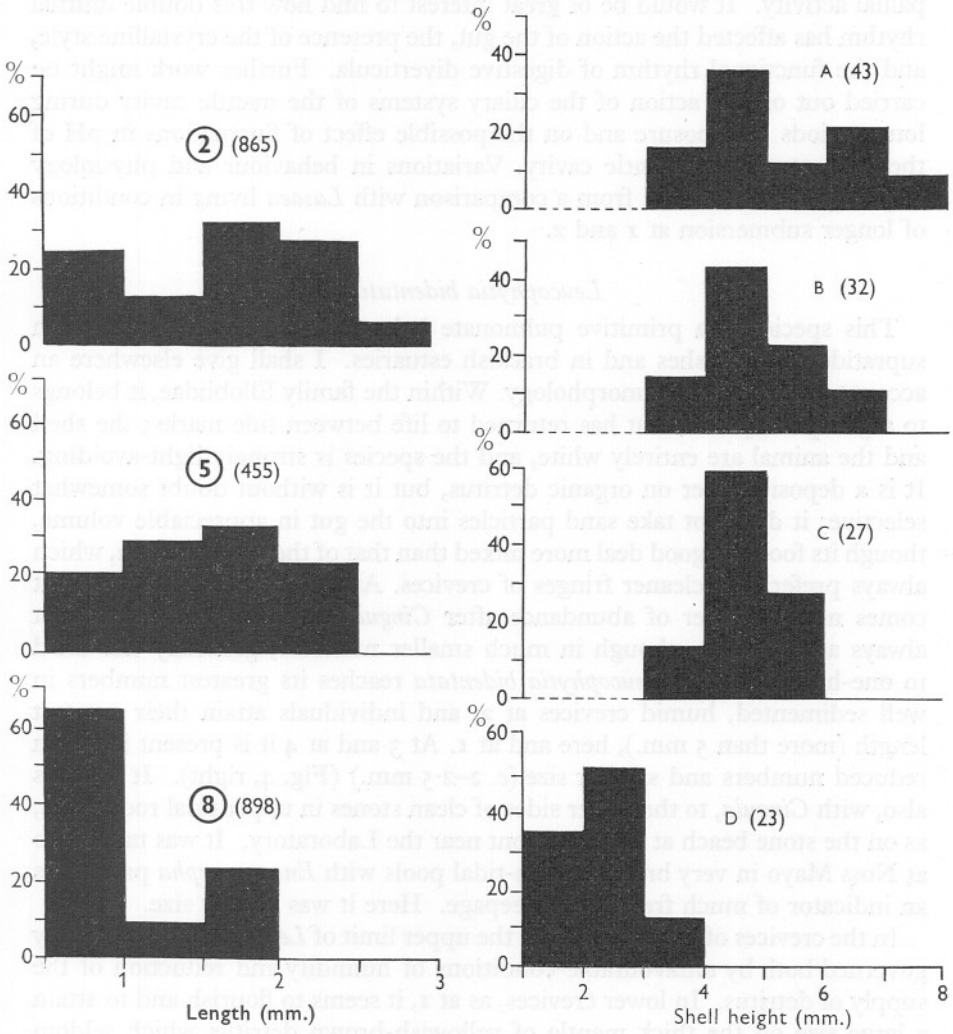


Fig. 4. Left. Histograms showing the percentage distribution of five size-groups of *Lasaea rubra*, in typical samples from locations of types **2**, **5** and **8** (actual numbers in samples are bracketed). Right. Histograms showing the size composition of four samples of *Leucophytia bidentata* (actual numbers are bracketed). A, B and C are from deep shaded crevices of type **2** at Wembury. Individuals below 3 mm in height were not measured for Wembury; repeated observations at **1** and **2** indicated that they form only a minor fraction of the population. Sample D is from shallow crevices in limestone conglomerate on the wave-exposed Hoe front. The level sampled was at the lower limit of the *Chthamalus* zone.

a tidal periodicity very strongly impressed on the whole of its feeding and pallial activity. It would be of great interest to find how this double diurnal rhythm has affected the action of the gut, the presence of the crystalline style, and the functional rhythm of digestive diverticula. Further work might be carried out on the action of the ciliary systems of the mantle cavity during long periods of exposure and on the possible effect of fluctuations in pH of the contents of the mantle cavity. Variations in behaviour and physiology might then be examined from a comparison with *Lasaea* living in conditions of longer submersion at **1** and **2**.

Leucophytia bidentata

This species is a primitive pulmonate belonging to a family centred in supratidal salt marshes and in brackish estuaries. I shall give elsewhere an account of its functional morphology. Within the family Ellobiidae, it belongs to a group of species that has returned to life between tide marks; the shell and the animal are entirely white, and the species is strongly light-avoiding. It is a deposit feeder on organic detritus, but it is without doubt somewhat selective; it does not take sand particles into the gut in appreciable volume, though its food is a good deal more mixed than that of the related *Otina*, which always prefers the cleaner fringes of crevices. Among the gastropods at **3** it comes next in order of abundance after *Cingula cingillus*, which it almost always accompanies, though in much smaller numbers, generally one-third to one-half as many. *Leucophytia bidentata* reaches its greatest numbers in well sedimented, humid crevices at **2**, and individuals attain their greatest length (more than 5 mm.), here and at **1**. At **3** and at **4** it is present in much reduced numbers and smaller size (*c.* 2–2·5 mm.) (Fig. 4, right). It spreads also, with *Cingula*, to the under sides of clean stones in upper tidal rock pools, as on the stone beach at the Hoe front near the Laboratory. It was taken also at Noss Mayo in very brackish high-tidal pools with *Enteromorpha* present as an indicator of much fresh water seepage. Here it was of tiny size.

In the crevices of the area studied the upper limit of *Leucophytia* is evidently governed both by unfavourable conditions of humidity and reduction of the supply of detritus. In lower crevices, as at **1**, it seems to flourish and to attain a large size on the thick mantle of yellowish-brown detritus which seldom dries out at low tide. Glynne-Williams & Hobart also reported it as a low-tidal crevice animal at Anglesey. It is undoubtedly very much better able to withstand submersion than *Otina*, and evidently employs the supra-anal lobe of its pallial skirt in respiration. The 'pulmonary' pallial cavity was never found to be filled with water. Unlike *Otina*, *Leucophytia* has no preference for wave splash or well oxygenated water; it crawls feebly, and has no power to resist the dislodging action of water movements. Specimens survived for several weeks in the still water of a tidal tank with no other aeration than a twice-daily exposure to the atmosphere.

Otina otis

This interesting little mollusc is also a representative of the most primitive of the marine pulmonates. Its distribution on British coasts appears to be limited to the west and south-west, and Glynne-Williams & Hobart do not record it from Anglesey. Its British and west European distribution is to be discussed elsewhere. At Wembury it is one of the most discriminating animals in its choice of environment. It is almost entirely confined to crevices and their immediate neighbourhood, within a rather short tidal range. At its

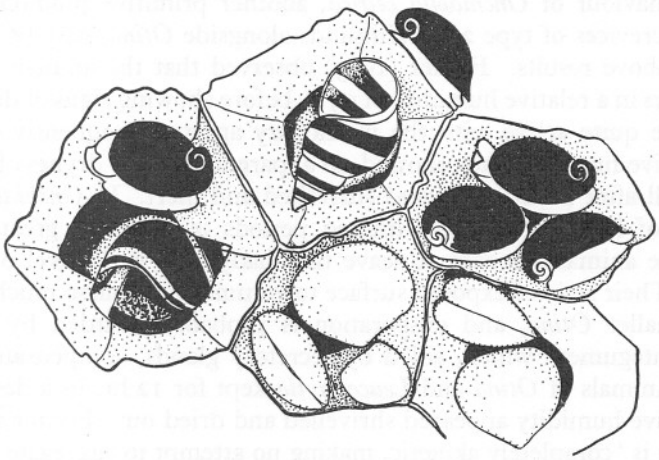


Fig. 5. A group of dead *Chthamalus stellatus* shells removed from within the lip of a crevice of type 4, and viewed from underneath to show the occurrence within them of *Otina otis* and *Littorina neritoides* (black), *Cingula cingillus* (banded) and *Lasaea rubra* (white).

maximum occurrence at 2 it avoids the more heavily silted conditions in the deeper parts of the crevice, and creeps out upon the *Spirorbis-Ralfsia* zone, though it often intermingles with *Leucophytia* farther in. A favourite habit is to creep farther out on to the barnacle fringe in shaded spots, and to nestle in empty *Chthamalus* shells like *Littorina neritoides* (Fig. 5). It is above all a lover of clean conditions, and is never found in situations where excess detritus is present. Of all the molluscs, save the littorines, it has the highest lower limit, and it does not accompany *Leucophytia* down to the silted conditions at 1. It is best developed at 2, but unlike *Leucophytia* it can live favourably at 3 and it is present in greater numbers than *Leucophytia* at 4. Its mode of life is not unlike that of a limpet; with its strong radula it scrapes up wave-washed diatoms and algal filaments growing at the mouth of the crevice. Farther down in the crevice it is able to secure richer detritus, but its stomach contents are always finely graded, with a high proportion of diatoms, and seldom include particles of coarse detritus.

Both *Otina* and *Leucophytia* are usually present in crevices at 4 where they can evidently tolerate several hours of lowered humidity. Sometimes also at

3 the saturation deficiency may approach that of 4. Perhaps both species are rather better able to withstand a shorter period of greater saturation deficiency at 4 than a longer one of lesser deficiency at 3. However, a period of humidity as low as 80% at 4 may represent a condition to which *Otina* and *Leucophytia* are not exposed for significantly long periods. Both take the fullest advantage of moist cracks where the humidity may remain locally high, and the humidity conditions of a single crevice may show wide local variations, with sheltered regions of high humidity which the thermocouple used did not reach.

The behaviour of *Onchidella celtica*, another primitive pulmonate which occurs in crevices of type 2 at Whitsands alongside *Otina*, may be compared with the above results. Fretter (1943) observed that the animals 'lived for several days in a relative humidity of 95% before showing signs of desiccation. They were quite active with the pulmonary aperture frequently open.' In 90% relative humidity they showed no apparent effect of dryness for 3 days. In 80% all after 2 days appeared shrivelled and inert. In *Onchidella celtica* tolerance of low humidity for limited periods is evidently great: in mid-August the animal was seen to leave the shady rock and travel over sunny surfaces. Their ratio of exposed surface to volume is of course much less than in the smaller *Otina*, and evaporation is probably retarded by the thick leathery integument which, aided by secretory glands, compensates for loss of shell. Animals of *Otina* and *Leucophytia* kept for 12 hr. in a desiccator at 90% relative humidity appeared shrivelled and dried out. Fretter states that *Onchidella* is 'completely akinetic, making no attempt to aggregate in regions of optimum humidity when placed in a humidity gradient of 75–95%.' *Otina*, on the other hand, reacts quickly to the equally unfavourable extremes of either a fall in the humidity of the atmosphere or of complete immersion. Jeffreys has observed (1869) that 'the animal is a restless little creature and when put into sea water crawls directly out of it.' The same active response is made when a crevice is opened and a group of *Otina* are subjected to the evaporating power of warm air. They crawl quickly into the shelter of a deeper part of the crevice, or, if exposed for more than 2–3 min., again become inactive, cease to crawl and clamp the shell tightly against the substratum.

The upper limit of *Otina* at *c.* 15 ft. C.D. is probably governed not by reduction of food supply but by prolonged saturation deficiency. Its lower limit at EHWN is fixed by its intolerance of long submersion and its dislike of silt. Still water or submersion for more than 3 or 4 hr. seem adverse. *Otina* does not survive in the still waters of a tidal tank, preferring the broken and well oxygenated water received from wave-splash. Both pigmentation of the shell and the streamlined limpet shape indicate that *Otina* is well at home on the clean-swept outer fringe of the crevice, and is well adapted to resist both wave attack and light. Its habit of thigmotaxis, with a tendency for a group of animals to cluster together in contact, or to nestle in a barnacle shell, is probably in part an adaptation for reducing water loss by evaporation. In

addition, the habit of negative geotaxy, clinging by preference to the upper slate of a crevice, may assist it to take advantage of the atmospheric air longer retained in this part of the crevice.

Discussion

Ecological factors

Any discussion of the causes affecting the distribution of crevice-dwelling animals in relation to their environment must necessarily be tentative and inconclusive. We know little at present of the mechanisms by which shore animals respond to changes in their surroundings, and distributional data are at present available only for the few localities studied in detail by the writer, and previously by Glynne-Williams & Hobart. In the absence, however, of any attempt, before these two papers, to assess the importance of factors operating in crevice habitats, the writer felt justified in advancing the ideas induced by the present study. Their preliminary nature will be evident; and their greatest use may be to form a basis of more exact future work.

Of the ecological factors with a direct effect on the fauna of crevices, the principal ones are probably relative humidity and the presence of adequate food supply, whether in the form of organic deposits or of wave-borne micro-organisms. Other factors may act to a large extent indirectly by their influence on one or other of the first two, though perhaps the effects of temperature and the dislodging power of wave action may also be direct. The most important factors influencing relative humidity are probably percentage of daily exposure, maximum longest period of exposure during a tidal cycle, maximum air temperature, incidence of breezes and amount of shade and rate of drainage of the crevice. The last two will, of course, vary complexly with the whole topography of the surrounding rock mass. With the contrasts at Wembury between southerly and northerly aspects, with crevices overthrust towards the north, both sets of factors—shelter and the structure of the rock mass—may be combined in one or other of their two sets of extremes. For the majority of the fauna both factors are in their most favourable combination on the north, and most rigorous towards the south. It would be possible in rocks overthrust towards the south, with deep crevices thus facing south, to examine the effects of separating rather than combining these two factors. Relative humidity, temperature and duration of exposure are easily measured (see above), and on the moisture content and organic detritus in crevices, quantitative data have already been given by Glynne-Williams & Hobart.

In the presence of a saturated atmosphere in crevices, the distribution of animals is likely to be governed chiefly by food supply. Here the controlling factors are probably the direct action of wave swell and splash, bringing suspended micro-organisms within the range of suspension feeders, and shifting detritus from the crevices. The influence of the surrounding rock structure is here very variable with situation, and very complex.

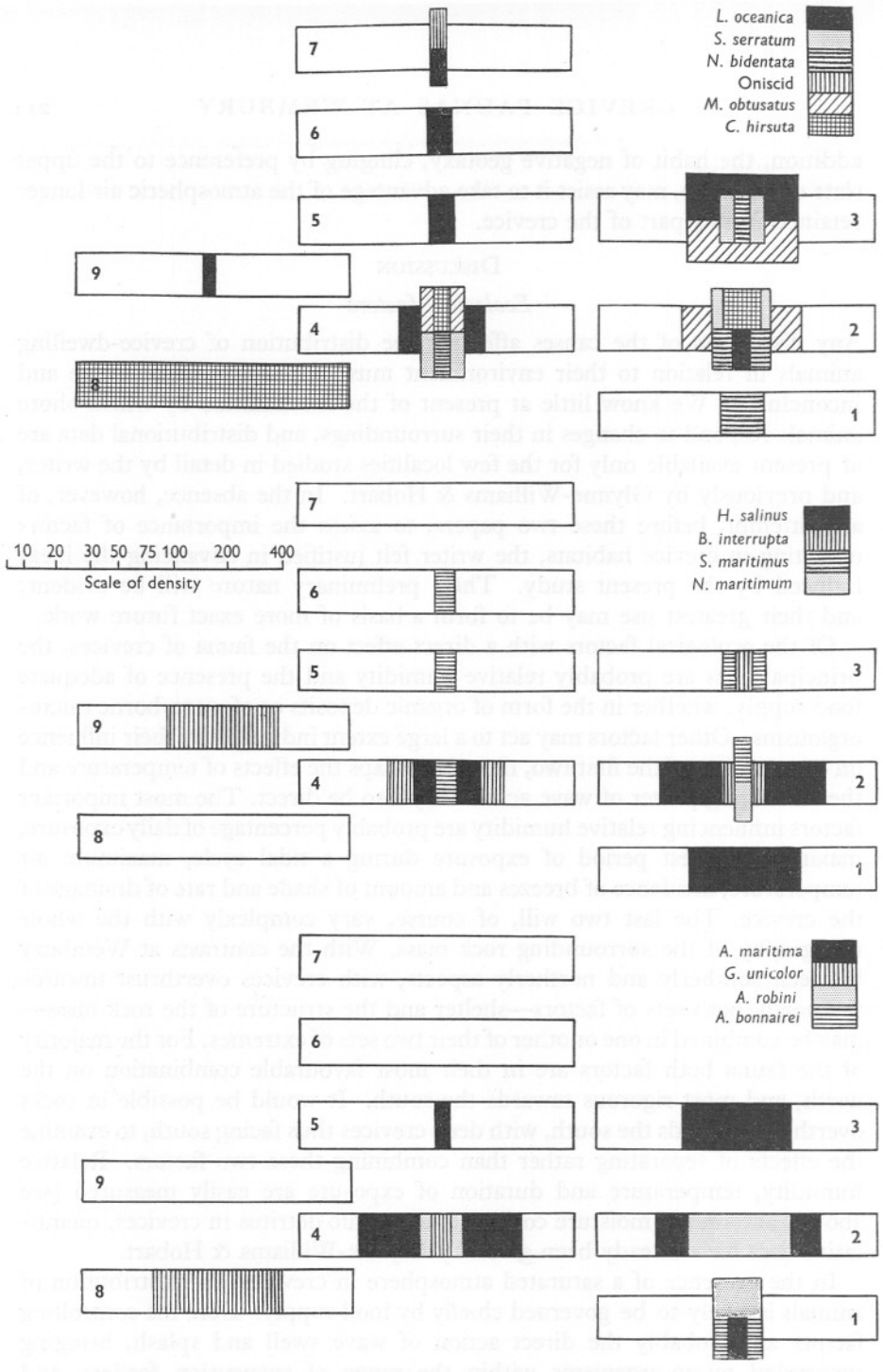
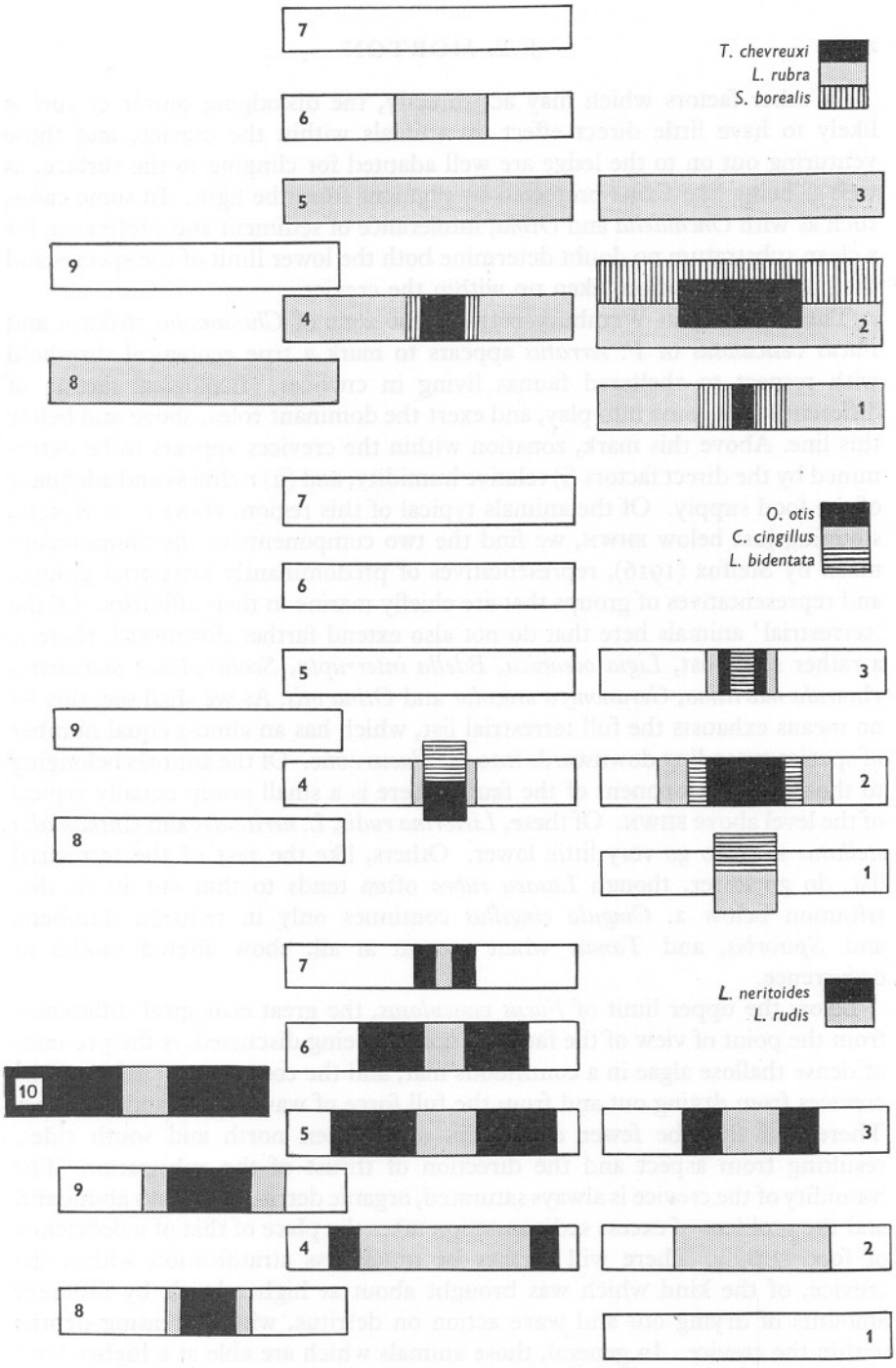


Fig. 6. Diagrams showing the quantitative distribution of twenty-two species of animals in crevices and related habitats at Wembury. The species are the following: *Ligia oceanica*, *Sphaeroma serratum*, *Naesa bidentata*, terrestrial oniscid, *Marinogammarus obtusatus*, *Campecopea hirsuta*; *Hydrogamasus salinus*, *Bdella interrupta*, *Scolicoplanes maritimus*, *Neobisium maritimum*; *Anurida maritima*, *Geranomya unicolor*, *Aëpus robini*, *Aëpophilus bonnairei*; *Tanais chevreuxi*, *Lasaea rubra*, *Spirorbis borealis*; *Otina otis*, *Cingula cingillus*, *Leucophytia bidentata*; *Littorina neritoides*, *Littorina rudis*. They have been arranged in



six groups, mostly according to approximate taxonomic relationships. Each horizontal panel represents a numbered location according to the classification adopted in the text. They are arranged in approximate sequence according to conditions of shade and height for easy reference to Fig. 1. Location **10** is included only in the case of the littorinids. The scale of densities adopted is shown inset. The area shaded for each species is to be regarded as extending across the middle of the panel, and the smaller areas as superimposed on, and not interrupting, the larger.

Of other factors which may act directly, the dislodging power of surf is likely to have little direct effect on animals within the crevice, and those venturing out on to the ledge are well adapted for clinging to the surface, as well as being like *Otina* protected by pigment from the light. In some cases, such as with *Onchidella* and *Otina*, intolerance of sediment and preference for a clean substratum no doubt determine both the lower limit of the species and the horizontal position taken up within the crevice.

The boundary at Wembury between the zone of *Chthamalus stellatus* and *Fucus vesiculosus* or *F. serratus* appears to mark a true ecological threshold with respect to sheltered faunas living in crevices. Ecological factors of different kinds come into play, and exert the dominant roles, above and below this line. Above this mark, zonation within the crevices appears to be determined by the direct factors (i) relative humidity, and (ii) richness and adequacy of the food supply. Of the animals typical of this region, above *c.* 10 ft. C.D., stopping just below EHWN, we find the two components of the fauna recognized by Stelfox (1916), representatives of predominantly terrestrial groups, and representatives of groups that are chiefly marine in their affinities. Of the 'terrestrial' animals here that do not also extend farther downward, there is a rather short list, *Ligia oceanica*, *Bdella interrupta*, *Scolicoplanes maritimus*, *Anurida maritima*, *Geranomya unicolor* and *Otina otis*. As we shall see, this by no means exhausts the full terrestrial list, which has an almost equal number of species extending downwards into the *Fucus* zone. Of the animals belonging to the marine component of the fauna, there is a small group equally typical of the level above EHWN. Of these, *Littorina rudis*, *L. neritoides* and *Chthamalus stellatus* seem to go very little lower. Others, like the rest of the terrestrial list, do go lower, though *Lasaea rubra* often tends to thin out in its distribution below 2. *Cingula cingillus* continues only in reduced numbers, and *Spirorbis*, and *Tanais* when present at all, show altered modes of occurrence.

Below the upper limit of *Fucus vesiculosus*, the great ecological difference, from the point of view of the fauna at present being discussed, is the presence of dense thallose algae in a continuous mat, and the consequent shelter of the crevices from drying out and from the full force of wave action and scouring. There will thus be fewer differences as between north and south sides, resulting from aspect and the direction of thrust of the substratum. The humidity of the crevice is always saturated, organic detritus is always abundant, and the problem of excess sedimentation takes the place of that of a deficiency of food supply. There will further be much less stratification within the crevice, of the kind which was brought about at higher levels by different amounts of drying out and wave action on detritus, with increasing depths within the crevice. In general, those animals which are able at a higher level to tolerate the heavily sedimented parts of the crevice are able to extend below the *Fucus* upper level, at least in reduced numbers. There is thus an overlap

into this zone of a group of animals extending downwards below the *Fucus vesiculosus* upper limit, but reaching their most typical development and greatest numbers above that line. Among these may be placed *Leucophytia bidentata*, *Cingula cingillus*, *Lasaea rubra* and in small numbers *Tanais chevreuxi*. Conversely, on both sides of the line but dwindling on extent into crevices in the barnacle zone is a group of animals containing *Amphitrite gracilis*, *Cirratulus cirratus*, *Perinereis marioni* and probably most of the worms, with the exception of *Spirorbis borealis*, *Lineus longissimus* and *Eulalia viridis* which, in crevices, are more at home in the barnacle zone.

For the terrestrial element that does not extend below the lower barnacle limit, namely *Otina otis*, *Bdella* sp., *Scolicoplanes maritimus* and *Neobisium maritimum*, (though the latter is very sparse in numbers and it is difficult to be exact about its distributional limits) the limiting factor is probably increased sedimentation of the crevices which is especially important in restricting *Otina*. For land-derived crevice animals as a whole there does not seem to be a general lower barrier at this zone. *Aëpus robini* and *Aëpophilus bonnairei* exist happily at Wembury at the level of 1, and Glynne-Williams & Hobart have shown their extent to or beyond MLWS at Anglesey. They no doubt retain aerial respiration as do the mite *Hydrogamasus salinus* and the thysanuran *Anurida maritima*; reliance on aerial conditions is probably possible at much lower levels and with much shorter exposures between tides than at EHWN. This group of insects, and of the mites—at least *Hydrogamasus salinus*, are thoroughly intertidal. Their extent into this zone is by no means dependent on a precarious compromise with marine conditions, and they are content with a short intertidal exposure for the renewal of air supplies. The arachnid *Neobisium*, the chilopod *Scolicoplanes*, the mite *Bdella*, and probably also the tipulid *Geranomya* are less well adapted for conditions in the lower intertidal zone. But, on the whole, the uppermost zone—locations **3**, **4**, **5** and **6**—is characterized in largest numbers not by members of the terrestrial element but by marine animals (*Lasaea rubra*, *Littorina rudis*, *L. neritoides* and *Ligia oceanica*) which have evolved a tolerance of longer exposure.

Of the upper limits of range of the species recorded in the present survey, the most significant is at the *Fucus vesiculosus–Chthamalus stellatus* boundary. A large and varied fauna of type 1 location—not in detail listed here—cuts out between crevices 1 and 2. Crevices 2 never contain, for example, ophiuroids, pilumnid or porcellanid crabs, *Anomia* or *Hiatella*. The most interesting disappearance here is that of the bug *Aëpophilus bonnairei* which is not a high-tidal form at all. An upper limit for a further group of species lies between crevices of types 2 and 3. The animals disappearing here are *Spirorbis borealis*, *Cirratulus cirratus*, *Amphitrite gracilis*, *Tanais chevreuxi*, *Hydrogamasus salinus*, *Aëpus robini* and *Neobisium maritimum*. *Leucophytia* is here greatly thinned out, and we find almost the last of *Otina* and *Cingula*.

Above the level of 3 and for the most part in 4, the fauna, with the exception

of *Scolicoplanes*, is not on the whole terrestrial in composition, but consists of an enterprising filter-feeding and suspension-feeding marine element like *Chthamalus* and *Lasaea*, or like the Littorinas, which are equipped with a strong scraping radula. Here they are almost entirely free of predator pressure, if this is ever indeed a significant factor, which is perhaps to be doubted in this area, with the exception of *Nucella lapillus* in relation to *Chthamalus* at its lower boundary. Animals of the terrestrial element are less adept than those above listed at obtaining their food suspended in sea water. For the most part, both for the sake of an adequate food supply and for protection from desiccation, they must go farther down in the intertidal zone and enter more fully into tidal conditions. Here they have almost all become carnivores or scavengers. The reasons for the falling out of the terrestrial element, at the highest levels surveyed, are probably connected with both food supply and humidity, disadvantages which hard-shelled, filter-feeding or radula-browsing animals have overcome.

Ecological Succession

Crevices between slates are in constant course of opening up by the loosening and ultimate detaching of a block of slate, usually the underlying one, by the action of water and weather. We have an accurate idea of the way in which a crevice may widen during the process of weathering, and though it was not possible in two seasons to detect the succession of the fauna at a single location, we can, by observing the zones present in crevices of different depths, build up a picture of the changes that must take place in the composition of a crevice fauna. Intertidal crevices provide a good example from the sea shore of an ecological succession in the words of Allee's definition (1949), 'the progressive sequence of replacement of communities over a given point, area or locality'. A crevice is first colonized as a small chink on the rock surface affording shelter to animals, and ultimately becomes deeply thrust into the rock mass before it is eliminated by the breaking away of the slate. The changes thus brought about in the substratum by weathering may be referred to as a 'physiographic succession'; the entire series of stages from bare rock through the deepening crevice with a final return to bare rock, form a 'sere'. The changes in the composition and relationships of the fauna may be regarded as constituting together the 'ontogeny' of the community. At its fullest development a crevice in Dartmouth Slate will reveal the five or six rather well defined zones illustrated in Fig. 2. As stages in succession we may recognize several phases—of *colonization* with the opening up and penetrating of a new crevice by successive groups of animals, new zones becoming represented with greater depth; of *maturity* as represented with complete horizontal zonation as in Fig. 2; and of *regression* by the gradual opening up and drying out of the crevice, leading to the final dropping of the slate and the re-occupation of the bare rock surface by *Chthamalus* and *Pygmaea*. It is

difficult, without long-term observations, to estimate the rate at which succession may proceed. It is probable, however, that with the relatively rapid weathering of a foliaceous rocky substratum, the sequence of stages in Dartmouth Slate may move faster than is usual on rocky shores. The human investigator with his hammer and chisel becomes at times a biotic factor that appreciably speeds up the course of succession.

The diagram (Fig. 7) attempts to sum up the course of events at the level of a type **2** crevice, where the mature fauna is able to develop its fullest zonation. For comparison with the stages in a complete sere, we may refer to the other types of location, where the final stages represent stages intermediate in the succession at **2**. Here the ecological conditions are seldom favourable to give expression to all the stages of a complete sere. Thus, each of the types **3**, **4**, **5**, **6**, **7** and **8** broadly correspond to stages passed through in the development of a crevice at location **2**. At stage (i) in the suggested succession, a small chink is established in the slate, and barnacles at once extend into it, together with *Littorina neritoides* and *L. rudis*. *Pygmaea* also takes advantage of the shaded surface overhanging the crevice above, though it normally reaches only just to the typical level of **2**. A comparison of this stage may be made with locations of types **7** and **8**. Wherever *Pygmaea* grows a little detritus lodges deeper to it, so that at (ii) *Lasaea rubra* is able to nestle in a protected niche, with still a few *Littorina neritoides* as before, even in the slightest chink, but generally with a predominance of *L. rudis*. *Scoliocoplanes maritimus* can lodge itself deeply in the extending crack, and *Ligia* shelters here and runs about freely on the exposed surface. Comparison may be made between this stage and the final condition at locations **5** and **6**.

At (iii) the crevice has pushed back a little farther, more organic material has accumulated together with an admixture of fine sand, and *Tanais* becomes established in its galleries to form the zone lying deepest, with, immediately outside it, *Lasaea*, now accompanied by *Cingula* and a few *Leucophytia* (cf. location type **4**). At (iv) the weathering has extended deeper. The *Tanais* zone tends to be pushed back as always to the narrowest, most recently opened parts, though there is sometimes, as in the complete succession, a zone of muddy sand or inorganic weathering products inhabited by deposit-feeding worms whose tentacles extend through gaps in the *Tanais* zone. Farther outwards in the crevice there is now room for members of the zone (C) of Fig. 2, with *Leucophytia*, *Cingula*, *Sphaeroma*, *Naesa* and most numerous of all, *Lasaea rubra*. Still farther out becomes established the innermost lighted zone (B), which is open enough not to be clogged with detritus. Here there is an encrusting mantle of *Spirorbis* and *Ralfsia*, and *Otina* is best developed here as well as extending into zone (C).

At maturity the crevice is established with its full zonation as shown at Fig. 2. Regression of zonation takes place upon further opening up and drying out of the crevice, by the action of greater wave-scouring, and penetra-

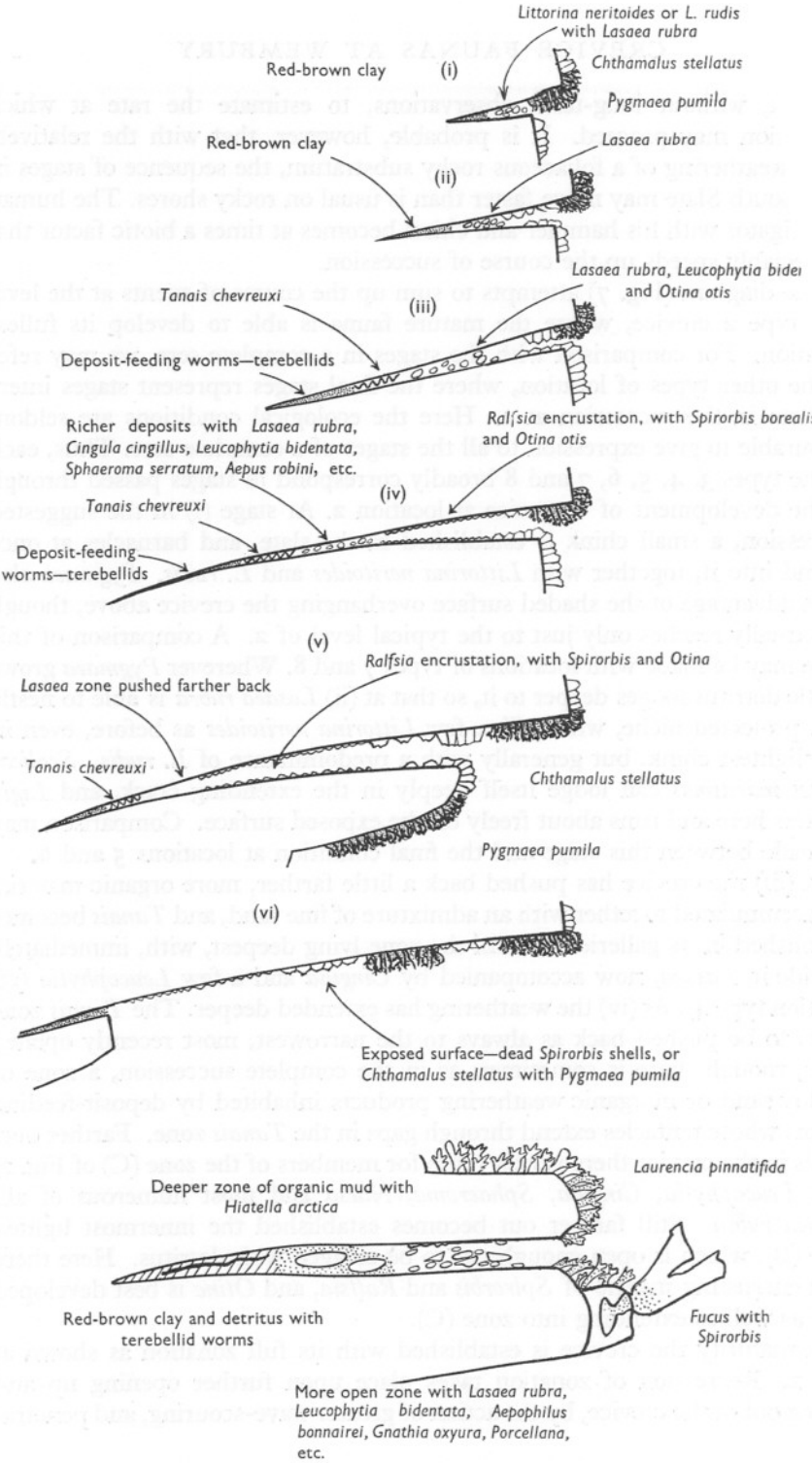


Fig. 7. Scheme to illustrate the course of ecological succession, (i) to (vi), as shown by crevice faunas at a type **2** location at Wembury. The lowest figure shows for comparison a crevice in the fucoid zone (location **1**).

tion of the heat of the sun. The level of zones (B) and (C) is all the time pushed farther back, while at the bottom of the crevice where the slates are almost in contact is a fragmentary or complete zone of *Tanais* (D). Sometimes extending behind the *Tanais*, at times intermixed with (C), is the zone (E) inhabited by deposit-feeding terebellids.

With still greater loosening of the slate (vi) the crevice may become too open to wave-scouring to retain rich deposits, and during exposure to air the humidity of its atmosphere may fall. In such a crevice *Ligia*, *Littorina rudis* and sometimes *Gibbula umbilicalis* of the outer zone are able to push in more deeply, and the zone (C) of *Lasaea* moves towards the bottom of the crevice, with *Leucophytia*, *Otina* and *Tanais* tending to be lost. As a final stage of weathering, the whole lower slate may break away, and the under surface of the topmost slate may then sometimes remain encrusted with dry *Spirorbis* shells, or—at a higher level—acquire barnacles and tufts of *Pygmaea*.

Locations of type 1, as has been pointed out, fit less well into such a system as is outlined above. These crevices have a longer submergence, and are more permanent, with a more stable and less obvious zonation within. The chief ecological feature is the much greater silting-up and the permanent water-logging of the deposit. The communities consist of a narrow zone of *Laurencia*, spreading into the mouth in place of *Pygmaea*, an open silted zone with porcellanid crabs, ophiuroids, etc. (see p. 201), and finally a zone of compacted sand and detritus where *Hiatella* and deposit-feeding worms are typical.

Comparison with other localities

Glynne-Williams & Hobart (1952) discussed the faunas of crevices in green mica schists. Both here and at another locality examined in the Plymouth area, the Dartmouth Slate reef at Whitsand Bay, the mode of foliaceous weathering and the succession and composition of the fauna seem to be essentially the same as at Wembury. Two unexplained local differences at Whitsand Bay are the presence there, alone in the Plymouth area, of *Onchidella celtica*, and the replacement of *Cingula cingillus* by its white variety *rupestris*. At the two Dartmouth Slate localities, the crevices are probably on an average deeper than at Anglesey, which may account for the greater range of horizontal zonation. Five inhabited zones could be recognized, of which the first two probably best correspond to Glynne-Williams & Hobart's 'outer zone', the remainder to their more heavily silted 'inner zone'. Crevice faunas have also been examined at Rum Bay and Jennycliff Bay (Staddon Grits), Drake's Island (igneous intrusion), and Kingsand (felsite). Here, the large blocks of rock develop narrow vertical joint planes, and though the fauna is similar to fragments of the Wembury zonation, the full succession seems to be seldom developed.

The chief feature of the fauna lists for the Plymouth area and for North Wales is the detailed similarity in composition and in the dominant species

represented. Thus for the intertidal fauna, *Lasaea rubra*, *Littorina rudis*, *Cingula cingillus*, *Leucophytia bidentata*, *Ligia oceanica*, *Campecopea hirsuta*, *Sphaeroma serratum*, *Anurida* (*bisetosa* in North Wales, *maritima* here), *Cyrthydrolaelaps hirsutus*, *Bdella interrupta*, *Hydrogamasus salinus*, *Neobisium maritimum*, *Scolicoplanes maritimus* and *Eulalia viridis* are all characteristic of both regions. *Aëpus robini*, *Aëpophilus bonnairei* and *Gnathia oxyuraea* are equally characteristic at a lower level. This list may probably be a familiar one on most British shores of similar formation, and, as remarked by Glynne-Williams & Hobart, the greater number of its species are those not commonly found outside crevices. We find, as it were, a 'crypto-zonation' in crevices, equally characteristic with the zonation on exposed surfaces. With the damping down of differences in wave action, light and evaporation, zonation in crevices may be more uniform over considerable vertical heights than the surface zonation. Nevertheless, with differences in aspect and in depth of crevices, many gradations are brought about, and—in particular, the influence of aspect and topography—makes the concept of 'critical levels' of less value, with its demarcation of zones more strictly in terms of tidal level.

One or two important absences of Plymouth animals from the Anglesey list of crevice fauna may be noted. *Spirorbis* is not stated to play any part in crevice communities, and there is no mention at all of *Littorina neritoides* at higher levels. Its lack of mention is probably due to the smaller emphasis placed by Glynne-Williams & Hobart on the distinctive features of crevices in the upper part of the zonation. *Otina otis* and *Tanais chevreuxi* are evidently entirely absent at Anglesey. *Tanais* seems roughly to be replaced ecologically by *Apseudes talpa*, which was not found at all in crevices at Plymouth.

Otina otis has a rather restricted geographical distribution, being a north-west European and British species only. Jeffreys (1869) gives Normandy, Brest, Quiberon, Piriac and Loire Inférieure for its French distribution, and in England the Devon and Cornish coasts. It extends to south Wales, south and north Eire and Northern Ireland. Moore (1937) records it on the Isle of Man. *Otina otis* is evidently a southern species and its range would seem in Britain to follow fairly closely that of *Chthamalus stellatus* with which it lives in fairly intimate association. It is apparently absent, however, from the north Scottish coast, accompanying *Chthamalus* only as far as the Firth of Clyde. At Carmel Head and North Stack, Anglesey, *Chthamalus* was found by Southward (1951) at its minimal distribution for the Irish Sea, and no *Otina* at all were noticed by Glynne-Williams & Hobart. The range of *Otina* in the English Channel a little exceeds that of *Chthamalus*, and a record by Jeffreys of a dead shell from Northumberland seems to require reinvestigation.

It is possible to suggest a tentative scheme of crevice zonation, based on the Plymouth results, which may with some modifications, as at Anglesey, be found to hold good over a large part of British shores where the nature of the rock mass is suitable to it. The following regions can be defined in terms of

approximate tidal levels, though these are subject to wide variation, and the sequence of zones is always most complete on the shaded overhanging side, and modified as has been shown on the unshaded side.

(i) Above EHWS, where *Littorina neritoides* is dominant and often alone.

(ii) From MHWS to MHWN, where *L. rudis* enters into the zonation with *neritoides* and *Lasaea rubra* become abundant and dominant. *Ligia oceanica* is also highly typical, and associated with *Chthamalus stellatus* appears the marine pulmonate *Otina otis*.

(iii) MHWN to the bottom of the *Chthamalus* zone, where the Littorinas drop out, and a numerous deposit-feeding element appears. *Lasaea rubra* is completely dominant; deposit feeders like *Leucophytia bidentata*, *Cingula cingillus* and *Otina otis* are most abundant here, and *Spirorbis*, *Eulalia viridis*, *Aëpus robini* and *Hydrogamasus* are typical.

(iv) A special habitat at the same level, the tufts of *Pygmaea pumila*, still dominated by *Lasaea rubra* which is accompanied by *Geranomya unicolor* and *Campecopea hirsuta* in vast numbers.

(v) A zone of changed conditions below the *Fucus vesiculosus* upper limit, where *Otina* and *Tanais* disappear and *Cingula cingillus* thins out. There is a continued dominance of *Lasaea rubra*, abundance of *Leucophytia*, *Aëpus* and *Hydrogamasus*, and a new appearance of *Aëpophilus*, *Porcellana*, *Hiatella*, *Gnathia* and many other low-tidal forms.

SUMMARY

This paper presents the results of a study of the animals dwelling in crevices and related habitats on the upper portion of an intertidal reef of Dartmouth Slates at Wembury. The oblique overthrust of the slates towards the north provides a series of habitats on the north and south aspects differing in respect of exposure to the sun and to wave action, and in the depth of the crevice and the composition of its substrate. A series of ten types of microhabitat has been recognized, and an account given of the quantitative distribution and mode of occurrence of the typical species of animals present at each type of location. The nature is discussed of the horizontal zonation within a typical crevice under the most favourable conditions on the shaded side. Fuller ecological notes are provided for several of the ecologically more important species, especially the molluscs *Lasaea rubra*, *Leucophytia bidentata* and *Otina otis*. Of the environmental factors, temperature and relative humidity during the time of exposure have been measured at intervals throughout a single intertidal period. A tentative discussion is given of some of the ecological factors which may be of limiting importance in these habitats, and a scheme is presented of the probable course of ecological succession with the weathering of the rocky substratum and the progressive deepening of the crevice.

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Printed in Great Britain

THE NERVOUS CONTROL OF LUMINESCENT RESPONSES IN POLYNOID WORMS

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(Text-figs. 1–10)

CONTENTS

Introduction

Some preliminary observations on luminescence in polynoid worms have been presented in a previous paper (Nicol, 1953). These animals produce light in scales (elytra) which cover the dorsal surface of the body. The source of the light lies in a layer of unicellular epithelium on the lower surface of the scale. Histologically, this tissue consists of columnar cells (photocytes), characterized by the presence of coarse eosinophilic granules in the cytoplasm. The nervous supply of the elytrum derives from a nerve trunk which ascends the stalk or elytrophore and proceeds to a ganglion in the centre of the scale. From this ganglion nerves radiate peripherally and proceed to the photocytes, and to sensory receptors on the dorsal surface and at the margin of the scale (Bonhomme, 1942).

The normal response of a scale consists of a series of brief flashes, each about 80 msec. in duration, which appear over the entire photogenic area of the scale. The flashes usually start at a frequency of about 9/sec., but the rate soon falls off to about 1/sec., and is sometimes maintained for a minute. In rhythmic flashing of this pattern it is observed that the first few flashes gradually build up to maximal intensity, and then fall off in height. This

progressive decrease in intensity of consecutive flashes is due to gradual exhaustion of luminescent material, and if the response proceeds long enough, the flashes ultimately become very weak, or disappear. When the response stops short of complete exhaustion, progressive fatigue can be produced by repeating the stimulation at suitable intervals.

Owing to the quick and repetitive character of the flashes, it has been possible to secure considerable quantitative data for physiological analysis of the response mechanism, which will be considered in the present paper.

Material and Methods

The luminescent responses of six species of polynoids occurring in the Plymouth fauna have been described previously, namely *Lagisca extenuata*, *Gattyana cirrosa*, *Harmothoë lunulata*, *Polynoë scolopendrina*, *Acholoë asterícola* and *Malmgrenia castanea* (Nicol, 1953). Of these *Acholoë* and *Polynoë* proved most valuable for physiological study and were used extensively in the present investigation. The procedure has already been described. The scales were removed from the animals after narcotizing with magnesium chloride, and were stimulated electrically. Luminescent responses were recorded with a photomultiplier cell, electronic amplifier, and oscilloscope. Other procedures are described under the relevant sections.

Observations

The quick flashes which can be obtained by electrical stimulation of the scale are not affected in magnitude by variations in the strength of the stimulus. In scales which tend to flash rhythmically it has been found that a gradual increase in voltage has no effect until threshold is reached, when the scale starts to flash repeatedly at light intensities quickly rising to a maximum. Since such a response leads to exhaustion, it cannot be repeated in the same scale for the study of the effects of subsequent stimuli. Apparently, however, there is no gradual increment in response according to the strength of stimulus, but rather a maximal outburst when the effective strength is reached.

Although rhythmic flashing is the usual response to a single shock, occasionally an elytrum gives only a single flash to one stimulus. This is observed in elytra which have been stimulated previously by electric shocks and in some way fatigued, and also occurs in some elytra which have not yet been subjected to electrical stimulation. These latter preparations may well be from animals which have been excited during collecting and handling, or in which the elytral ganglion has been injured. It is difficult, however, to avoid contingencies of this kind. Preparations which will give only one flash per stimulus can also be secured by removal of the ganglion. This is described in a later section.

With scales that respond by a single flash to a stimulus it has been observed that once threshold is reached, further increase in stimulus strength brings about no increment in the intensity of the *quick* flash. This result is in agreement with the conclusion previously reached, that the luminescent flashes are produced by nervous stimulation, for excitation of the efferent nerve fibres is an all-or-nothing phenomenon.

When the stimulus strength is raised greatly, or the pulse duration is increased, a second type of response is obtained. This takes the form of a bright, prolonged glow, which quickly rises to a peak in about $\frac{1}{2}$ sec., and lasts up to 10 sec. (Fig. 1A). Such a response frequently leads to complete exhaustion of the photogenic material contained in the scale, and subsequent stimuli are ineffective. In some preparations a pattern of rhythmic flashing is superimposed on the bright glow, or can be induced by repetitive stimulation (Fig. 1B). It is concluded that in this type of response, partial or complete depolarization of the photocytes is achieved by direct stimulation of the latter, and that the effect of nervous impulses may intervene and influence the primary response. When scales which are being strongly stimulated are watched under the microscope it can be seen that the entire photogenic area lights up with a bright prolonged glow, or part of it does so, and rhythmic flashes proceed peripherally from a central glowing region. In the latter event only part of the photogenic area has been excited directly, and nervous stimulation is affecting the remainder of the photogenic field. For experimental purposes the strength of stimulation has been kept at a minimum in order to avoid direct excitation of the luminescent cells, but a certain amount of direct depolarization appears in a few of the records.

The Effect of Repetitive Stimulation

By using scales which respond initially by a single flash to a shock it is possible to analyse the effects of repetitive stimulation.

Single flash responses, one per stimulus, are illustrated in Fig. 1C–E (*Acholoë*). The experiment recorded in Fig. 1C, D will be considered in detail. It will be noticed that the overt responses to the first two stimuli (time interval 6 sec.) are barely perceptible (direct depolarization), but the response to the third stimulus is a definite flash. Continued stimulation, at a rate of 45/min. (interval 1·3 sec.) resulted in discrete flashes, one per stimulus, but after the fourth stimulus double flashes appeared, the beginning of repetitive flashing. All subsequent responses to a single stimulus consisted of several flashes. The responses also became much smaller with repetition, as the result of fatigue. A somewhat similar pattern is shown in Fig. 1E. Here the response to the first stimulus was small (direct depolarization), the next six responses increased gradually in height, and occasionally there was a tendency for repetitive flashing.

The increase in height of successive responses with repetitive stimulation

is a noteworthy feature of these records and is documented in Figs. 1 C–E and 3 D and in the accompanying graph (Fig. 2), based on a photographic record.

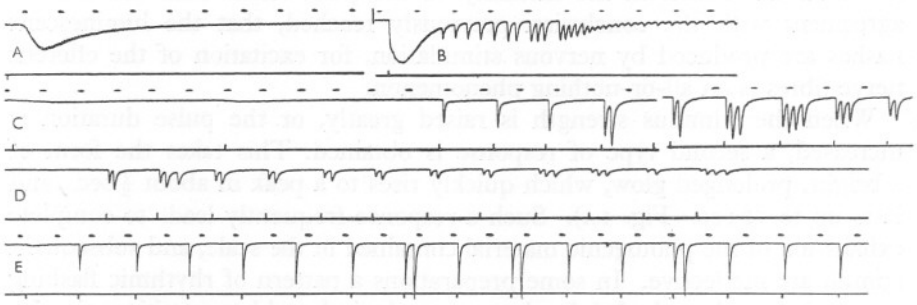

Fig. 1. A, protracted luminescent responses (elytrum of *Lagisca extenuata*), resulting from direct stimulation of the luminescent cells. Time scale, 1/sec. Effective stimulus, lower left. B, protracted luminescent response (elytrum of *Acholoë astericola*), on which are imposed rhythmic flashes after a delay of 1·8 msec. Time scale, 1/sec. Effective stimulus, lower left. C, D, single flashes and rhythmic flashing induced in an elytrum of *Acholoë* by single shocks and repetitive stimulation. Time scale above, 1/sec. Stimuli indicated on lower line. E, luminescent responses in an elytrum of *Acholoë*. Single flashes and repeated flashes induced by repeated shocks at a slow rate of 42/min. Time scale 1/sec. Stimuli shown on lower line.

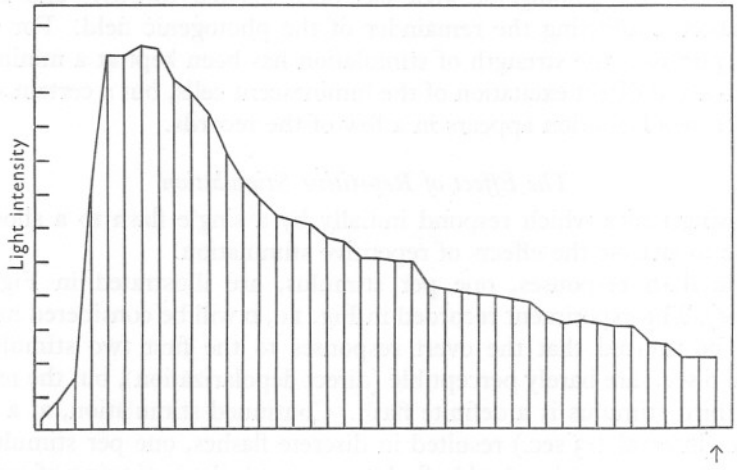

Fig. 2. A plot showing the change in intensity of successive responses in an elytrum of *Acholoë* stimulated by a burst of impulses at a frequency of 5/sec. Ordinates, light intensities in arbitrary units. Each vertical line represents a separate light flash. Arrow indicates end of stimulation.

Three factors are revealed in these records: (i) the response to the first stimulus may be small, but subsequent stimuli will evoke bright flashes; (ii) repetitive stimulation brings about an increase in the magnitude of

successive flashes; (iii) finally, repetitive stimulation will evoke the onset of rhythmic flashes or flickering.

The effects obtained with repetitive stimulation can be compared advantageously with the course of events in rhythmic flashes evoked by a single stimulus. In the latter form of response it is also observed that the initial flashes increase in intensity (Nicol, 1953). Occasionally, when a stimulus produces only a single flash, two or more stimuli will start the scale flashing, again with progressive increment in light intensity (Fig. 3A–C, *Lagisca* and *Harmothoë*). Changes in the frequency of rhythmical flashing and the intensity of the flashes, however, are distinct phenomena, although the former is capable of influencing the latter.

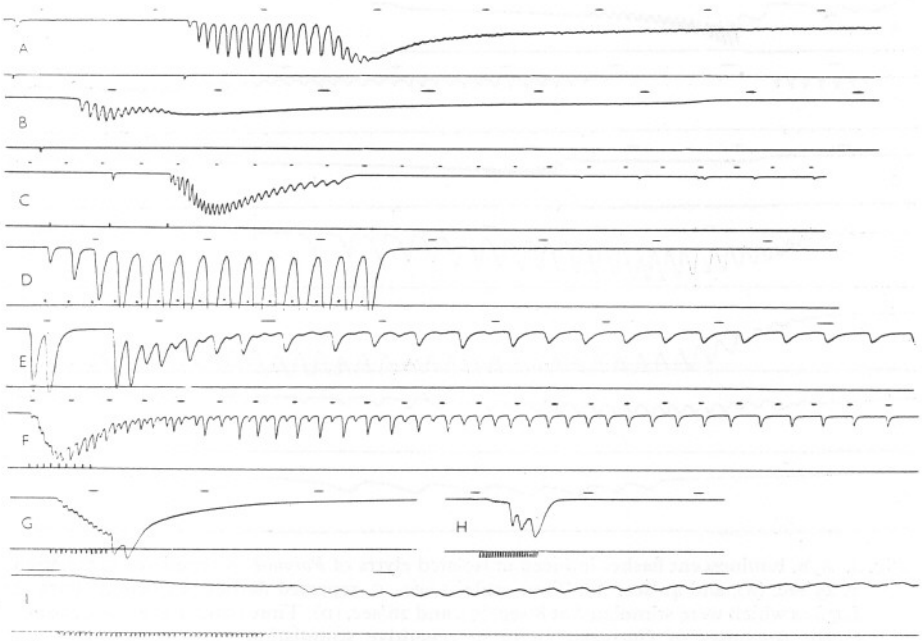

Fig. 3. A, luminescent responses of an elytrum of *Lagisca*, a rhythmic flashing induced by two consecutive impulses at an interval of 1·5 sec. B, flashing induced by a single stimulus. Time scale 1/sec. Stimuli shown on bottom line. The response in these records terminates in a prolonged glow. C, luminescent responses of an elytrum of *Harmothoë lunulata*. Repetitive flashing induced by three consecutive stimuli at a frequency of 42/min. Time scale, 1/sec. Stimuli shown on bottom line. D–I, luminescent responses of elytra of *Acholoë*, stimulated by series of impulses at different frequencies. Time scale above each record, 1/sec. Stimuli shown on lower line. Frequencies of stimulation are: D, E, 6/sec., E is a continuation of record D, and shows delayed rhythmic discharge; F, 6/sec.; G, 24/sec.; H, 36/sec.; I, 70/sec.

Summation of Luminescent Responses

When elytra are stimulated at low frequencies, below 7/sec., separate and distinct flashes are obtained (Figs. 1E, 3D, F). At frequencies somewhat

greater than this the separate flashes begin to fuse together, and the degree of fusion becomes more and more pronounced at higher frequencies. In *Acholoë* the separate flashes are still perceptible at frequencies of about 25/sec. (Fig. 3G), but are practically erased at frequencies reaching 40/sec. or more (Fig. 3H, I). Similar results were obtained by stimulating isolated scales of *Polynoë* (Fig. 4A, B), *Lagisca* (Fig. 4C, D), and *Gattyana* (Fig. 4E–H). Since the time taken to reach maximal intensity in a single flash is in the region of

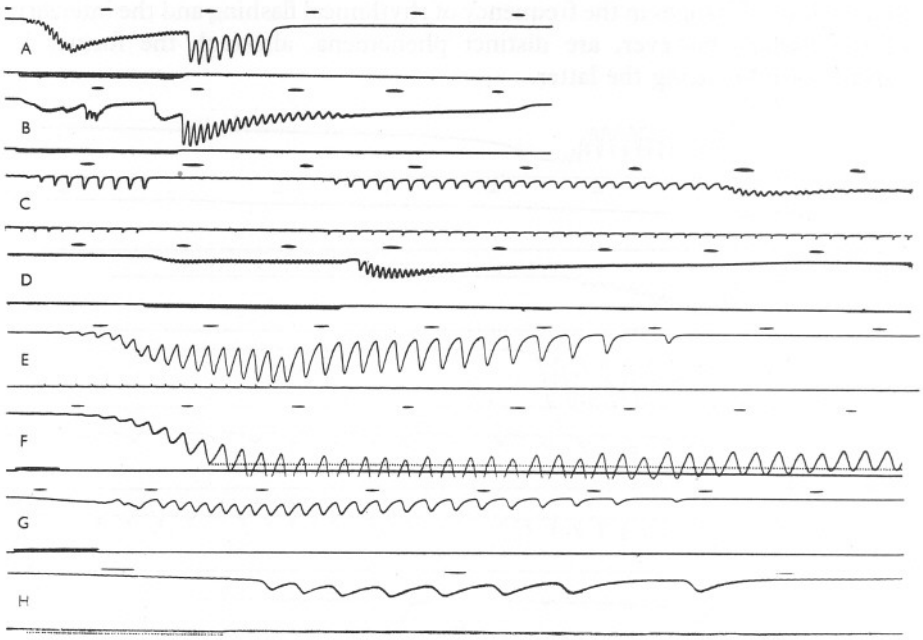

Fig. 4. A, B, luminescent flashes induced in isolated elytra of *Polynoë* by repetitive stimulation at 25/sec. (A), and 42/sec. (B). Time scale, 1/sec. C, repeated flashes in isolated elytra of *Lagisca* which were stimulated at 8/sec. (C), and 26/sec. (D). Time scale, 1/sec. E–H, luminescent responses of *Gattyana* elytra to repetitive stimulation. E, 8/sec.; F, 38/sec.; G, 66/sec.; H, 120/sec. Time scale above each record, 1/sec.

40 msec. in *Acholoë*, it would be expected that a frequency of 25 or more impulses/sec. would be needed to bring about complete fusion of the separate responses.

These results, which were produced by repetitive stimulation, again can be compared with records of protracted rhythmical flashing induced by one or a few stimuli. When rhythmic flashing is occurring at a slow rate (up to about 5/sec.), the separate responses are discrete and each one returns to base-line (zero intensity) (Fig. 3A, B). When rhythmic flashing is taking place at higher rates than this, the separate flashes summate to various degrees, depending upon the intervals between consecutive flashes. For example, in

Fig. 3C (*Harmothoë*), rhythmic flashing shows a frequency of 9/sec. (interval 100 msec.), and there is a progressive build-up of intensity. Scales rarely flash rhythmically at a faster rate than this, which is insufficient to produce complete fusion.

If the frequency is gradually raised during a period of stimulation, the rate of flashing closely follows the rate of stimulation (Fig. 5A).

At high rates of stimulation the luminescent responses show irregularities in both frequency and amplitude. When the frequency is greater than 35/sec., the scale may not respond to every stimulus, but may begin flashing at some rate slower than the frequency of stimulation. In Fig. 5B (interval 21 msec.),

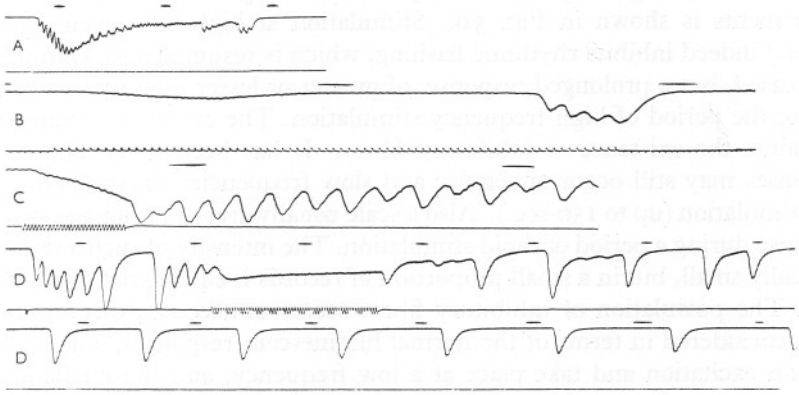

Fig. 5. A, increasing the frequency during a period of stimulation of the scale of *Acholoë*. The frequency of stimulation rises from an initial level of 28/sec. to 42/sec., and the flashes closely follow the rate of stimulation. Time scale above, 1/sec. B, repetitive stimulation (47/sec.) of an elytrum of *Acholoë*. There is initially a small smooth response, lasting 0·8 sec., followed by 4 flashes at a slow rate independent of the rate of stimulation. Time marks above, 1 sec. intervals. C, stimulation of an elytrum of *Acholoë* by alternating current at a frequency of 100 cycles/sec. During stimulation the luminescent response shows a slow rise, followed by rhythmic flashing at a slow rate (10/sec.) once electrical stimulation has ceased. Time marks above, 1 sec. interval. D, E, the effect of high-frequency stimulation on rhythmic flashing in an isolated elytrum of *Acholoë*. Rhythmic flashing was induced by a single impulse, and the scale was then stimulated by a burst of a.c. lasting 1·6 sec. at a frequency of 50 cyc./sec. Time scale above, 1/sec.

for example, the scale shows initially a small smooth response, but ultimately begins flashing at a slow rate, much below the frequency of stimulation. During stimulation the scale responds either by discrete flashes, or a prolonged glow, according to the frequency, but once the stimulation ceases, the scale may continue to respond by rhythmical flashes at a slow frequency (Figs. 3G, I, 4A, B, E–H). That is, once the excitation at a fast rate is removed the now excited scale continues to respond normally at its own characteristic frequency. A third noteworthy feature is that the amplitude of the luminescent response is often small at higher frequencies of stimulation (either definite flashes or smooth response curve). Once stimulation has ceased, however, the

continued rhythmic responses of the scale often proceed at a much greater amplitude (Figs. 3G, 4A, B, F–H).

Several possible explanations can be advanced for these observations. The first is that stimulation at high frequencies produces polarization in the tissues, and is responsible for the small prolonged responses. This can be discounted since a.c. stimulation produces the same effects as condenser shocks (Fig. 5C). The second explanation is that the photocytes are supplied both by excitatory and inhibitory fibres, and that the effect of the latter predominates at high frequencies. This has been investigated by stimulating elytra into luminescence and then subjecting them to a short bursts of a.c. or condenser shocks at high frequencies during the period of rhythmic flashing. An example of these experiments is shown in Fig. 5D. Stimulation at high frequencies (above 50/sec.) indeed inhibits rhythmic flashing, which is resumed once stimulation has ceased, but a prolonged response, of greater or lesser intensity, continues during the period of high-frequency stimulation. The evidence accumulated is against the existence of inhibitory fibres. It has been noted above that responses may still occur at regular and slow frequencies during periods of fast stimulation (up to 150/sec.). Also a scale usually gives a prolonged smooth response during a period of rapid stimulation. The intensity of such a response is usually small, but in a small proportion of records it equals that of a normal flash. The postulation of inhibitory fibres seems unnecessarily complicated when considered in terms of the normal luminescent responses, which occur only on excitation and take place at a low frequency, and the mediation of such responses is probably effected solely by impulses in excitatory nerve fibres.

It is probable that these results can be best explained on the basis of a refractory period, either of the efferent nerve fibres, or of the photocytes. The latent period of the luminescent response is of the order of 31–21 msec. in *Polynoë* (Nicol, 1953), which includes the refractory period of the nerves. The refractory period of the luminescent response has been measured directly by arranging a Keith Lucas spring rheotome to deliver paired condenser shocks at suitable intervals. With this technique it has been determined that the refractory period lies between 9 and 16 msec., and a second impulse, falling within this interval, is ineffective. These figures give upper limits of 62–110 stimuli/sec. at which nervous stimulation would still be effective. At high frequencies, when successive impulses fall within the refractory period of the nerve, it would be expected that every second or third or subsequent stimulus would be effective, but such form of response has only occasionally been recorded, and that irregularly (Figs. 3H, 5B). Slight protracted responses (faint glow) have been obtained at frequencies as high as 150 pulses/sec., possibly resulting from some slight direct excitation of the photocytes, but actually the luminescent flashes usually show pronounced reduction in amplitude at frequencies from 20 to 40/sec. A peculiar feature is that even the

response to the first stimulus, at high frequencies, is minute. It appears, therefore, that some terminal mechanism is incapable of responding fully or is blocked at high frequencies, and this effect may occur either at the nervous terminals, or in the photocytes themselves. Without a new approach, and additional data, the problem cannot be pursued further.

Interpretation of the Increment in Light Intensity

It has been ascertained that an increase in light intensity occurs during repetitive stimulation or during spontaneously rhythmical flashing. During prolonged stimulation and rhythmic flickering, however, the individual flashes gradually decrease in intensity and this is ascribed in part to fatigue of luminescent ability. The question is now posed whether the initial increase in the intensity of successive light flashes is due to summation of effector responses, or to some elevation in the level of excitation which in turn controls the level of the effector response.

It has been noted above that under rapid stimulation, or during rapid rhythmic flashing, the separate responses may follow each other so quickly that a new flash may begin before the previous flash is extinguished and recovery of that flash is completed (e.g. Figs. 3C and 4A). If the successive responses were of equal magnitude and each response were superimposed on the preceding one before the latter had time to return to zero, then a gradual build-up in light intensity should result. A similar result should obtain even if successive responses are of decreasing amplitude, so long as each falls during the rising phase of the inflexion or the early period of decay of the preceding response curve. The arithmetic relations are sufficiently obvious not to need further elaboration. This will partly explain the progressive increment in light intensity which can be seen in many of the records.

In these records of repeated responses, however, it is apparent that another factor is operating in addition to summation of the light intensities of separate flashes. Often the response to the first stimulus is very small, and subsequent responses are many times greater than the initial response, and increase rapidly in magnitude. Also, the peaks of the first few responses may show a stepwise increase in intensity which is too great to be due entirely to partial summation of individual responses. When the light intensities of the first few responses are measured from the beginning of the inflexion to the peak of each flash, it is frequently found that a given response is greater than the one immediately preceding it.

When the responses are occurring at a slow rate (either rhythmic flashing, or as the result of continued stimulation at a low frequency), separate flashes may be observed, each of which decays to zero before another begins (Figs. 1C, D, 3A, B, D, E). Under these conditions, as previously indicated, the initial few responses increase successively in height.

The relative amount of increment shown by successive responses varies

considerably from specimen to specimen, and of course changes in the same specimen according to its physiological condition and the rate of flashing. When the initial response is large, the following responses increase gradually and progressively in height, but when the initial response is minute, then the increase of intensity in the succeeding first few responses is very striking (Figs. 1C, D; 3A, B, D, E; 4E).

It is concluded from these results that each stimulus establishes a protracted state of facilitation, and, when the successive stimuli are arriving at suitable intervals, the corresponding facilitatory states are additive and heightened excitation results. This in turn controls the intensity of the response up to a certain limit determined by the physico-chemical conditions of the luminescent reaction. The effective duration of the facilitatory state is remarkably long, and it has been possible to characterize it as follows.

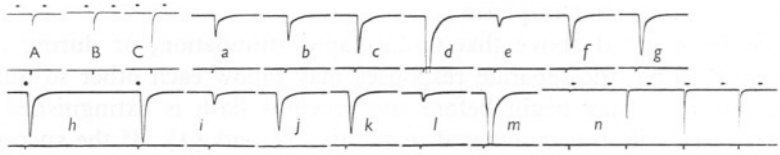

Fig. 6. A–C, single flashes from an elytrum of *Polynoë* stimulated at various intervals. Stimuli, single condenser shocks. A, initial flash; B, second flash after an interval of 4 min; C, third flash after an interval of 3 min. Time scale, 1/sec. *a–n*, the effect of paired stimuli delivered at various intervals in scales of *Polynoë* which responded by giving a single flash per stimulus. Frames *a* to *m* from a single scale; *n* from another scale. Time intervals between stimuli in consecutive frames as follows: *a–b*, 2 min.; *b–c*, 1 min.; *c–d*, 30 sec.; *d–e*, 2 min.; *e–f*, 15 sec.; *f–g*, 15 sec.; *g–h*, 15 sec.; *h–i*, 2 min.; *i–j*, 10 sec.; *j–k*, 10 sec.; *k–l*, 2 min.; *l–m*, 1 min. The two stimuli in *h* were separated by an interval of 1 sec.; the consecutive stimuli in *n* were separated by intervals of 1·5 sec. Time scale, 1 sec., for frames *a* to *m* shown in frame *h*; time scale for frame *n*, 1/sec., above.

Duration of the Facilitatory State of the Luminescent Response

In a previous communication (Nicol, 1953, fig. 12) it has been shown that the effect of a previous stimulus, as revealed in the increase of the light intensity of a succeeding flash, may last as long as 6 sec. in *Acholoë*. A detailed study of this phenomenon is documented in Fig. 6 from records of single flashes in *Polynoë scolopendrina*. In a fresh preparation subjected to repeated stimuli at various intervals, the effect of the first of two stimuli lasts as long as 4 min. (Fig. 6A–C). After this interval, a second stimulus gives a measurably larger response, amounting to an increment of 33% (Fig. 6B). When a scale has been repeatedly stimulated and fatigue sets in, the response elicited after several minutes shows no increment, or may even be slightly less than a previous flash (cf. *a*, *b*, and *d*, *e*, in Fig. 6). This could be due to fatigue in the system of neuro-effector transmission, but it is simpler to regard it, tentatively, as resulting from progressive exhaustion of luminescent material. The assumption is made that the intensity of a response is a function of the

level of excitation and the quantity of luminescent material available in the photocytes. Since the residual facilitation after a protracted interval is small, the heightened response which it would be expected to provoke is cancelled by the concomitant decrease in luminescent material available for the manifestation of the response.

As the interval between two consecutive impulses is shortened, the second response increases relative to the first response in magnitude (Fig. 6, *b–k*). This is shown with special clarity in frame *n* (stimulation interval, 1·5 sec.). In the pair of records shown in frames *l* and *m*, the second stimulus set off a double flash, with an interval of about 30 msec. between the two components, and with this interval the second peak of the double flash showed an

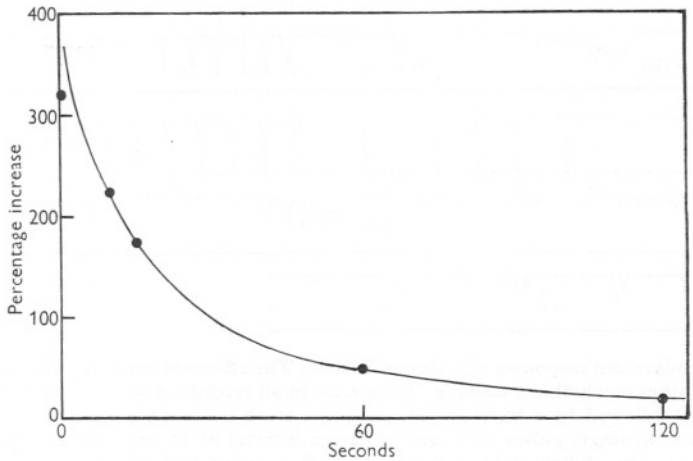

Fig. 7. A curve based on measurements of the light intensity produced by the second of a pair of flashes in a series in which the paired stimuli were delivered at varying intervals. Percentage increment in the intensity of the second response over the first is plotted against time between the two stimuli (sec.) Elytrum of *Polynoë*.

increment of over 300%. Data from a scale have been plotted in Fig. 7, which shows that the excitatory effect of a previous stimulus decreases with time, and that the rate of decay of facilitation or excitation follows an exponential curve. If the material basis of this facilitation be thought of as a substance released or liberated at or in the effector cells by the arrival of a nervous impulse, then the curve of decay of facilitation shown in Fig. 7 suggests a simple monomolecular reaction effecting the removal of the substance concerned and converting it into an inactive form. On the same basis, the increment of excitation in successive responses, at brief intervals, as recorded by the rise in intensity of sequential flashes, would be due to gradual accumulation of an excitatory or complementary substance at a rate faster than it could be removed.

Rhythmic Flashing

In very excitable preparations a single shock initiates prolonged rhythmic flashing (see Nicol, 1953, for records of typical responses). Other scales give only a single flash per stimulus, and there are intermediate cases in which a single stimulus gives rise to a few flashes, or which require a number of impulses to start rhythmic flashing (Figs. 1C, D; 3A, B, C).

From an examination of the many records collected from different animals, it became apparent that the onset and duration of rhythmic flashing are dependent on the previous history and the excitatory state of the scale. It also appeared that rhythmic flashing shows a condition of progressive fatigue akin to that revealed in diminution of intensity during the course of repeated flashing. An attempt has been made to analyse the factors involved as follows.

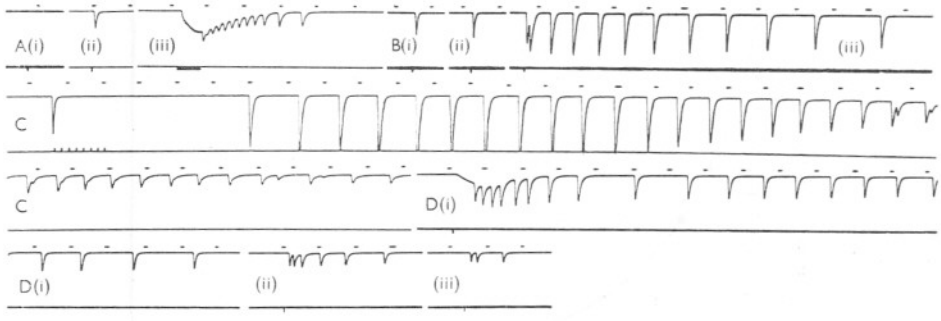

Fig. 8. Luminescent responses of scales of *Acholoë*. The effects of single stimuli and repeated stimulation on rhythmic flashing. Time scale in all records, 1/sec. A, single pulses in (i) and (ii) followed by a burst in (iii) (0·75 sec. at 22/sec.). 12 sec. between (i)–(ii), and (ii)–(iii). B, single pulses in (i) and (ii) at an interval of 10 sec., showing increment in light intensity, followed by a third pulse after an interval of 1·6 sec. in (iii), which resulted in prolonged rhythmic flashing. C, rhythmic flashing induced by a burst of impulses, with a pause of 5 sec. between the first and succeeding flashes. D, reduction in the number of flashes in consecutive periods of response. Stimulation, a single pulse in each of frames (i), (ii) and (iii).

In preparations which respond by rhythmic flashes, there are sometimes long intervals, extending to 15 sec., between consecutive flashes or groups of flashes (Figs. 3D, E; 8C). In these cases the scale suddenly starts to flash rhythmically, often at a fast rate, several seconds after the response apparently has ceased. This indicates that the residual excitatory condition connected with the rhythmicity of the response can last for surprisingly long periods.

When a single pulse fails to evoke rhythmic flashing, several pulses or a burst of pulses will frequently do so. The excitatory condition responsible for rhythmic discharge, consequently, can be influenced by repetitive stimulation. The level of underlying excitation is augmented by repeated stimulation, resulting in an increase in the *number* of rhythmic flashes which occur after the stimulus or period of stimulation has terminated (Figs. 1C, D; 8A, B).

In an attempt to determine how long a stimulus will continue to influence the onset of rhythmic flashing, suitable scales were stimulated by pulses separated by intervals extending up to 2 min. Rhythmic flashing was evoked by stimulating at frequencies as slow as 1 per 12 sec. As noted above, rhythmic flashing has appeared spontaneously after quiescent periods of up to 15 sec. after a previous display has subsided. My data, therefore, set a limit of 12–15 sec. for maximal duration of the effective excitatory state governing the onset of rhythmic flashing.

Finally, it has been observed that when a scale is stimulated on successive occasions into rhythmic flashing, each successive period of rhythmic flashing is of shorter duration, until eventually only one flash results from a stimulus, and a burst of impulses is then necessary to evoke a rhythmic discharge. A gradual fatigue or diminution of excitatory potentiality thus takes place with repetition, and this is manifested as a reduction in the number of flashes resulting from a single stimulus or given number of stimuli (Fig. 8D).

The presence of a ganglion in the elytrum suggested that this structure might be responsible for the rhythmic character of the flashing observed in severed scales. Experiments to test this hypothesis were carried out as follows. Elytra were narcotized with $MgCl_2$, and were cut into two under a dissecting microscope in such a way that one half of each scale contained the elytrophore stalk and ganglion, and the other lacked this structure. The preparations were then washed out in sea water and the two halves of each scale were stimulated separately. Specimens of *Acholoë* and *Polynoë* were used in these experiments. With a long series of bisected scales it was observed that half scales containing a ganglion responded to a single shock by a series of rhythmic flashes or a single flash, whereas half scales lacking a ganglion never gave rhythmic flash responses (Fig. 9A). It is concluded that the elytral ganglion is involved in mediating the rhythmical flashing which is the characteristic response of normal elytra.

The occurrence of continued and rhythmic flashing long after the effective stimulus (tactile or electrical) has ceased denotes the existence of some repetitive excitatory mechanism governing its evocation. From several lines of evidence it is possible to cull the more likely explanations of this phenomenon, and to outline its main features. This evidence points towards repetitive discharge in nerve cells as the responsible factor involved.

Rhythmic flashing is not due to the recurrence of some excitatory condition at or in the peripheral effector cells for the following reasons. In the majority of records the flashes show great regularity in their timing and characteristics, each flash consisting of a smooth 'unimodal' curve, and subsequent flashes appearing at regular intervals. It seems highly improbable that a mass of isolated units (glandular cells) could maintain a rhythm of cyclical activity, as the result of an initial triggering stimulus, so as to produce such a series of

discrete and uniform synchronous responses. The possibility of the light flash from one cell or group of cells affecting the others in turn, and so cyclically, is ruled out by the fact that the luminescent cells are not sensitive to illumination.

The crucial experiment consists of recording from fragments of elytra. As reported in the previous section, scale fragments lacking the ganglion give only a single flash to each electrical stimulus, whereas fragments containing the elytral ganglion flash rhythmically when subjected to a single stimulus. This ganglion lies in the centre of the elytrum and contains a number of nerve cells. It receives a nerve trunk from the elytrophore and gives rise to nerve fibres which radiate out over the elytrum and proceed to the photocytes.

Continued flashing might be due to repetitive firing in nerve fibres supplying the luminescent cells, but the fact that a fragment of a scale lacking the ganglion, but still retaining its peripheral nerve fibres, fails to flash repetitively, rules this out. The evidence thus implicates the ganglion cells in the control of rhythmic flashing.

Without some more direct method of recording, the exact mechanism must remain a subject for speculation. The rhythmic flashing may be due to regular oscillations in the excitatory state of nerve cells lying in the elytral ganglion. According to this hypothesis the excitatory oscillations would be initiated by some stimulus (electrical, nervous, mechanical), and nerve impulses would be generated in the efferent fibres at the excitatory peaks of the cycle. A second hypothesis assumes the existence of oscillatory nervous arcs whereby nervous impulses can continue firing in closed circumscribed circuits within the ganglion, and in this central locus each circular sweep of impulses fires the efferent nerve trunks. The process involves a very interesting instance of rhythmic nervous activity in a peripheral ganglion at a low phyletic level, and is worthy of further investigation.

The flashes often appear uniform enough to represent synchronous or nearly synchronous activation of all the photogenic cells in the scale. Occasionally there is a slight wobble in the response spikes which may represent slight differences in the timing of nervous impulses arriving in different regions of the scale. A uniform and regular pattern of flashing is the usual form of response in the scales of all species, but occasionally there is asymmetry in the pattern of rhythmic flashing. Sometimes there are alternate periods of strong and weak flashes, at other times the pattern is more irregular, with variable periods between spikes of different amplitude (Figs. 3D, E; 9B). It has been suggested previously that these asymmetrical responses are due to the separate activity of two or more neuro-effector units firing at different rates and acting out of phase, so that responses at one time are distinctly separable, and at others partially or wholly summated (Nicol, 1953).

The Effect of Drugs on the Luminescent Response

Drug action has been explored in a number of luminescent species, and in view of the peculiar conditions of nervous regulation obtaining in the luminescent responses of polynoids, it seemed desirable to determine whether the action of certain drugs would throw any light on the processes involved. The following drugs were tested: adrenaline, acetylcholine, nicotine, eserine, atropine, curare, and strychnine. These had little or no effect on the luminescent responses of isolated scales, as the following summaries show.

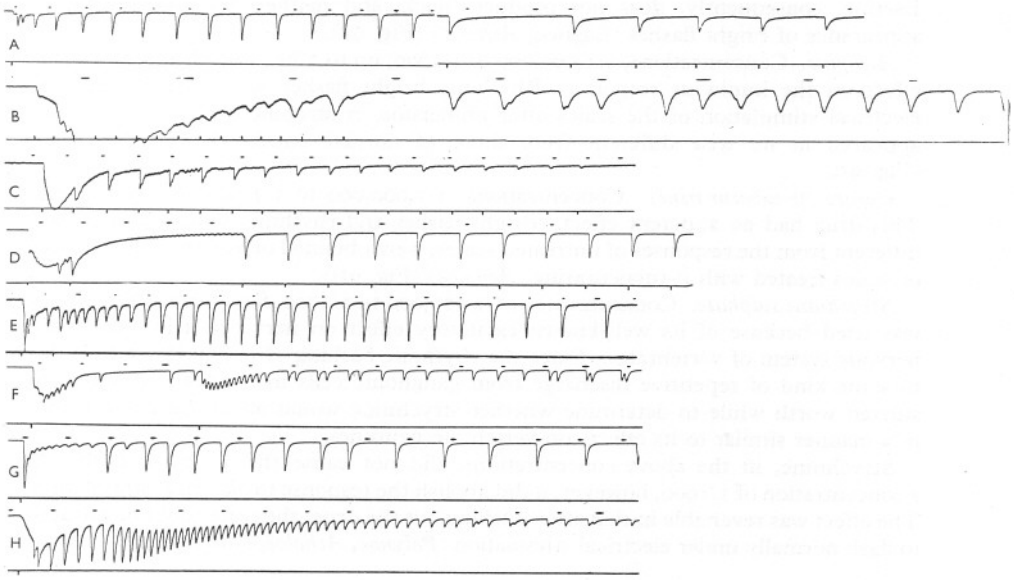

Fig. 9. A, stimulation of half scales of *Acholoë*. Left, rhythmic flashes in part of an elytrum containing a ganglion. Stimulation consisted of a single pulse. Right, single flashes, one per stimulus in the other portion of the same elytrum. Time scale, 1/sec. B, rhythmic flashing induced in an elytrum of *Acholoë* by a burst of impulses. Time scale, 1/sec. C–H, effects of drugs on the luminescent responses of isolated scales of *Acholoë*. Scales were immersed in solutions of the drugs for the times stated and then subjected to electrical stimulation (single or repeated shocks). Time scale, 1/sec. C, adrenaline 1/10,000, 20 min.; D, acetylcholine 1/10,000, 120 min.; E, nicotine 1/100,000, 70 min.; F, eserine 1/100,000, 50 min.; G, atropine 1/100,000, 3 hr.; H, D-tubocurarine, 1/10,000, 30 min.

Adrenaline. Concentrations, 1/1,000,000 to 1/10,000 for 30–60 min. This drug failed to excite isolated scales, and when treated scales were stimulated electrically, they still responded rhythmically and gave bright flashes very similar to those produced by normal untreated preparations (*Acholoë*) (Fig. 9C).

Acetylcholine. Concentrations, 1/1,000,000 to 1/1,000 for $\frac{1}{2}$–$2\frac{1}{2}$ hr. Solutions of the highest concentrations were adjusted to pH 8·2 with sodium acetate and sodium hydroxide. This drug failed to induce luminescence in isolated scales. At concentrations of 1/1,000,000 to 1/10,000, scales still gave bright flashes and repetitive discharges when subjected to electrical stimulation, and the response recorded appeared normal

when compared with those of untreated scales. At a concentration of 1/1000, some scales failed to respond, or gave reduced responses (*Acholoë*, *Polynoë*) (Fig. 9D).

Nicotine. Concentrations of 1/1,000,000 to 1/1000 for 30–75 min. Scales did not flash when placed in solutions of nicotine. Electrical stimulation of treated scales produced responses, single bright flashes and rhythmic flashes, entirely similar to those of untreated scales (*Acholoë*, *Lagisca*) (Fig. 9E).

Eserine. Concentrations, 1/1,000,000 to 1/10,000 for 30–60 min. Scales placed in solutions of eserine did not luminesce and electrical stimulation was employed to determine whether the drug had any effect on the characteristics of the response. At all strengths the scales still responded with bright flashes and rhythmic discharges. Eserine, consequently, does not produce block, and neither does it prevent the appearance of bright flashes (*Lagisca*, *Acholoë*) (Fig. 9F).

Atropine. Concentrations, 1/1,000,000 to 1/1000, up to 3 hr. This drug was without effect on the luminous response. Rhythmic bright flashes were still obtained on electrical stimulation of the scales after immersion in atropine solutions, and these appeared in no wise different from those of normal untreated scales (*Acholoë*) (Fig. 9G).

Curare (D-*tubocurarine*). Concentrations, 1/1,000,000 to 1/10,000 for 30–60 min. This drug had no apparent effect. Bright flashes and rhythmic flashing, in no way different from the responses of untreated scales, were obtained on electrical stimulation of scales treated with D-tubocurarine (*Acholoë*) (Fig. 9H).

Strychnine sulphate. Concentrations, 1/1,000,000 to 1/1000, for 30 min. Strychnine was tried because of its well-known excitatory effect on nerve cells in the central nervous system of vertebrates. Since the rhythmic flashing of polynoid scales is due to some kind of repetitive discharge from ganglionic cells in the scale, it was considered worth while to determine whether strychnine would affect these nerve cells in a manner similar to its effects on vertebrate neurones.

Strychnine, in the above concentrations, did not cause the scales to flash. At a concentration of 1/1000, however, it did abolish the response to electrical stimulation. The effect was reversible in that after washing out the drug, the scale could be induced to flash normally under electrical stimulation (*Polynoë*, *Acholoë*, *Gattyana*).

It is noteworthy that none of these drugs, with the exception of strychnine, was effective in abolishing the luminescent flashes which can be induced by electrical stimulation. Since the quick flash-response is due to nervous excitation, it seems reasonably certain that atropine and curare are unable to block neuro-glandular transmission in the scales of these animals. Neither acetylcholine nor adrenaline was effective in inducing a luminous response in the scales, and this would appear to exclude these substances as excitatory agents. Adrenaline is ruled out as an inhibitory agent since normal luminescent responses can still be elicited after its application; and there is no evidence that acetylcholine and eserine had any pronounced depolarizing action, in view of the quick flashes which attended electrical stimulation of scales which were treated with these substances. Strychnine, in high concentrations, is known to block axon conduction, and in the present experiments its inhibitory effect was probably achieved through this route (Heinbecker & Bartley, 1939; Coppée & Coppée-Bolly, 1941).

The effect of pharmacological agents on luminescent tissues has been

explored to only a very limited extent, and comparative data are fragmentary. Adrenaline fails to elicit luminescence in the polychaete *Chaetopterus* (Nicol, 1952*a*), and in the decapod crustacean *Systellaspis* (Harvey, 1952). It apparently decreases the sensitivity to mechanical stimulation in the ctenophore *Mnemiopsis* (Chace, 1941). There is no evidence from any other source that adrenaline is normally produced in these animals, and this may be correlated with the absence of any pronounced physiological action. Chromaffine cells (believed to secrete adrenaline) have been described in the Aphroditidae (in *Aphrodite aculeata*), however, but the present evidence shows that adrenaline is not involved in the luminescent responses of polynoids (Gaskell, 1914). In contrast, adrenaline forms a powerful stimulant to light production in fire-flies (Coleoptera). The action appears to be an indirect one, on the tracheae and cells, and not direct on the luminescent cells. In consequence, the tracheoles are dilated and the photogenic cells receive a greater supply of oxygen, which in turn regulates the oxidative luminescent process and the intensity of light emitted. Since adrenaline, so far as known, does not occur naturally in insects, this effect appears to depend on fortuitous sensitivity to adrenaline (Creighton, 1926; Emerson & Emerson, 1941).

Quite different is the situation in teleosts where adrenaline is normally produced by suprarenal tissue, and shows a sympatheticomimetic effect on many visceral activities (Nicol, 1952*c*). Injections of adrenaline into *Porichthys notatus* and *Echiostoma ctenobarba*, two species of teleosts bearing photophores, cause them to luminesce (Greene & Greene, 1924; Harvey, 1931, 1952). This is suggestive evidence that adrenaline, which is a blood-borne hormone, may normally be involved as a chemical mediator as well in the luminescent response of these species. It is not unlikely that the serially arranged photophores in the head and trunk of teleosts are innervated by post-ganglionic neurones of the sympathetic nervous system, via recurrent grey rami, and cranial and spinal nerves. Branches of these nerves are known to innervate photophores in *Argyropelecus* and *Lampanyctus*, but fibre-pathways have not been worked out (Handrick, 1901; Ray, 1950). At least the positive response to adrenaline suggests that nerve fibres supplying the photophores may be adrenergic in nature (Nicol, 1952*c*).

Turning now to acetylcholine and other parasympatheticomimetic drugs, we find much less information available. Both acetylcholine and nicotine evoke luminescence in *Chaetopterus*, and the response to electrical stimulation is augmented by eserine (Nicol, 1952*a*, *b*). In *Mnemiopsis*, Chace (1941) found that eserine increases the sensitivity to mechanical stimulation, and also increases the duration of the luminescent flashes. Acetylcholine, in high concentrations (1 in 3000), enhances this effect. Muscarine and pilocarpine (1% solutions in sea water) have a strong excitatory effect on the luminescent responses of *Ophiopsila annulosa* (Ophiuroidea). Atropine appears to have an inhibitory effect, as judged by responses to mechanical stimulation

(Mangold, 1907). Luminescence in the millipede *Luminodesmus sequoiae* is not inhibited by curare, but there is no evidence that light production is under nervous control in this species (Davenport, Wootton & Cushing, 1952). During a cruise on the R.R.S. *Discovery II* in 1952, I have ascertained that neither *Pelagia* nor *Systellaspis* lights up after treatment with acetylcholine in various concentrations (10^{-6} to 10^{-4}).

I have collected these scattered observations dealing with the effects of drugs on luminescent responses in order to expose any similarities and trends which may exist. There is obviously much variation in the effects of the several drugs listed above on different species. This is probably to be expected on several grounds. Luminescence, as one type or category of effector response, is often equated with others such as muscular contraction, chromatophore activity, glandular secretion, etc. The mechanisms of luminescence are markedly different in various species, however; indeed, several different mechanisms sometimes co-exist in the same species. In some species a luminescent secretion may be expelled, in others light is produced as the result of intracellular oxidative processes. In still other species the light is continuous, and muscular mechanisms or chromatophore-screens control the emission. With this heterogeneity of mechanisms it is not surprising that external agents should have extremely diversified effects on different luminous organisms. These several mechanisms are in turn regulated by the nervous system, and the details of innervation vary with the morphological complexity of the animal and the character of the tissue innervated. The effects of various pharmacological agents on the nervous systems of different animals are extremely varied, and this is reflected in the luminous responses. In evaluating the significance of drug action on the luminescent responses of any given species, it is advantageous to know the effects of a particular drug on other effector tissues and on the nervous system of that organism. Evidence from related fields of physiology and morphology may then indicate certain excitatory and effector processes which may be operative in the organism under investigation, and which can be further tested in terms of the luminescent response. Investigations of this kind have rarely been attempted.

The Effect of Cyanide

Luminescence is basically an oxidative reaction, and depends on oxygen. For this reason the effect of cyanide, a respiratory inhibitor, was tried on isolated scales (*Acholoë*, *Harmothoë*). KCN, in concentration 0·0001 M for 1 hr. failed to prevent luminescence following electrical stimulation. In concentration 0·001 M, KCN abolished luminescence in 45–60 min. It seems probable, from the concentrations and times required to abolish the luminescent response, that KCN is acting by slow poisoning of efferent nerves. Cyanides, generally, have little effect on luminescent responses and reactions of animals (see Harvey, 1952).

The Effect of Unbalanced Salt Solutions

In order to test the effect of different ions on the luminescent responses of polynoids, isolated scales were immersed in isosmotic solutions of selected salts. All experiments were carried out with scales of *Lagisca*, *Acholoë*, and *Gattyana*. At least four elytra were tested in each solution. The pH was adjusted to 8·2 with cresol red, unless otherwise stated.

Isotonic salt solutions having the following salt concentrations were used.

	g./l.		g./l.
NaCl	31·56	$MgCl_2$	73·20
KCl	40·26	Choline chloride	65·64
$CaCl_2$	39·95		

Freshwater. When dropped into distilled water, scales invariably luminesce. This takes the form of either a prolonged glow, or repeated flashing.

Solutions of simple salts

KCl. Isosmotic solutions of KCl always produce a bright prolonged glow which persists until the scale is exhausted.

NaCl. In solutions of isosmotic NaCl, scales respond by slow or rapid repetitive flashes, which continue for some time and are followed by a steady glow.

$CaCl_2$ (pH 7·9–8·2). There is some doubt about the effect of this salt. In a few scales it evoked an initial brief flash, followed by repetitive flashing, but in the majority of scales its application was not attended by a luminescent response. It appeared to enhance the sensitivity of scales, however, since these were easily excited into luminescence by slight mechanical agitation after being placed in a solution of $CaCl_2$.

$MgCl_2$. No apparent effect.

Mixtures of isotonic salt solutions

The proportions of the different salt solutions are given in volumes.

NaCl 25 + *KCl* 0·6. Scales responded by rhythmic flashing.
NaCl 25 + *$CaCl_2$* 1. No response observed.
NaCl 25 + *$MgCl_2$* 5. No response observed.
NaCl 25 + *KCl* 0·6 + *$CaCl_2$* 1. No response observed.
NaCl 25 + *KCl* 0·6 + *$MgCl_2$* 5. No response observed.
NaCl 25 + *KCl* 0·6 + *$CaCl_2$* 1 + *$MgCl_2$* 5. No response observed.
KCl 25 + *$CaCl_2$* 0·6. Scales responded by a bright prolonged glow.
KCl 25 + *$MgCl_2$* 5. Scales responded by a bright prolonged glow.
KCl 25 + *$CaCl_2$* 0·6 + *$MgCl_2$* 5. Scales responded by a bright prolonged glow.
$MgCl_2$ 25 + *$CaCl_2$* 1. No response.

Mixtures of isosmotic salt solutions and choline chloride

Choline chloride was employed as a physiologically inert substance to replace some one or other salts in the test solution.

Choline chloride. This substance by itself produced no luminescent response.
NaCl 75 + *choline chloride* 25. Scales flashed rhythmically in this solution.
KCl 2 + *choline chloride* 98. No response observed.
KCl 10 + *choline chloride* 90. Scales responded with a quick flash followed by a prolonged glow.
$CaCl_2$ 3 + *choline chloride* 97. In some preparations occasional weak flashes were observed after a considerable interval, but the majority of scales gave no overt response.

NaCl 75 + *KCl* 2 + *choline chloride* 23. This solution evoked a few quick flashes followed by a prolonged glow.

NaCl 75 + $CaCl_2$ 3 + *choline chloride* 22. This solution evoked slow rhythmic flashing.

NaCl 75 + $MgCl_2$ 15 + *choline chloride* 10. No response observed.

NaCl 75 + $CaCl_2$ 3 + $MgCl_2$ 15 + *choline chloride* 7. No luminescent response observed.

KCl 2 + $CaCl_2$ 3 + *choline chloride* 95. One scale out of six gave rhythmic flashes.

KCl 2 + $MgCl_2$ 15 + *choline chloride* 83. No response observed.

KCl 2 + $CaCl_2$ 3 + $MgCl_2$ 15 + *choline chloride* 80. A faint persistent light was observed.

NaCl 75 + *KCl* 2 + $CaCl_2$ 3 + *choline chloride* 20. No light observed.

NaCl 75 + *KCl* 2 + $MgCl_2$ 15 + *choline chloride* 8. No light observed.

$CaCl_2$ 3 + $MgCl_2$ 15 + *choline chloride* 82. No observable response.

These responses may be summarized by observing that KCl in excess (4 g./l. or more) produces a prolonged steady glow; NaCl in amount equivalent to that occurring in sea water, evokes rhythmic flashing; $CaCl_2$ has little effect by itself; and $MgCl_2$ acts as an anaesthetic. In a mixture of NaCl and KCl the scales respond initially by rhythmic flashes and then give a prolonged glow. The addition of Mg abolishes the rhythmic flashing called forth by Na, but not the prolonged glow due to K. Ca likewise reduces or abolishes the effect of Na, but not of K, at least in excess. As is usually found in experiments of this kind, the addition of further ions restores the balance; thus, neither Na + K + Ca nor Na + K + Mg evokes a luminescent response.

The depressant effect of Mg on excitable tissues is well known, and its anaesthetic action calls for no further comment here. Ca and Mg individually, and together, act as ionic antagonists for Na, and either opposes the excitable effects of Na + K. Antagonistic effects of this kind on excitable tissues have been frequently observed among marine invertebrates, and many examples can be found in research publications.

In an investigation of the effects of cations or combinations of cations, two apparent factors are involved, viz. the ability of the cations to excite tissues, and the ability to modify excitability as tested by stimulation. With the exception of Mg ion, only the former action has been tested in the present experiments. From the nature of the effects produced, certain conclusions can be drawn. K ion, in excess, is known to have a strong depolarizing effect on excitable tissue (nerve, muscle), and the prolonged glow which is the result of immersing elytra in an isosmotic solution of K is evidence of a direct depolarizing effect on the photocytes (Hodgkin & Huxley, 1945; Calma & Wright, 1947). In effect, it is very similar to the bright prolonged response which follows strong stimulation of a scale, also ascribed to direct excitation of the luminescent cells.

Sodium ion also has a stimulatory effect on nerve, and its role in the transmission of the nervous impulse has recently been discussed by Hodgkin (1951).

In the present experiments it is noteworthy that Na, initially at least, acts on the nerve and not directly on the photocytes, as revealed by the rhythmic discharge which its application elicits. The depolarizing action of K on the photocytes is antagonized by Na, and these two ions together stimulate the efferent neurones, producing rhythmic discharge and flashing. Neither Ca nor Mg, it may be noted, counteract the depolarizing effect of K (0·52–0·43 M) on the photocytes, but each is effective in reducing or preventing the stimulatory effect of Na ion on the efferent neurones supplying the photocytes.

Different cations have been tested on a variety of luminescent metazoans, and many scattered observations are now available, by no means easy to interpret. The scyphomedusan *Pelagia noctiluca* becomes luminescent in a solution of KCl, and, on poisoning with Ca, spontaneous luminescence appears over the whole surface. In the absence of Mg, i.e. in a solution of Na, K and Ca, *Pelagia* passes into a state of hyperirritability, and flashes of light appear on the bell (Heymans & Moore, 1923, 1924). The pennatulid *Cavernularia habereri* luminesces in isosmotic KCl but not in NaCl. Moreover, luminescence is evoked by all solutions containing K, and combinations of K plus one, two, or three other salts, viz. NaCl, $CaCl_2$ and $MgCl_2$. The other three ions, in the absence of K, fail to induce luminescence. It is rather surprising that a solution containing $NaCl + KCl + CaCl_2 + MgCl_2$ should evoke luminescence. Since equal quantities of isosmotic solutions of the four salts were used in making up the test solution, the resultant concentration of K would be *c.* 14 times that in sea water, and the excitatory effect produced could be ascribed to K in excess. Unfortunately, the hydrogen-ion concentrations of the salt solutions do not appear to have been regulated, and the relatively high acidities of the unbuffered salts may themselves have produced excitatory effects (King-Li-Pin, Tchang-Si, Tai-Lee & Liu-Yu-Su, 1936). It is reasonable to conclude that K has a similar stimulatory effect on *Cavernularia* as on *Pelagia*, but whether on the nervous system, or directly on the photogenic cells, is unknown. The ctenophores *Mnemiopsis* and *Beroë* also luminesce when treated with isosmotic solutions of KCl and $CaCl_2$, but not NaCl and $MgCl_2$, results similar to those obtained with *Pelagia* (Moore, 1925).

In *Chaetopterus variopedatus*, a luminescent polychaete, isosmotic KCl causes bright luminescence, and NaCl, though still effective, produces a fainter glow. *Chaetopterus* is a species which discharges a luminescent secretion by some contractile process, and it is not unlikely that K produces its effect by exciting the glandular cells directly. In this animal Ca and Mg fail to inhibit excitation by K, but they reduce or abolish the stimulatory effect of Na, possibly by blocking Na depolarization of efferent nerve fibres supplying the photocytes. When Ca or Mg is added to a solution of NaCl and KCl, luminescence fails to appear, or is greatly reduced (Nicol, 1952*a*).

Some additional data are available. Mangold (1907) observed that isolated spines of *Ophiopsila annulosa* will luminesce in strong NaCl solutions. Shōji

(1919), who studied the effects of ions on the luminescence of the squid *Watasenia scintillans*, was concerned with the persistence of luminescence in the mantle-photophores, and retention of irritability to stimulation. His tables indicate that luminescence and irritability persist for about the same length of time in solutions of NaCl, KCl, and $CaCl_2$, but several times longer in solutions of $MgCl_2$. These solutions were osmotically equivalent to one another but only one half of the values for sea water ($\Delta - 0{\cdot}93^\circ$ C.). It is not clear in these experiments to what extent the several ions initially excited luminescence. With combinations of salts it appears that Mg is most favourable for continued luminescence. Luminescence and irritability persisted longest in combinations lacking Ca (i.e. Na + K + Mg). The photophores soon ceased to glow in isosmotic solutions containing Ca (i.e. Na + K + Ca), but continued to respond to stimulation for long periods in balanced salt solutions (Na + K + Ca + Mg) and in solutions lacking Ca (Na + K + Mg). Other experiments are shown in which different anions were tested. The results indicate that luminescence and irritability continue much longer in solutions of Na_2SO_4 and $MgSO_4$ than in NaCl and $MgCl_2$. These various results are rather puzzling but they show that the effects of different ions on the luminescent response are bound to be very complex. The persistence of luminescence and irritability in solutions of $MgCl_2$ and $MgSO_4$ for 3–4 hr. is extraordinary, in view of the general anaesthetic action of these salts on marine invertebrates. It is still uncertain whether squid photophores are subject to direct control, and these results may reveal only duration of vitality.

Discussion and Conclusions

It appears to be firmly established from this and earlier studies that the luminescent responses of polynoids are under nervous control. The nerve fibres supplying the photocytes have been revealed by histological means, and their activity demonstrated by experimental methods. These nerve fibres are excitatory nerve fibres, and appear to be the only ones supplying the photocytes (Bonhomme, 1942; Nicol, 1953).

In the normal intact animal the chain of neural events leading to the appearance of luminous flashes appears to involve: excitation of peripheral receptors or sensory endings, which abound on the dorsal surface of the elytra, and are probably distributed over the general surface of the body as well; initiation of impulses in afferent fibres running into the nerve cord; reflex transmission of impulses in efferent fibres to a peripheral ganglion lying in the elytrum; relaying of nervous impulses into terminal efferent fibres which radiate outwards from the elytral ganglion to the photocytes (Pflugfelder, 1933; Bonhomme, 1942).

Some of the evidence for this reflex arc is indirect, but there seems little reason to doubt that it represents one method by which the luminescent

response is evoked. When any localized region of the body is stimulated mechanically, luminescence appears not only in the elytra of the region directly stimulated, but in more distant segments as well. Excitation is thus initiated by peripheral stimulation, and is transmitted longitudinally along the nerve cord. Electrical stimulation of the nerve cord also evokes normal luminescent flashes.

Turning now to the efferent pathways, we find that the normal response of a scale is a long series of discrete rhythmic flashes. Evidence has been presented that flashing is controlled by a ganglion situated in the centre of the elytrum. In an entire scale, or portion of a scale containing the ganglion, a single shock sets the scale flashing, as the result of rhythmic discharge from the ganglion. It may well be that, in the intact animal, one or a few impulses are sent to the elytral ganglion from the nerve cord, and initiate rhythmic discharge and flashing. Of this we have no knowledge as yet.

A second method of stimulation, operating under natural conditions, is traumatic injury. Transection of the body evokes flashing posterior to the cut, but not anterior. I have previously hypothesized the existence of synaptic resistance in longitudinal pathways through the nerve cord to explain this phenomenon. Owing to the sensitivity of these animals and the ease with which luminosity is exhausted, it may prove difficult to get information on this problem.

Autotomy or removal of a scale also starts it flashing. Excitation follows from injury potentials established when the nerves are severed. Persistent discharge in nerves after injury is not unknown in other animals. It forms the basis of Parker's hypothesis concerning caudal bands in fish (Parker, 1948). Adrian (1930) has demonstrated the occurrence of a persistent discharge of impulses in mammalian nerves after injury. The persistent discharges arise from the injured ends of the nerve fibres, the permanent depolarization of the injured region acting as a stimulus to the intact part of the fibre. The frequency of discharge is high, and the time relations indicate that the nerve fibres are responding to an excitation which outlasts the refractory period of the fibre; the refractory period thus determines the rate of discharge. There are several reasons for believing that the same process is not operating in autotomized polynoid elytra. First, rhythmical flashes (following electrical stimulation) can be induced only in a preparation containing a ganglion; bits of scale lacking a ganglion respond by a single flash to each stimulus. The ganglion, accordingly, is necessary for luminescence. Secondly, the rate of flashing is relatively slow, 10 to 1 per sec., far below the rate which would be determined by the refractory period of nerve, if this were the controlling factor.

It seems that a stimulus, be it injurious, mechanical, nervous, or electrical, excites the pre-ganglionic nerve fibres[1] and the nerve cells. A prolonged

[1] There is no clear-cut evidence, as yet, for the existence of synapses in the elytral ganglion. T-shaped neurones, for example, could be involved.

excitatory state is set up in the elytral ganglion, which far outlasts the duration of a single impulse, and is responsible for the initiation of impulses in the efferent fibres. These events are reflected in the luminous flashes which appear rhythmically for some time after stimulation. It appears as if the pre-ganglionic stimulus, on exciting the ganglionic cells, sets up a rhythmic oscillatory state in the latter, with a rather slow period of discharge, beginning at about 0·1 sec. and increasing to 1 sec. Presumably at the peaks of the cycle, excitation rises to a sufficient level to fire the post-ganglionic fibres, and send off impulses to the photocytes.

A search of the literature reveals no strictly comparable system, although analogies exist. Rhythmic ganglionic discharges of an inherent nature have been recorded from the brain and nerve cord of different invertebrates (Bullock, 1947), and occur in certain isolated peripheral ganglia, e.g. cardiac ganglion of *Limulus* (Armstrong, Maxfield, Prosser & Schoepfle, 1939; Bullock, Burr & Nims, 1943). A constant background of spontaneous discharge is characteristic of certain peripheral receptors, e.g. of vestibulo-lateralis system of fishes, and the frequency of such discharge may be rather low, around 5 impulses/sec. (Suckling & Suckling, 1950; Lowenstein & Roberts, 1950). Caudal photoreceptors have been identified in the nerve cord of the crayfish *Cambarus*, which discharge at a high frequency when illuminated. Of particular interest is the observation that after illumination ceases, an after-discharge persists for several seconds, during which the impulses decline to a spontaneous level (Prosser, 1934). Recordings from the isolated visceral ganglion of *Aplysia* have revealed the existence of spontaneous rhythmic oscillations of potential, arising in nerve cell bodies. These oscillations, followed by discharges in efferent nerves, show rather long periods, about 420 msec. (minima, 70 msec.) (Arvanitaki & Cardot, 1941).

As the above résumé reveals, other instances are known in which rhythmic oscillations of potential, sometimes evoked by external stimulation, lead to nervous discharges, and the frequencies are often within the range encountered in polynoid flashing (1–15/sec.). By postulating the occurrence in ganglionic neurones of rhythmic oscillations of potential corresponding to the rhythmic flashes, one can suggest explanations for certain characteristics of the rhythmic process. We have seen that long pauses, several seconds in duration and far exceeding the length of a normal period, sometimes intervene between successive trains of flashes (e.g. Fig. 8C). It is not unlikely that these quiet hiatuses indicate periods of subliminal potential oscillations, below the threshold for firing efferent fibres. Owing to the fact that the luminescent flashes show progressive fatigue leading to extinction, it is difficult to secure much information about the maximal duration of the underlying excitatory process responsible for the rhythmic flashes; certain favourable preparations suggest a maximal duration of 1–2 min. at least for supraliminal activity.

Curves showing variations in flash frequency with time and succession

have been presented in a previous paper (Nicol, 1953). The frequency is high at first, rapidly falls off to a steady level of approximately 1/sec., and after a variable period, often about 1 min., decreases to extinction. Now if the time relations of this rhythmic flashing are a true index of underlying potential oscillations, then we can conclude that an external stimulus or nervous impulse excites the nerve cell bodies, and initiates rhythmical oscillations and discharge. At first high, the frequency of oscillation quickly falls off during the first few seconds. It has also been discovered that with repeated stimulation, the number of rhythmic flashes which can be induced by a single stimulus declines until finally only a single flash is induced by one stimulus. In the latter event rhythmic flashing can be evoked again by delivering a burst of high frequency pulses. It appears as if the excitatory state of the neurones is itself subject to fatigue. After repeated periods of stimulation the number of rhythmic oscillations falls off as well, until only a single discharge is evoked. These speculative remarks indicate the need for electronic recording of action potentials.

Peripheral ganglia, associated with the appendages, have been described in other errant polychaetes. In *Nereis* (Nereidae) and *Hermione* (Aphroditidae), there are parapodial ganglia at the bases of the parapodia, and in the latter animal these connect with cirrus ganglia near the cirri. Sensory nerves and motor nerves to parapodial muscles pass to and from these centres (Schneider, 1902; Hanström, 1928). According to Maxwell (1897) the parapodial ganglia of *Nereis* act as local reflex centres for parapodial movements. It is possible that the elytral ganglia of polynoids are derived from parapodial or cirrus ganglia, such as those existing in *Hermione*.

The increase in height of subsequent luminescent responses indicates the existence of some facilitatory phenomenon. The progressive increment in intensity of consecutive responses is analogous to staircase in the vertebrate heart, or tension-rise in crustacean muscle. The prolonged facilitatory period, up to 4 min., would appear to preclude the operation of some persistent electrical potential. Neither is there any indication that chemical transmitters—acetylcholine and adrenaline—are involved in the luminescent response. There is some evidence for the existence of cholinergic nerve fibres among annelids, however, with the distinct possibility that acetylcholine is concerned in neuromyal transmission (evidence reviewed by Prosser, 1950). Evidence also exists for sensitivity to adrenaline in a few species (earthworm gut, vascular system of the leech) (Gaskell, 1914; Hanström, 1939; Ambache, Dixon & Wright, 1945). The ineffectiveness of atropine and curare on the luminescent response offers no conclusive evidence, since these two drugs fail to block neuromyal transmission in some other annelids (Nicol, 1952*d*).

It appears, then, that either nervous excitation of the luminescent gland cells is radically dissimilar from neuromyal transmission, or that the neuroglandular junction is inaccessible to the reagents used. Nachmansohn (1950) has

developed a hypothesis of this kind to explain differences between the effectiveness of methylated quaternary ammonium salts and tertiary amines, e.g. eserine. It may be noted that Bonhomme (1942) has asserted that the nerve fibres supplying the photocytes of polynoid worms may penetrate into the glandular cells. In this event a post-synaptic membrane, comparable to that between nerve and muscle, would not exist, and the drugs would have no extracellular interface upon which to act. The existence of distinct microscopic granules of luminescent material in the cytoplasm of the photocytes must mean that these cellular inclusions are provided with distinct intracellular interfaces. It is tempting to regard these interfaces as the site of control of the luminescent flash, by ionic exchange, release of energy-bearing substance, or other mechanism. Facilitation, then, may represent changes at these loci. The influence of the ionic environment, particularly of changes in sodium and potassium, is described on pp. 243–6. ATP, which has a positive effect on the luminescent reaction of certain animals, fails to revive luminescence in extracts of polynoid scales after the initial glow has died away (see Appendix) (Harvey & Haneda, 1951).

Luminescent responses, consisting of brief flashes, are recorded for other marine and terrestrial animals, and it is of interest to discover whether the same or similar regulating mechanisms may be operative in these animals. Certain calyptoblastic hydroids give off intermittent repeated flashes when mechanically stimulated, possibly representing repetitive discharge in the nerve net. Intermittent flashes are reported in nudibranch molluscs (*Kaloplocamus*, *Plocamopterus*), a pulmonate (*Dyakia*), euphausiids (*Nyctiphanes*), and decapod crustacea (*Sergestes*). The regulating mechanism in these animals has not been investigated. In cephalopods and fishes which give off discrete flashes the photophores are either innervated and subject to direct nervous control (myctophids or lantern-fish), or periodically uncovered by screening devices (fire-fly squid *Watasenia*; stomiatoid teleost *Astronesthes*). Rhythmic luminous responses (flashing) have been most studied in fire-flies (Lampyridae). In the majority of luminescent species the response takes the form of an intermittent glow, pulsation, or series of flashes. In *Photuris pennsylvania*, for example, males flash at a rate of about 3/sec., and each flash normally appears as a symmetrical response, lasting around 0·15 sec., and rising to a peak in half that time. There is still some uncertainty how these flashes are controlled, but much suggestive evidence exists that flashing is regulated by provision of oxygen through tracheal end-cells (see Buck, 1948, and Harvey, 1952, for review of literature). In Fig. 10 some flash-curves are presented of different species under various conditions. Luminescent flashes appear to develop a little faster in polynoids than in *Photuris*, and to be of slightly shorter duration. An apparent difference between the flash-curves of the two animals lies in the symmetrical appearance of the curve for *Photuris*, and the slower progress of decay in the curve for *Acholoë*.

The symmetrical appearance of the response of *Photuris* (likened to a distribution curve) probably depends upon summation of the flashes of a great many sub-units, all slightly asynchronous, while the photocytes in each scale of *Acholoë* flash more synchronously. The decay portion of the curve (exponential) in the latter animal, therefore, more nearly represents the course of decay of the luminescent reaction in each photocyte. Added to Fig. 10 are two curves for oxidation of *Cypridina* luciferin and luciferase, mixed prior to admission, and in the presence, of oxygen. In this animal, at least, interaction

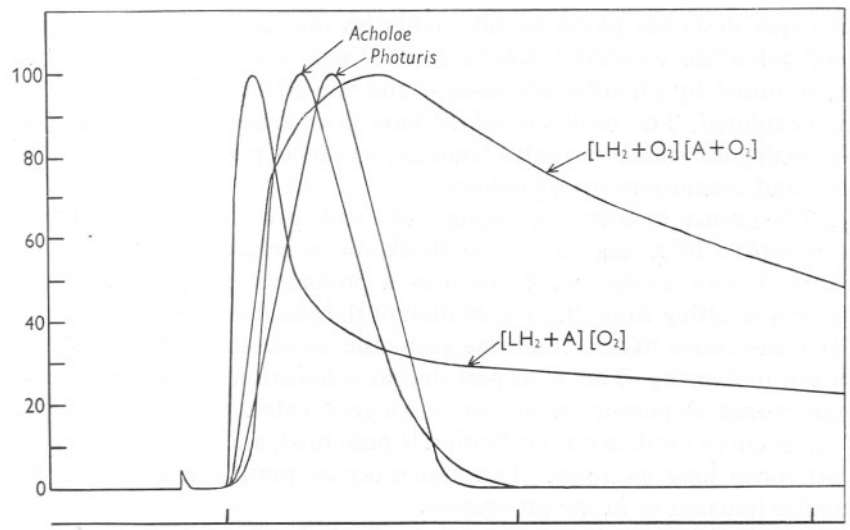

Fig. 10. Curves of luminescent responses and luminescent reactions from various sources. Luminescent responses of *Acholoë* (polynoid) and *Photuris* (fire-fly). The small pip at the left on the base line is the stimulus mark for *Acholoë* only. Two curves are shown for oxidation of *Cypridina* luciferin. Luciferin and luciferase mixed in presence of O_2 ($[LH_2+O_2]$ $[A+O_2]$), and a mixture of luciferin and luciferase to which O_2 is admitted ($[LH_2+A]$ $[O_2]$). Time scale below, intervals of 100 msec.

between luminescent substrate and catalyst is a more limiting factor than oxygen availability. In intracellular luminescence (Polynoidae), the rise of intensity must represent the time course of provision of reactant(s), and interaction of reacting materials (Snell, 1932; Chance, Harvey, Johnson & Millikan, 1940).

The luminescent responses of single scales of polynoids are usually simple flashes resulting from synchronous activity of all the photocytes, although a few records give evidence of asynchronous flashing of several units. From this it appears that a single neurone innervates all the photocytes (one neuro-effector unit) or, if more than one neurone is involved, that the several neurones show synchronous activity and fire in pace with each other. Asynchronous activity, occasionally recorded, confirms histological evidence that

several neurones exist in the elytral ganglion. Synchronous activity possibly depends on interaction of cell potentials, such as takes place between contiguous nerves (*Carcinus*) or adjacent nerve cells (*Aplysia*) (Katz & Schmitt, 1940; Arvanitaki, 1942).

Part of the expenses incurred in this research was defrayed by a grant-in-aid of scientific investigations from the Royal Society.

Summary

This paper describes physiological studies on the luminescent responses of several polynoids, especially *Acholoë* and *Polynoë*. Luminescent flashes have been recorded by photoelectric means, and the effects of various chemical agents explored. The results obtained have been related to, and integrated with, analogous studies on other animals, as far as possible. The principal results and conclusions are as follows.

(i) The normal luminescent response of a scale is a series of flashes, which can be evoked by a single electrical shock and is controlled by the nervous system. A very strong shock produces a prolonged glow, exhausting the scale, and resulting from direct excitation of the glandular cells.

(ii) Consecutive flashes, following each other at suitable intervals, show an increase in intensity. This is in part due to summation when the frequency is high enough to permit fusion, but is to a great extent attributable to facilitation. A curve for decay of facilitation is presented, and the effect is shown to last for as long as 4 min. Facilitation occurs peripherally at the neuroglandular junction or in the photocytes.

(iii) At high frequencies, above 25/sec., the flashes begin to fuse. A consequence of high-frequency stimulation is that the intensity of response is reduced, due to failure of transmission or to the existence of relative refractoriness peripherally.

(iv) Absolute refractory period (*Polynoë*) of the response lies between 9 and 16 msec.

(v) Rhythmic flashing is controlled by a peripheral ganglion in the elytrum, from which nerves radiate to the photocytes. Preparations lacking this ganglion give only a single flash to each stimulus.

(vi) At the beginning of a response the flash interval is small, around 100 msec.; this increases to 1 sec. during the course of a response; in some preparations long intervals, up to 15 sec. between successive flashes, have been noted. When preparations are subjected to consecutive periods of stimulation, the number of flashes evoked gradually falls off, suggesting fatigue of the neural mechanism regulating flashing.

(vii) Of a series of drugs tried, viz. adrenaline, acetylcholine, curarine, eserine, atropine, nicotine, and strychnine, all were without effect on flashing except the last, which blocked electrical excitation.

(viii) Isosmotic solutions of single salts and combinations of salts had the following effects: potassium caused a prolonged glow; sodium evoked rhythmical flashing; calcium increased excitability; choline chloride was without effect; magnesium acted as an anaesthetic; calcium and magnesium antagonized the excitatory effect of sodium. Potassium is thought to act directly on the photocytes, sodium on the nerves at first, and then on the photocytes.

(ix) ATP is shown to be without effect on extracts of the luminous tissues of polynoids, *Pholas* and *Polycirrus* (Appendix A).

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APPENDIX

EFFECT OF ADENOSINE TRIPHOSPHATE ON THE LUMINESCENCE OF CERTAIN MARINE ORGANISMS

Following the experiments of Harvey & Haneda (1951), I have tested the effects of adenosine triphosphate on luminescent extracts of several marine animals, viz. *Pholas dactylus*, *Polycirrus caliendrum*, *Acholoë astericola* and *Polynoë scolopendrina*. The luminescent tissues of these animals were ground up with sand in a little sea water (10 ml.), until the light disappeared. A solution of 5 mg. ATP was then added to this extract, or to the supernatant fluid after centrifugation. In none of these preparations did ATP revive luminescence.

I have to thank Dr G. Y. Kennedy of the Cancer Research Laboratory, University of Sheffield, for a sample of the barium salt of ATP. This was converted to the sodium salt by the procedure described in Carter (1942).

J. Mar. biol. Ass. U.K. (1954) 33, 257–264
Printed in Great Britain

A SPRING-LOADED BOTTOM-SAMPLER

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(Text-figs. 1–3)

Introduction

For routine sampling of the infauna of the sea bottom an apparatus is required which is simple, easily handled, and which can give samples of consistent volume from different types of bottom and in varying weather conditions.

The Petersen sampler and its modification, the van Veen, are simple and easily handled and give reasonably consistent samples from some types of soil. But on hard ground they are less satisfactory, and in rough weather they tend either to land unevenly on the bottom, thereby taking smaller samples of variable volume, or to release in mid-water with the rolling of the ship, so that no sample is taken.

A new sampler, which is in use at the Plymouth Laboratory, has been described by Holme (1949). We are glad to be able to acknowledge here the kindness of the Director and Mr Holme in arranging for us the early supply of one of these samplers. However, in spite of its improvements, it has certain disadvantages which are well summed up in the author's own words 'the area sampled ($\frac{1}{20}$ m^2.) is, however, rather small, and the apparatus rather heavy, and so difficult to work except in calm weather'. In using Holme's sampler from Aberdeen this last point, the difficulty of using it in anything but calm weather, was found to be its greatest disadvantage.

When bottom-fauna work has to be fitted into a wider programme, it is often impossible to wait for suitable conditions, and the need was still felt for a sampler which could be used with confidence in any weather. The apparatus described here was designed and constructed, by the first author, in an effort to produce a sampler which would give consistent results even in bad weather and on 'difficult' grounds. It was constructed at the Northern Engineering Works, Peterhead, and we wish to express our thanks to the proprietors for their interest and assistance.

The Sampler

The apparatus is shown in Fig. 1. It consists essentially of a spring-loaded bucket carried in a frame. In use, when the apparatus comes to rest squarely on the bottom, the springs are released and drive the bucket into the soil; hauling on the warp then closes the sampler.

The bucket, which is semicircular in cross-section, is hinged on an axle along the centre line, and is opened and closed by means of two arms (Fig. 3 A).

It is carried by an inverted U-shaped bridle which is attached at both ends of the axle. The horizontal bar of the bridle is bored so that the bridle and bucket can move vertically on a tubular guide (Fig. 1) fixed at the centre of the frame. This vertical movement is limited to 3 in. by stops. Two springs

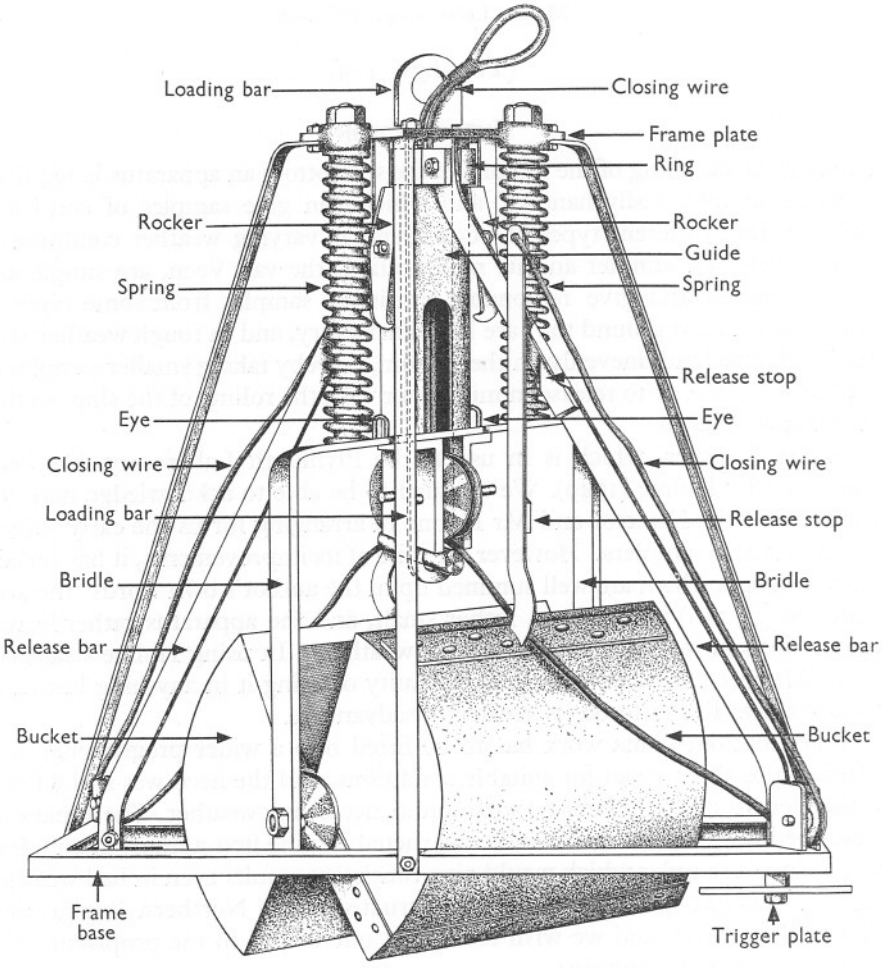


Fig. 1. Diagram of sampler, in the unloaded position (overall dimensions, 26 × 26 × 20 in.).

(each loading 50 lb./in.) are placed so that their lower ends bear against the bridle and their upper ends against the underside of the frame plate. Each spring is located by an internal rod on the bridle (Fig. 3A) which slides into a tube attached to the frame plate. To the middle of the bridle is secured a loading bar which passes up the centre of the guide and projects through the frame plate. In loading, this bar is pulled up by means of a loading lever,

and the bucket is raised against the pressure of the springs until two eyes on the bridle come within reach of two rockers attached to the frame plate. The rockers engage in the eyes and hold the bucket in the loaded position against the pressure of the springs. The faces of the rockers bearing in the eyes are set at such an angle that a horizontal component of the pressure tends to free the rockers from the eyes, releasing the bucket. This movement of the rockers is prevented by a ring against which the upper ends of the rockers bear when

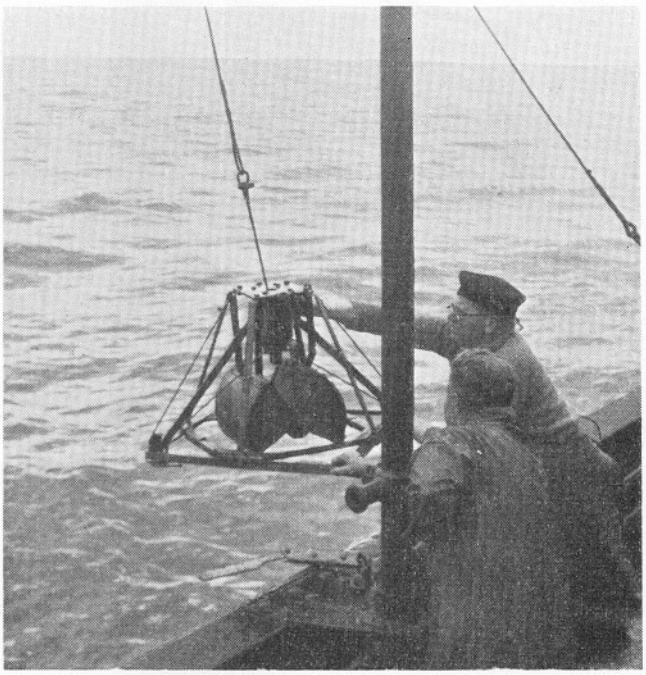
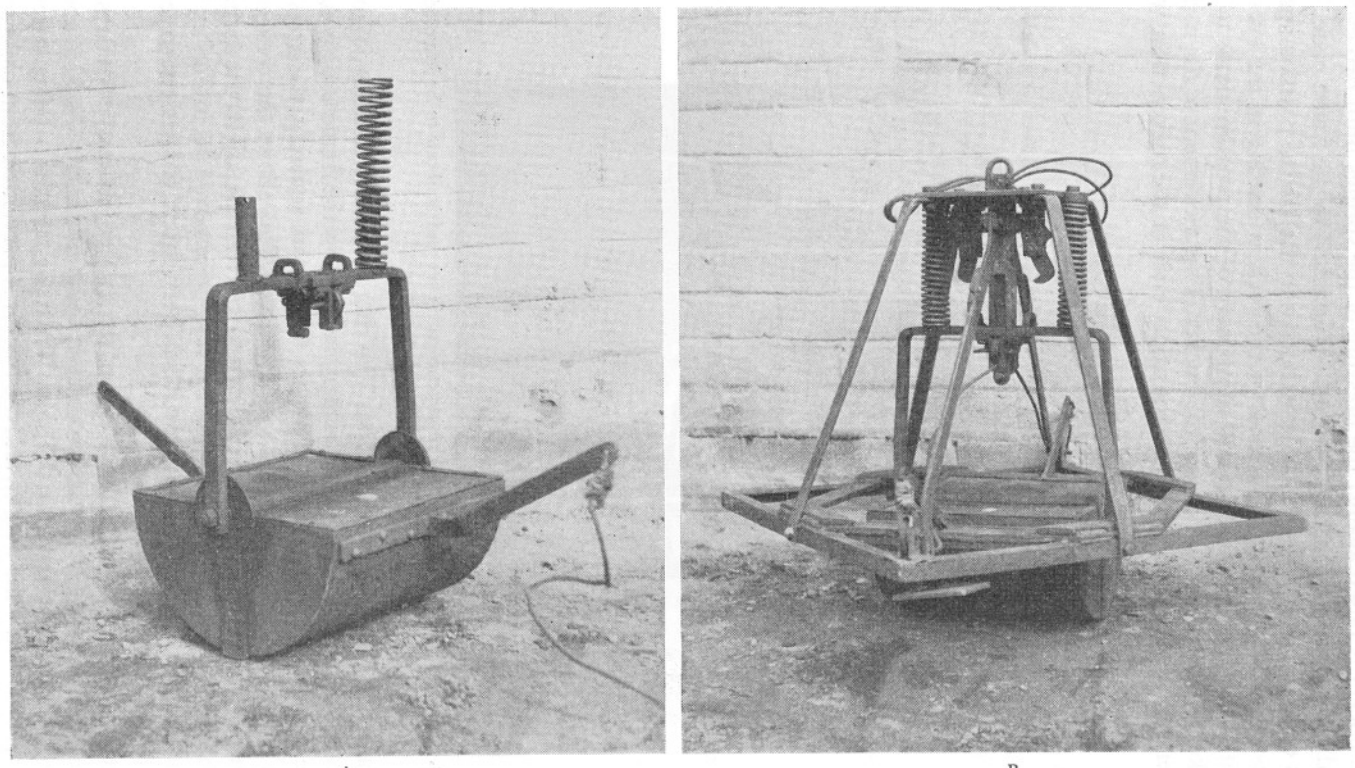


Fig. 2. The sampler in use at sea. The instrument is set and is about to be lowered. The loading bar has been removed.

the apparatus is in the loaded position. The ring is fixed by knuckle joints to two release bars which terminate in trigger plates below the frame at diagonally opposite corners. When the sampler strikes the bottom squarely, the pressure on both trigger plates causes the ring to move up. This allows the rockers to swing free of the eyes and the open bucket is forced into the soil by the springs. When the instrument is set the loading bar may be removed (as in Fig. 2), but when the sampler is in continuous use it has been found convenient to leave the bar in position.

A wire attached to the end of each arm of the bucket passes over pulleys on the frame and bridle, up through the centre of the guide, and is shackled above the frame plate to the warp from the ship. When the sampler is being lowered the closing wire bears the weight, and the bucket is prevented from closing by



A B

Fig. 3. A, the bucket, closed. B, the sampler, closed, with the loading bar in position.

stops on the arms bearing against similar stops on the release bars (Fig. 1). The release of the bucket when the sampler hits the bottom disengages the arms from the stops so that hauling on the warp first closes the bucket, then lifts the sampler off the bottom.

The bucket has a radius of $17\frac{1}{2}$ cm. and a length of 32 cm., and is constructed of $\frac{1}{8}$ in. (3·2 mm.) sheet metal. The area of bottom sampled is $\frac{1}{10}$ m.^{2}. The top of the bucket is covered with fine-mesh gauze. The arms which are riveted to the bucket are 28 cm. long and are of $1 \times \frac{1}{4}$ in. flat iron. The bridle is made from two pieces of $1\frac{1}{4} \times \frac{1}{2}$ in. flat iron bent at right angles, and welded to a $\frac{1}{2}$ in. plate 3 in. square which is bored to slide on the central guide. The guide is 10 in. long and of $1\frac{3}{4}$ in. diameter tube. It has a $\frac{3}{4}$ in. slot on each side extending upwards from the lower end for 5 in. The base of the frame consists of four bars of $1 \times \frac{1}{8}$ in. angle iron 26 in. long, and from each side of the frame an upright bar of $1 \times \frac{1}{4}$ in. flat iron runs to the frame plate, which is 8 in. square and $\frac{1}{4}$ in. thick. The angle iron used for the base proved to be too light, and was strengthened by cross-bars (see Figs. 2 and 3B). The loading bar is $1 \times \frac{1}{4}$ in. flat iron and has a loop welded to the top. The apparatus is 20 in. high and weighs 100 lb. (45 kg.).

Results

Previous work (Thamdrup, 1938) indicates that the van Veen is more efficient than the Petersen grab, and experience at Aberdeen with the van Veen, Petersen, and Plymouth samplers has shown that the van Veen was the most satisfactory over a wide range of conditions. The van Veen has thus been in general use at Aberdeen, and since it is obvious that any new sampler must be compared with the most efficient of the older models, tests have been carried out using the $\frac{1}{10}$ m^2. van Veen and the Smith sampler.

The samplers were used from the ship at anchor on various grounds and at different depths, the order of use being determined from a table of random numbers. The volumes, in litres, of material taken in each sample are set out below:

(1) Dornoch Firth, 9 m. depth, muddy sand									
Smith sampler	3·75	3·5	2·5	3·25				Mean 3·25	per $\frac{1}{10}$ m.^{2}
v. Veen sampler	4·0	3·5	3·0	2·5				Mean 3·25	
(2) Dornoch Firth, 13 m. depth, muddy sand									
Smith sampler	4·5	2·5	4·5	4·5				Mean 4·0	per $\frac{1}{10}$ m.^{2}
v. Veen sampler	2·25	2·5	2·25	2·0				Mean 2·25	
(3) Dornoch Firth, 24 m. depth, muddy gravel									
Smith sampler	4·75	4·75	5·0	4·0				Mean 4·6	per $\frac{1}{10}$ m.^{2}
v. Veen sampler	3·25	4·0	3·0	2·75				Mean 3·25	
(4) Dornoch Firth, 24 m. depth, mud									
Smith sampler	5·0	4·0	4·75	5·5				Mean 4·8	per $\frac{1}{10}$ m.^{2}
v. Veen sampler	3·0	3·0	3·5	3·25				Mean 3·2	
(5) Aberdeen Bay, 37 m. depth, hard sand									
Smith sampler	3·5	1·5	4·0	4·0	3·5	3·5	3·0	Mean 3·3	per $\frac{1}{10}$ m.^{2}
v. Veen sampler	2·0	3·5	2·5	2·0	1·5	0·5	3·5	Mean 2·2	

For test number (5) above, a detailed analysis was made and the frequencies of the various organisms taken are shown in Table I. In nearly every comparison the Smith sampler gave a higher population estimate, and statistical analysis showed that the difference between the two samplers in this respect was significant at the 1% level.

TABLE I

Aberdeen Bay. Depth 37 m. Bottom, hard sand. The mean numbers of animals taken in seven dips with the van Veen and Smith samplers respectively.

	van Veen sampler	Smith sampler
Gastropods	0·1	2·0
Nucula	20·4	23·0
Thyasira	1·1	1·3
Montacuta	2·7	1·6
Cyprina	1·3	2·3
Dosinia	11·1	13·6
Venus fasciata	2·3	3·9
Tellina fabula	5·6	6·1
Abra	3·9	6·1
Gari fervensis	1·3	1·1
Cultellus	0	0·4
Thracia	1·6	2·3
Other lamellibranchs	0·3	0·7
Ophiuroids	3·0	4·4
Echinoids	3·9	4·0
Crustacea	3·4	6·0
Aphroditidae	2·0	2·4
Goniada	2·9	3·3
Nepthys	1·7	2·6
Lumbriconereis	1·3	1·6
Other errant polychaetes	0·3	1·1
Ariciidae	2·9	5·1
Spionidae	0·7	2·0
Magelona	0·3	1·0
Cirratulidae	1·7	2·0
Chlorhaemidae	1·3	1·6
Other sedentary polychaetes	0·7	0·9

On two occasions the new sampler was compared with a $\frac{1}{5}$ m.^{2} Petersen grab, with the following results (volumes in litres):

(1) Smith Bank, 37 m. depth, fine sand

Smith sampler	4·0	6·0	5·0	4·0	4·0	4·0	3·5	4·0	5·0	Mean 4·4	per $\frac{1}{10}$ m.^{2}
$\frac{1}{5}$ m.^{2} Petersen sampler	3·0	4·0	5·0							Mean 4·0	per $\frac{1}{5}$ m.^{2}

(2) Off Smith Bank to northwest, 73 m. depth, mud

Smith sampler	2·5	2·0	4·0	5·0	2·5	2·0	Mean 3·0	per $\frac{1}{10}$ m.^{2}
$\frac{1}{5}$ m.^{2} Petersen sampler	2·5	3·0					Mean 2·75	per $\frac{1}{5}$ m.^{2}

It is of interest that the smaller sampler tended to take a larger volume.

Apart from such specific tests, an indication of the relative performance of the two samplers is given by figures collected during routine surveys over

a period of several months. The following comparisons are from recent data (volumes in litres):

(1) Smith Bank, 37–40 m. depth, fine sand

Smith sampler	52 dips	Mean volume 3·9	Range 1·0–7·0.
v. Veen sampler	165 dips	Mean volume 3·4	Range 0·25–6·0.

(2) Smith Bank, 37–40 m. depth, shell gravel

Smith sampler	9 dips	Mean volume 5·0	Range 4·0–7·0.
v. Veen sampler	15 dips	Mean volume 4·5	Range 0·5–6·0.

(3) Off Smith Bank to northwest, 62–73 m. depth, mud

Smith sampler	9 dips	Mean volume 3·0	Range 2·0–5·0
v. Veen sampler	9 dips	Mean volume 2·1	Range 0·5–3·5.

Discussion

The above tests show that the new sampler is more efficient than the van Veen in the areas visited. Perhaps the most significant feature, however, is not shown by these data. The opportunity to compare the two instruments usually arose during a general bottom-sampling programme. On several occasions the comparison had to be abandoned because bad weather prevented the van Veen from operating. On each occasion, however, it was found possible to complete the programme with the Smith sampler only, which continued to give samples of reasonably consistent volume even under the most trying weather conditions.

The success of the new sampler in bad weather is due to its inability to release until resting squarely on the bottom. If it strikes the bottom unevenly only one of the release arms is operated and, since the arms are connected to the ring by knuckle joints, movement of one arm merely causes the ring to pivot. Release cannot take place until the other arm is also raised, i.e. until the apparatus is settled on the bottom.

A distinctive feature of the new sampler is connected with its method of closing. A great part of the superiority of the van Veen over the Petersen grab is probably due to the leverage effect exerted by the arms in closing the jaws. This leverage effect is greatest when the van Veen is fully open, and decreases as the jaws close and the arms come together. In the new sampler, however, the pull on the arms is downward, and the leverage effect increases as the sampler digs into the ground and reaches a maximum as the jaws come together.

Another feature of the new sampler is the gauze covering on top of the bucket. Because of this the down-wave produced on descent is relatively small, and this may help to account for the high proportion of active surface-dwelling forms, such as euphausiids and amphipods which have been found in the new sampler. In surveys of Smith Bank, for example, the new sampler took on the average twice as many amphipods as the van Veen. Samplers with a solid upper surface must set up a considerable down-wave, and this will tend to wash small surface animals out of the sampler's reach. It is also important

that, since the bucket closes completely, no material is lost in hauling. Further, the mesh of the gauze covering is considerably finer than that of the gauze used in sieving.

In its present form, the theoretical maximum depth to which the new sampler can bite is 7 cm., and it is of interest to note that the depth to which it has in fact been digging, calculated from its average volume of 4 l., is 6·7 cm. It is felt that there is a considerable reserve of power in the sampler, and alterations are being contemplated which would give an increased depth of bite.

Once the routine of loading and operating the new sampler has become familiar, it can be used almost as quickly as the van Veen. Working from F.R.S. *Explorer* at a depth of 40 m. the average time taken to complete three dips with the van Veen was 14 min., and with the new sampler 15 min.,—a negligible difference.

SUMMARY

A new bottom-sampler is described. It covers a surface area of $\frac{1}{10}$ m.^{2} and digs to a depth of almost 7 cm. The sampler consists of a bucket which is driven into the soil by springs and is closed by the hauling in of the warp. The apparatus can sample only when resting squarely on the bottom, and was designed to work on hard sand and in bad weather. It has been shown to be successful in both these respects.

Specific tests and experience under various conditions show it to be more generally satisfactory than any of the other samplers used.

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J. Mar. biol. Ass. U.K. (1954) 33, 265–290
Printed in Great Britain

STUDIES ON THE CULTURE OF A MARINE DIATOM

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(Text-figs. 1–16)

Little is known of the biochemistry of diatoms, although many workers have reported growth experiments with the unicellular algae of the marine phytoplankton. Experiments have often been performed without due regard for the appropriate control of physical and chemical conditions. Many reports contain only incomplete data of the growth under a given set of conditions, and it is often impossible to say whether the effects recorded are upon the growth rate, the total crop, or both. Other studies have been reported which included the addition of organic matter to cultures which were only uni-algal and not bacteria-free. At the present time even the mere maintenance of stock cultures of the marine unicellular algae is perforce an empirical matter. Results in replicate cultures often show gross differences in growth that are apparent on inspection by eye alone, and insufficient information is available regarding the nature of these variations in growth to allow the rational development of improved culture media. Due therefore to a lack of suitable techniques, most of the results available are difficult, if not impossible, to interpret in terms of the biochemical activities of the algae.

Quantitative measurements of the growth of bacterial cultures have been widely used by Hinshelwood, Monod and others (Hinshelwood, 1946; Monod, 1942). The technique may be limited in its application, for example in the elucidation of metabolic pathways by the permeability of the cell wall to intermediates, but such studies can furnish much valuable information. Investigations have shown that the growth of bacteria as measured by an increase in cell numbers or cell material generally obeys certain simple rules. The growth can thus be defined by a number of growth constants which reflect the physiological behaviour of the cells. Similar considerations should apply to the growth of any unicellular organism. Comparison of the growth constants under various conditions should therefore provide some information about the suitability of these conditions for culture work, allow the rational development of improved culture media, and make possible fuller interpretation of the results of growth experiments.

It is normal practice at the moment to use natural sea water as a base for culture medium for the marine unicellular algae. This is usually enriched with further amounts of phosphate, nitrate and soil extract. The preliminary investigation reported in this paper has been restricted to experiments in medium of this type.

Definition of Terms Used

In this paper the terminology used by the previous workers on the kinetics of bacterial growth has been largely adopted. The nature of some of the growth phases obtained, however, is such as to lead to difficulties if the traditional nomenclature is followed. These exceptions will be discussed later as they

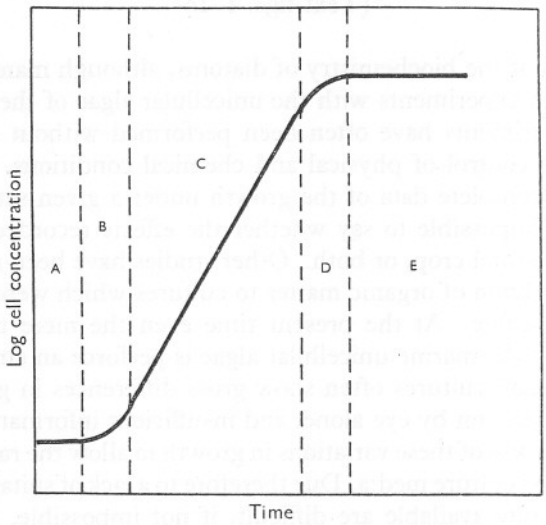


Fig. 1. An idealized growth curve showing the five phases of growth. A, initial stationary phase; B, phase of increasing growth rate; C, exponential phase; D, phase of decreasing growth rate; E, final stationary phase.

arise. The various growth phases are best described in terms of an idealized growth, as shown in Fig. 1. It is obvious that the growth may be expressed in terms of either the increase in cell numbers (cell concentration) or the increase of cellular material (cell density). Since the technique used for estimating growth measures cell concentration, in all that follows the term growth will be used to mean an increase in cell numbers per unit volume.

When cells of the test organism are inoculated into suitable culture medium cell division may or may not start at once. In the latter case there is usually an initial stationary period, during which no increase in cell concentration occurs, though there may be an increase in cell density. The initial stationary phase is followed by a phase of increasing growth rate when the rate of cell division increases with time up to a maximum value. The initial stationary

phase and the phase of increasing growth rate are often considered together and called the 'lag phase'. The lag phase is followed by a phase of regular exponential growth, the cell concentration increasing with time in geometrical progression according to the equation:

$$n = n_0 e^{kt},$$

where n_0 is the original cell concentration and n the cell concentration at time t, k being a constant. During this exponential growth the division rate of the cells remains constant. The extent of the exponential phase is limited by the utilization of nutrients or other modifications in the culture medium caused by the growth of the organism, and it finally leads to a phase of decreasing growth rate. During this phase the growth rate decreases with time until it becomes zero. This last phase is known as the final stationary phase.

It is convenient to depict growth graphically on a plot of $\log_2 n$ (or in some cases $\log_2 n/n_0$) against time. On a plot of this sort an increase of one unit on the ordinates corresponds to one cell division. The lag time is usually measured by a plot of $\log_2 n/n_0$ and extrapolating to zero when the intercept on the time axis gives the lag time. The exponential growth may be characterized by the mean generation time or the time taken for the cell concentration to double. The total growth is the difference between the initial and the final (i.e. during the final stationary phase) cell concentration. These three constants, the lag time, the mean generation time and the total growth, largely characterize the growth obtained under a given set of cultural conditions.

Methods

Organism Used and Maintenance of Stock Cultures

The diatom used in these studies was a bacteria-free strain of *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima* Allen & Nelson. The species was first isolated by Allen & Nelson (1910) and has since been maintained at the Plymouth Laboratory in the Miquel sea-water medium of Allen & Nelson. Unlike many other marine algae, this diatom will grow indefinitely in the absence of soil extract. Because of this, the organism was considered more suitable for the initial studies of the effect on growth of the basic physical and chemical factors than one needing the unknown factors of soil extract. A bacteria-free strain of the organism was obtained by treatment with penicillin and streptomycin as previously described (Spencer, 1952).

Stocks of the organism were maintained by periodic aseptic subculture on solid media (see later for composition). After good growth was obtained, the cultures were removed from the light and stored in the dark at 2° C. Such stored cultures show little loss of viability for periods of up to 6 weeks. Stocks were normally kept on slopes in rimless Pyrex boiling-tubes stoppered with

cotton-wool and illuminated in a north window. The algal growth obtained on solid medium is sometimes somewhat mucilagenous and individual colonies may be quite hard. In neither culture is the growth conducive to even spreading by means of a wire loop. It has been found convenient to subculture the stocks by scraping selected growth off a slope with a wire loop and suspending, by agitation, in sterile sea water until the latter is just observably turbid. Loop-fulls of this suspension may then be spread evenly with a wire loop on the surface of a slope. The resulting growth then consists of numerous discrete colonies. The solid medium used will allow a good growth of many marine bacteria and its use for the maintenance of stocks is useful, since any gross bacterial contamination will at once show itself. The absence of obvious bacterial colonies is nevertheless no proof of sterility. Routine periodic checks of freedom from bacterial and other contamination were carried out by inoculation into a range of bacteriological media (Spencer, 1952) of 1 ml. samples of subcultures in liquid medium.

Passage through a subculture on solid media has a marked morphological effect on the cells. The original stock culture of the organism contained both the normal and the tri-radiate type of cells with a preponderance of the latter (Wilson, 1946). The first visible growth on solid media consisted entirely of naked cells, but normal cells appeared and soon predominated. On subculture to liquid medium, all naked cells disappeared and the culture consisted entirely of normal cells. After many serial subcultures in liquid media the tri-radiate form has not re-appeared. These observations seem in the main to confirm the findings of Barker (1935).

Culture Media

For reasons stated before, the present work has been confined to an examination of growth in media made up from natural sea water. Freshly collected off-shore water (International Hydrographical Station E. 1) was filtered through a Whatman no. 3 filter-paper and then allowed to age by storage in the dark for about 1 month. This ageing process assists uniformity between various batches of media. Difficulty was often experienced due to the formation of a precipitate as a result of autoclaving. This precipitation may perhaps be due to several factors, but experience showed that by following the procedure outlined below it could nearly always be avoided. The water was initially collected and allowed to age in Pyrex glass bottles. Medium was made up with this aged sea water diluted to 75% with glass-distilled water to give a resultant salinity of *c.* 26‰. The sea water was enriched at this stage with 2·0 μg. % manganese (as manganese chloride) and the nitrogen source (normally sodium nitrate) at the required concentration. It was then dispensed into the culture vessels in use, and after plugging with cotton-wool these were sterilized by autoclaving at 5 lb. pressure for 30 min. After cooling and being allowed to re-equilibrate with the carbon dioxide of the air, the

medium was further enriched by additions of 10·0 μg. % iron (as ferric citrate-citric acid: Rodhe, 1948) and phosphate at the required concentration (usually as disodium hydrogen phosphate). These additions were made aseptically from sterile stock solutions of the required strength. In the initial experiments additional silica was added at this point. Experience, however, showed that this addition was without effect on growth at the population densities used. This is not surprising since it is certain that considerable solution of silica occurs during autoclaving (F. A. J. Armstrong, private communication). In all that follows the term 'basic medium' is used to indicate 75% sea water with additions of iron and manganese.

The solid medium used for the maintenance of stock cultures consisted of the basic medium (with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus) solidified with 1·5 g.% agar and further enriched with 0·5 g. % peptone (Oxoid brand, bacteriological peptone). Some growth is obtained on solid media with nitrate nitrogen as the sole nitrogen source, but the addition of peptone causes more prolific growth. Better growth is obtained on media containing both peptone and nitrate than with either alone. Ammonium nitrogen seems to be equivalent to nitrate nitrogen for growth on solid media.

Analytical quality reagents were used throughout.

Culture Apparatus

Details of the culture apparatus used are shown in Fig. 2. For the quantitative experiments, the organism was cultured in 25 × 150 mm. rimless Pyrex boiling tubes. The cultures were aerated by means of a small-bore Pyrex tube which passed to the bottom of the culture tubes and was drawn out to a fine jet. It was found convenient for aseptic handling and for optical determinations of culture density to have the aerating tube held for its entire length against the side of the culture vessel. This was achieved by sealing about 1 in. of the top internal surface of the culture vessel to the aerating tube using 'Araldite' cement. The tubes were plugged with cotton-wool. A small guard filter containing cotton-wool was attached by rubber tubing to the aerating tube of each vessel. The open end of the guard filter containing cotton-wool was attached by rubber tubing to the aerating tube of each culture vessel. The open end of the guard filter was protected by a small inverted test-tube during autoclaving or storage before use.

The culture vessels were cleaned by prolonged scrubbing in hot soap and water followed by continual rinsing in cold tap water and finally by two rinses with hot distilled water. After much use the culture vessels often became coated with a greasy substance which was very resistant to the normal washing procedure. This could usually be removed by boiling the culture vessels in a soap-and-soda solution followed by prolonged rinsing in hot water.

The culture tubes were held in a rack and immersed in water to within 1 in.

of their tops in a glass-sided tank. This was situated in a constant temperature room without natural light. The water on the bath was rapidly circulated and thermostatically controlled at any desired temperature with an accuracy of $\pm 0{\cdot}2^{\circ}$ C.

Compressed air was well washed by passage through two Henrichi gas-washing bottles and then filtered by passage through an aspirator completely filled with cotton-wool. This was frequently autoclaved. The sterile air was

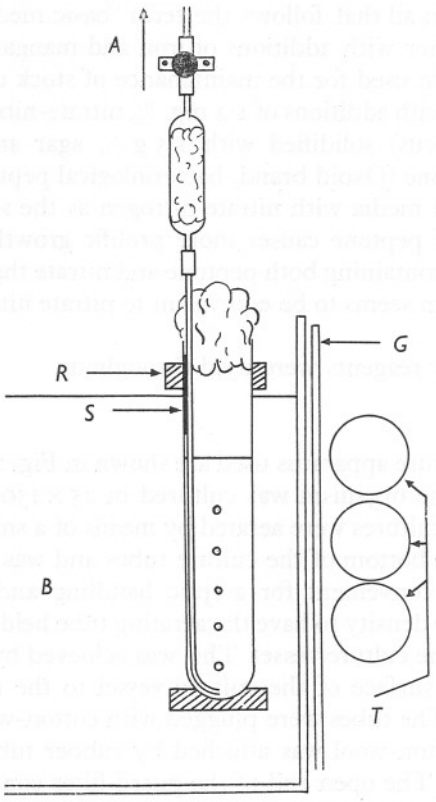


Fig. 2. Diagram of the culture apparatus used for the quantitative culture of *N. closterium*. *A*, to air supply; *B*, constant temperature bath; *G*, opal glass screen; *R*, rack; *S*, araldite seal; *T*, 80 W fluorescent tubes.

then passed into a glass manifold which ran out over the culture vessels in the constant temperature bath. The outlets of the manifold were connected by rubber tubing to the individual guard tubes on each culture vessel. The supply of compressed air was controlled by a master pin valve and a screw clip on each manifold outlet. Air was bubbled through each tube at a constant rate of about 50 ml./min.

Illumination was provided by three 80 W 5 ft. fluorescent tubes. These were fixed one above the other at one side of the glass tank and extended its whole length. The intensity of illumination of the cultures could be varied by inserting opal glass screens between the lights and the side of the tank.

No attempt has been made during the present studies to investigate systematically the effects of changing the lighting conditions. Examination of the emission spectrum of 'day-light' fluorescent tubes showed them to be poor in light in the red region of the spectrum. Since chlorophyll has an absorption band in this region it was thought advisable to include some red light in the illumination. This was done by using two 80 W 'day-light' tubes and one 80 W red fluorescent tube. Preliminary experiments showed that if the culture vessels in the constant temperature bath were placed at a distance of about 3 in. from the lamps they received an incident illumination of approximately 12,000 lux. It was shown that this intensity of illumination was such that decreasing it by 25% had no effect on growth up to cell densities equivalent to a limiting initial nitrate-nitrogen concentration of 1·2 mg. %. The degree of agitation provided by aeration proved to be sufficient to prevent mutual shading by the cells at the cell concentrations used. The conditions were therefore considered to be light saturated with respect to growth.

Estimation of Growth

Several previous workers have considered the use of optical-density measurements of algal cultures as a means of estimating growth (Rodhe, 1948; Gross & Koczy, 1946; and Osterlind, 1949). For the present studies a Harvey absorptiometer (Harvey, 1948) has been modified to project a parallel beam of light through the culture. The optical density of cultures was read, using the culture vessels as cuvettes. These were inserted into the light path of the absorptiometer and held in a specially made Perspex cell containing water. This fitted into a V-shaped channel in the absorptiometer and was automatically aligned. The base of the cell contained a circular depression to hold the bottom of the culture tube, the removable lid to the cell having a circular hole of the same diameter as the culture tube. The depression and hole were made so that the culture vessel was held vertically. Access to the Perspex cell was by a coincident hole in the case of the absorptiometer, which could be covered by a sliding shutter when not occupied by a tube. Each culture vessel was marked so that it was placed in the Perspex cell in exactly the same position each time, with the aerating tube to the side and out of the way of the light path. The light path was cut down to a circular beam 6 mm. in diameter by means of a stop on the incident light side of the Perspex cell. This served to minimize any disturbing effect from the curved surface of the culture vessel and eliminate interference with the light beam by the aerating tube.

To correct for individual differences in the culture vessels the following procedure was adopted for measuring the optical density of cultures. The

Perspex cell containing water was used throughout as a fiduciary standard. The culture vessel containing the complete medium was placed in the absorptiometer and the small optical density (E_0') due to the tube and contents measured. The medium was inoculated with cells and the optical density again determined (E_0''), the difference between these two values giving the initial optical density of the culture (E_0). The value of E_0' for each tube was subtracted from all subsequent optical density readings of the culture to give the true value of E at time t.

Initial experiments largely confirmed the findings of earlier workers in that the optical density is approximately proportional to the number of cells per unit volume (cell concentration) up to an optical density of about 1·0. But, again as in the earlier work, it was found that there is no constant relationship from culture to culture between the optical density per unit thickness of culture and cell concentration. The relationship varies, amongst other things, with the physiological state of the cells and their content of chlorophyll. Preliminary experiments had demonstrated that a suspension of the organism shows no sharp absorption peaks in the visible when examined with a Unicam spectrophotometer. However, experience showed that the best results are obtained by using red filters (Chances OR 1). Investigations with various cell suspensions of equal cell concentration showed that there is a considerable variation in the ratio between the loss of light due to scattering and the loss of light due to absorption. By varying the optical system it was found possible to arrange that the optical density as measured approximated to that due to absorption alone. This was done by using a small stop on the incident light side of the Perspex cell and a large stop which exposed the whole of the active area of the photocell on the transmitted light side of the Perspex cell. The Perspex cell was fitted in the absorptiometer as close as possible to the photocell. These conditions ensure that nearly all the light which is scattered at an angle of less than 90° to the incident is received by the photocell. On the other hand, with the Perspex cell situated at some distance from the photocell and with a stop in the incident light the same size as that in the transmitted light path, all scattered light is lost and the optical density as measured includes the light lost by both absorption and scattering. The relationship between the optical density of a growing culture of *N. closterium* due to absorption and scattering, the optical density due to absorption alone, and the cell concentration is shown in Fig. 3.

It will be seen that a growth curve drawn from optical density measurements in the 'near' position approximates to that obtained by using cell concentrations. The rate of increase of this component of optical density is comparable to the rate of increase of cell concentration. This is not so with the curve drawn from the optical density measurements in the 'far' position. The relationship between the two optical density measurements and the cell concentration at various points during the growth is shown in Table I.

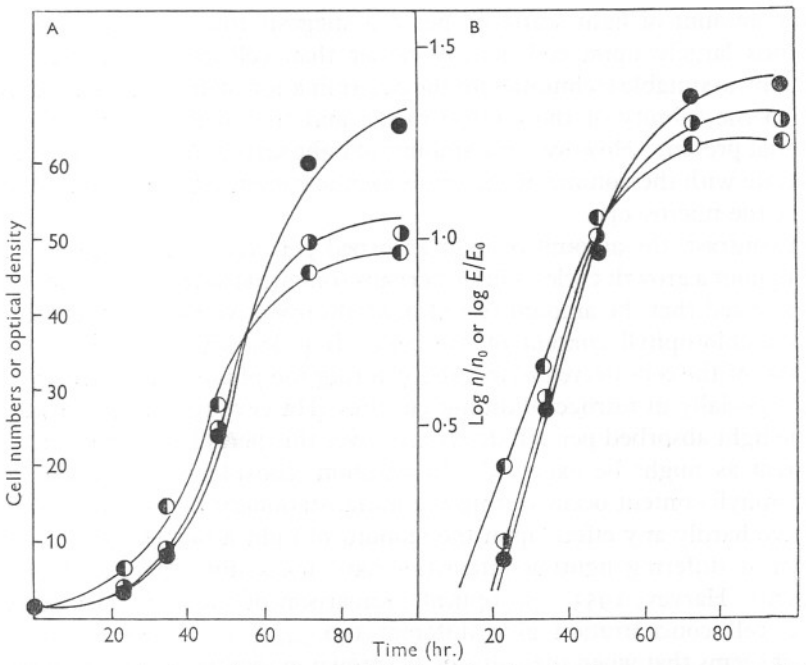


Fig. 3. The growth of a typical culture of *N. closterium* showing the growth curves obtained by using: (*a*) cell concentration, –●—●–; (*b*) optical density measurements in the 'near' position, –◑—◑–; (*c*) optical density measurements in the 'far' position, –◐—◐–. In A the ordinates represent arbitrary values of cell concentration and optical density calculated as follows: cell concentration, numbers per ml. $\times 10^{-5}$; optical density in the 'near' position, $E \times 2{\cdot}5 \times 10^2$; optical density in the 'far' position, $E \times 3{\cdot}7 \times 10$. Optical densities measured using Chances OR 1 filters. In B are given the corresponding logarithmic plots.

TABLE I. VARIATION IN THE LIGHT SCATTERED PER CELL AND THE LIGHT ABSORBED PER CELL THROUGHOUT THE GROWTH OF A CULTURE OF *NITZSCHIA CLOSTERIUM*

	Ratio O.D. per 10^6 cells per ml.					
Optical system	0 hr.	23 hr.	34 hr.	48 hr.	72 hr.	96 hr.
Optical density in 'near' position (approximates to light absorbed)	0·040	0·046	0·044	0·042	0·035	0·032
Optical density in 'far' position (approximates to light absorbed + light scattered)	0·268	0·490	0·420	0·310	0·202	0·199

The amount of light scattered per cell varies greatly throughout a growth cycle. It increases markedly during the commencement of exponential growth. Thereafter it declines steadily until the onset of the phase of decreasing growth rate when it exhibits a rapid decrease. The significance of measurements of $O.D._{(scattered\ light)}$ must remain speculative. However, the variations

in the amount of light scattered per cell suggests that this optical property depends largely upon cell density rather than cell concentration. Unfortunately no suitable techniques for the determination of dry weight are available due to the salinity of the culture media and the small amount of cellular material present. However, the amount of light scattered per cell does at least correlate with the volume of the protoplasmic content of the cells as observed under the microscope.

In contrast, the amount of light absorbed per cell is much more constant throughout a growth cycle. This is perhaps somewhat surprising since it might be expected that the amount of red light absorbed would be greatly affected by the chlorophyll content of the cells. It is known that the chlorophyll content of the cells decreases markedly during the phase of decreasing growth rate especially in nitrogen-limiting cultures (Harvey, 1953). Some variation in the light absorbed per cell does occur over this period, but the effect is not as great as might be expected. In addition, considerable increases in the chlorophyll content occur during the initial stationary phase, but these seem to have hardly any effect upon the amount of light absorbed per cell. Cells grown at differing lighting intensities have markedly different chlorophyll contents (Harvey, 1953) and optical comparison of such cell suspensions of equal cell concentration give different $O.D._{(absorbed\ light)}$ values. Nevertheless, it seems that when the cultures are grown in identical lighting conditions and, providing iron is in ample supply (Rodhe, 1948), the variation in the component $O.D._{(absorbed\ light)}$ per cell due to variations in the chlorophyll content are largely outweighed by other more constant factors. With the organism used, the overall size of the frustule seems to vary little throughout a growth cycle. Possibly it is this part of the cell which is the major factor controlling $O.D._{(absorbed\ light)}$.

To summarize, measurements of $O.D._{(absorbed\ light)}$ provide a method of comparing the cell concentrations of cultures of the organism used. Comparisons must be made between cultures in the same physiological states, grown in identical lighting conditions, and cultured with an adequate supply of iron. It is perhaps unwise to apply the method to any but bacteria-free cultures of the organism because of possible interference by bacterial growth. The cells of the culture must be evenly distributed and this necessitates continual agitation throughout growth, since microscopical examination shows that very considerable agitation is needed to break up all the cell clusters in a culture which has grown undisturbed for any time. The formation of precipitates in the culture medium must be avoided. This may be difficult since precipitation which is undetectable to the naked eye can occur in the course of the growth of a culture in the media used.

It must be emphasized that these considerations apply only to cultures of *N. closterium* and may not be applicable to algal cultures in general. It is known that the chlorophyll per cell decreases markedly during the phase of

decreasing growth rate, especially in nitrogen-limiting cultures (Harvey, 1953). During this phase, and the subsequent exhaustion phase, the optical density increases more slowly than the cell concentration, and in consequence there is no strict proportionality between the optical density and the cell concentration. During these phases of growth variations of up to 23% have been observed in the cell concentration corresponding to a given optical density.

Growth Experiments

The Exponential Growth Phase

It has been found possible using the cultural conditions described to obtain regular exponential growth of *N. closterium*, and under these conditions the mean generation time has remained remarkably constant over many subcultures.

Effect of Changed Cultural Condition

Before regular exponential growth can be obtained, the cells must be adapted to the physical and chemical conditions of the medium. Any change in these factors causes a period of irregular growth which may extend over many generations. Several factors, a change of which causes a period of irregular growth, have been investigated and will be discussed below. Fig. 4 shows some typical examples of irregular growth compared with the regular exponential growth obtained with cells adapted to the cultural conditions.

Curve I shows the typical regular exponential growth exhibited by cells adapted to the cultural conditions by previous growth in the normal basic medium enriched with additions of 0·6 mg. % nitrate-nitrogen and 0·6 mg. % phosphate-phosphorus per litre and grown with continuous illumination and aeration at 19° C. Curve II shows the nature of the growth obtained on subculture of these cells at 9° C. under otherwise identical conditions. Curve III is the growth obtained when cells adapted to the conditions of curve I are subcultured into medium containing 0·3 mg. % phosphate-phosphorus per litre, other conditions being unchanged. A similar irregular growth obtained on changing the cultural conditions is shown in curve IV. In this case cells grown on solid media were subcultured to the normal basic media under conditions identical to those under which the growth in curve I was obtained.

The irregular growth obtained on changing certain cultural conditions is generally characterized by periods during which no cell division occurs or of a much reduced division rate, alternating with periods of division at a greater rate than exhibited by adapted cells under the same conditions. That the phenomenon is essentially a lack of adaptation to changed cultural conditions is indicated by the observation that continued subculture in the new conditions finally leads to regular exponential growth being established.

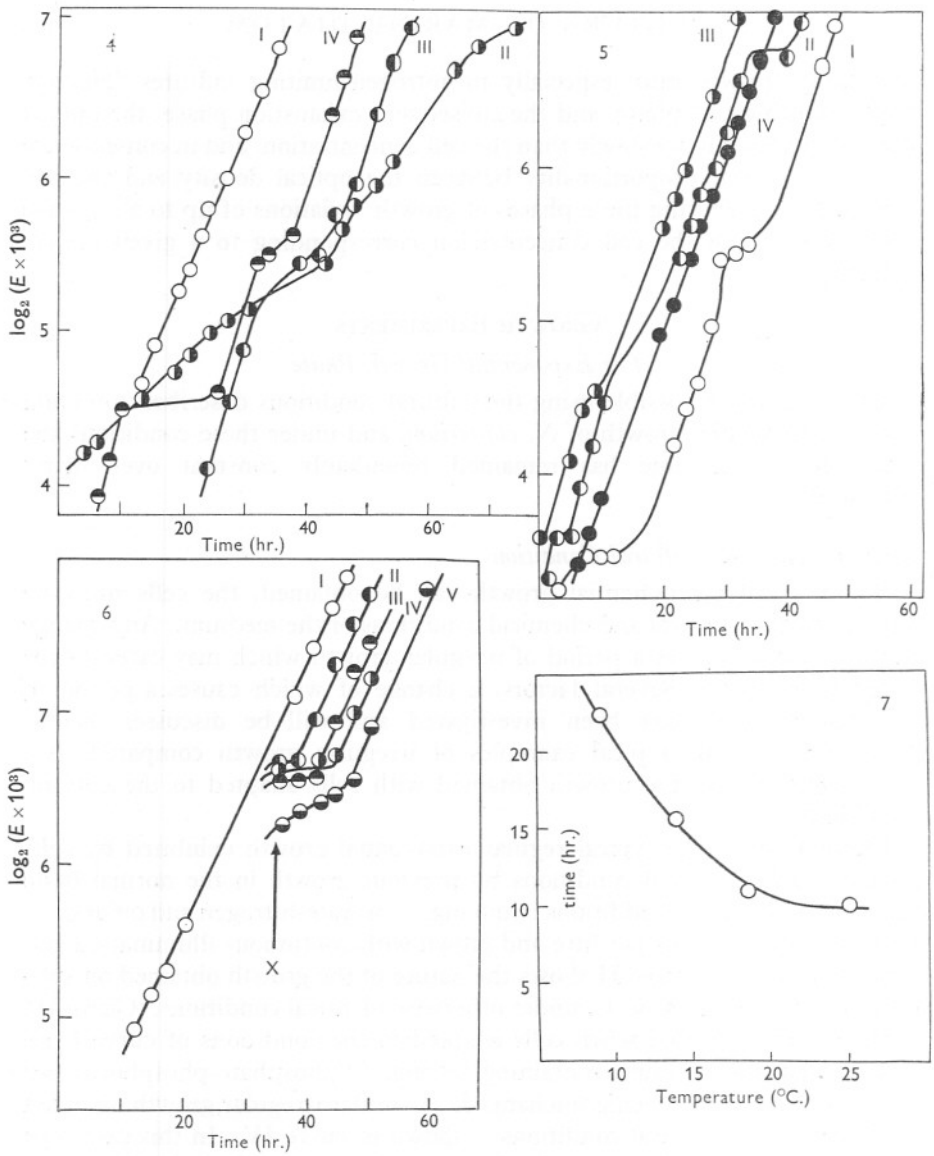


Fig. 4. Typical growth curves obtained with *N. closterium* by adapted (curve I) and by unadapted cells (curves II, III and IV). For details of culture conditions see text.

Fig. 5. The adaptation to changed cultural conditions. Parent culture grown on usual solid medium at room temperature in natural light from a north window and transferred to basic liquid medium with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration. Curves I–IV, second to fifth subcultures respectively.

Fig. 6. Replicate cultures in the basic medium with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus, grown to logarithmic phase at 19° C. with continuous illumination and aeration. After 34 hr. growth (*X*) cultures subjected to various periods of darkness and then re-illuminated.

Fig. 7. Variation of the mean generation time with temperature of cultures grown in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus with aeration and continuous illumination.

Fig. 5 shows the gradual adaptation to changed cultural conditions of cells which have been grown on solid medium in a north window in natural light at room temperature on subculture to basic liquid medium at 19° C. with constant illumination and aeration. The first subculture under the new conditions is not shown in the diagram. In this case five successive subcultures (together representing 20 to 25 divisions) had to be made before the cells became adapted to their new environment. This was one of the longest adaptation periods observed; sometimes as little as four divisions were sufficient to obtain regular exponential growth. In general the length of the adaptation period varies directly with the magnitude of the change in the cultural conditions. Once adaptation has been achieved the regularity of the exponential growth depends only upon the constancy of the prevailing cultural conditions.

When a culture of the organism is growing exponentially, the growth rate represents the resultant velocity of the whole series of reactions whereby cell substances are synthesized. The growth rate corresponds to the overall rate of a steady state system, the velocity constant of this system depending upon the resultant of the velocity constants of all the reactions involved. During such growth all the properties of the cell are constant, and any change in the cultural conditions may necessitate a change in the concentration of intermediates and enzymes. This re-organization may prevent regular exponential growth from being re-established immediately. It is possible that the irregular growths obtained are explicable in these terms.

The periods of very rapid growth alternating with periods of growth at a much reduced rate that are characteristic of unadapted cells suggest that the processes of cell division are in some way inhibited, whereas the synthesis of cell material may continue. In consequence, when cell division can occur it does so at a more rapid rate than normally by utilizing intermediates that have accumulated during the period of low division rate. The periods of rapid growth are soon succeeded by growth at the normal or at a suboptimal rate, presumably as the factors controlling cell division again come into play.

Effect of Dark Periods

The lighting conditions were such that the cells can be considered light-saturated with respect to growth. Under these conditions, periods of darkness cause an almost complete cessation of growth (Fig. 6). In the typical experiment shown re-illumination after up to 12-hr. periods of darkness allows an immediate resumption of regular exponential growth at the same rate as before. The experiment shown in Fig. 6 was performed with quintriplicate cultures all inoculated at the same time. The variations in cell concentrations after 36 hr. in cultures I, II, III and IV are in the same ratio as, and explicable by, variations in the size of the inocula. This is not so with curve V, the growth of which was apparently retarded by some unknown factor. It will be

seen that during the period in the dark this culture exhibited a slow but marked growth until the cell concentration reached a value comparable with that of the other cultures before the illumination was stopped. It would seem that if growth is held back by inhibitory factors or deficiencies which primarily affect metabolic channels other than those involved in photosynthesis, some cell division can occur in subsequent dark periods. Presumably this occurs by utilization of 'excess' carbon skeletons formed during prior periods of photosynthesis.

It is interesting to compare these results with those obtained in similar experiments by Rodhe (1948). He observed a decrease in the optical density of cultures during dark periods and an initial 'growth' on re-illumination at a very rapid rate. This gradually dropped to a much slower rate. In the experiments reported in this paper no such effects during or after dark periods have been observed. There must have been respiratory losses during the dark periods, and the reason that these did not show up in optical-density measurements during the present studies can only mean that the optical density as measured is a function of cell concentration rather than cell density. On the other hand, it seems that Rodhe's optical-density measurements must have been a function of cell density. If this were so, the more rapid 'growth' he observed after dark periods could represent an initial rapid synthesis of cell material only, the division rate of the cells remaining unaffected.

Effect of Temperature

The exponential growth rate depends upon the temperature. The results of a preliminary investigation on the variation of growth rate with incubation temperature is shown in Fig. 7. The mean generation time falls rapidly with increasing temperature, reaching a minimum for these cultural conditions at about 20° C. Further increases in temperature have little effect on growth rate but no inhibitory effect is observed up to 25° C. All inocula for the experiments summarized in Fig. 7 were taken from a parent culture grown in media of identical composition at 19° C. A 10° C. change in temperature causes a period of irregular exponential growth which lasts for about four divisions. No adaptation period is required on subculture from 19 to 14° or 25° C. Between 9 and 20° C. the Arrhenius Law is approximately obeyed as might be expected. It is to be expected that further increase in temperature above 25° C. will lead to a rapid increase in the mean generation time followed by a complete cessation of growth resulting from denaturation of proteins and disorganization of the cell.

Effect of Carbon Dioxide Tension

For regular exponential growth to continue, chemical as well as physical conditions must be constant. Under normal cultural conditions the concentration of nutrients is not constant due to their continued utilization during

growth, and division is stopped soon after the supply of one or more nutrients becomes exhausted. In the absence of aeration, the rate of diffusion of carbon dioxide into sea-water medium at a pH of about 8·6 is sufficiently slow to cause the supply of carbon dioxide for photosynthesis frequently to become limiting. This is clearly shown in Fig. 8 in which typical growths obtained with and without aeration are shown. It will be seen that, in the absence of aeration, the growth rate is soon affected and quickly drops almost to zero. In unaerated cultures the pH may rise to a value of 9·4 or higher before growth is stopped. The inhibition of growth in the unaerated culture is immediately reversed when aeration is recommenced.

It is apparent that aeration is necessary in cultures of the cell concentration used if the supply of available carbon for photosynthesis is not to drop below growth saturating levels. However, at least up to cell concentrations corresponding to a limiting nutrient concentration of 1·0 mg. % nitrate-nitrogen, it seems that bubbling with air will keep the concentration of available carbon sufficiently high to maintain regular exponential growth. A limiting nutrient concentration of 1·0 mg. % nitrate-nitrogen corresponds to a cell concentration of about 15×10^6 cells per ml. pH values in the region of 9·0 often occur in the later stages of regular exponential growth in aerated cultures. It is impossible to say from these results whether growth in non-aerated cultures is primarily inhibited by a shortage of available carbon or by the adverse effect of high pH. There is no evidence to suggest that at these growth rates and cell concentrations it is necessary to increase the partial pressure of carbon dioxide in the aerating gas to prevent this nutrient from becoming rate limiting. However, utilization of available carbon is sufficiently rapid to cause the pH to rise, and it may be advisable sometimes to use a higher partial pressure of carbon dioxide to obtain greater control of pH.

Effect of Other Nutrients

For maximum growth rate the concentration of the nutrients other than carbon dioxide must be such that all enzymic and other active centres are saturated with their respective substrates. No effect on the initial growth rate can be observed by reducing the nitrate-nitrogen concentrations or the phosphate-phosphorus concentrations down to 10 μg. %. Similarly, increases in the concentration of iron or manganese have no effect on the exponential growth rate. Consequently, it seems that the concentrations of these substances normally used are sufficient to saturate all active centres. It would be expected that, as the exponential growth rate becomes dependent upon a nutrient concentration at lower values, a hyperbolic relationship will exist between nutrient concentration and growth rate. However, special methods will be needed to determine the growth rate at these very low nutrient concentrations, since the total growth under these conditions is insufficient

to permit an accurate determination of growth rate by the techniques used in the present studies.

The Total Crop

The Onset of the Phase of Decreasing Growth Rate

The total growth in any medium will be limited by the onset of one of several conditions. Amongst the factors which may cause cessation of exponential growth and the onset of the phase of decreasing growth rate, Monod (1949) lists the following: (i) the exhaustion of an essential nutrient, (ii) the accumulation of inhibitory metabolic products, and (iii) changes in the ionic equilibrium of the medium caused by the growth of the organism, for example pH. The culture conditions outlined above include an adequate supply of carbon in photosynthetically available form and thereby some degree of control of pH and there is no evidence that the supply of iron or manganese is limiting. The total growth should therefore be controlled only by the initial concentration of either nitrate or phosphate. Fig. 9 gives the latter part of a typical growth curve, showing the gradually decreasing growth rate typical of the exhaustion of a nutrient. The culture in this experiment was adapted to the prevailing conditions. It may be demonstrated that the decline of exponential growth is due to exhaustion of nitrate or phosphate since further additions of the limiting nutrient allow a resumption of growth. In this particular case the limiting nutrient was nitrate, but similar curves may be obtained in phosphate-limiting cultures. It will be seen that the growth rate gradually decreases but does not finally become zero. The subsequent slow regular increase in the optical density of such cultures continues for a considerable time and has been followed for as long as 72 hr. after the onset of this phase. Cell counts have been conducted on cultures exhibiting this phenomenon, and the slow increase in optical density indeed represents a continued slow division. The rate of cell division during this phase depends to some extent upon the cell concentration, there being a tendency for it to be greater with higher cell concentrations.

This slow regular increase of cell concentration after the phase of decreasing growth rate may be due to either the continual addition to the media of limiting nutrient via the aerating gas (e.g. ammonia), or to autolysis of senile cells regenerating limiting nutrient in a form available to the remaining viable cells. Because of this slow cell division it is considered undesirable to call this final phase the stationary phase. It will therefore be referred to as the exhaustion phase.

Adaptation to Increased Limiting Nutrient Concentration

In experiments in which the growth of the diatom has been followed in cultures containing an adequate and constant amount of phosphate and varying amounts of nitrate another form of adaptation to changed cultural

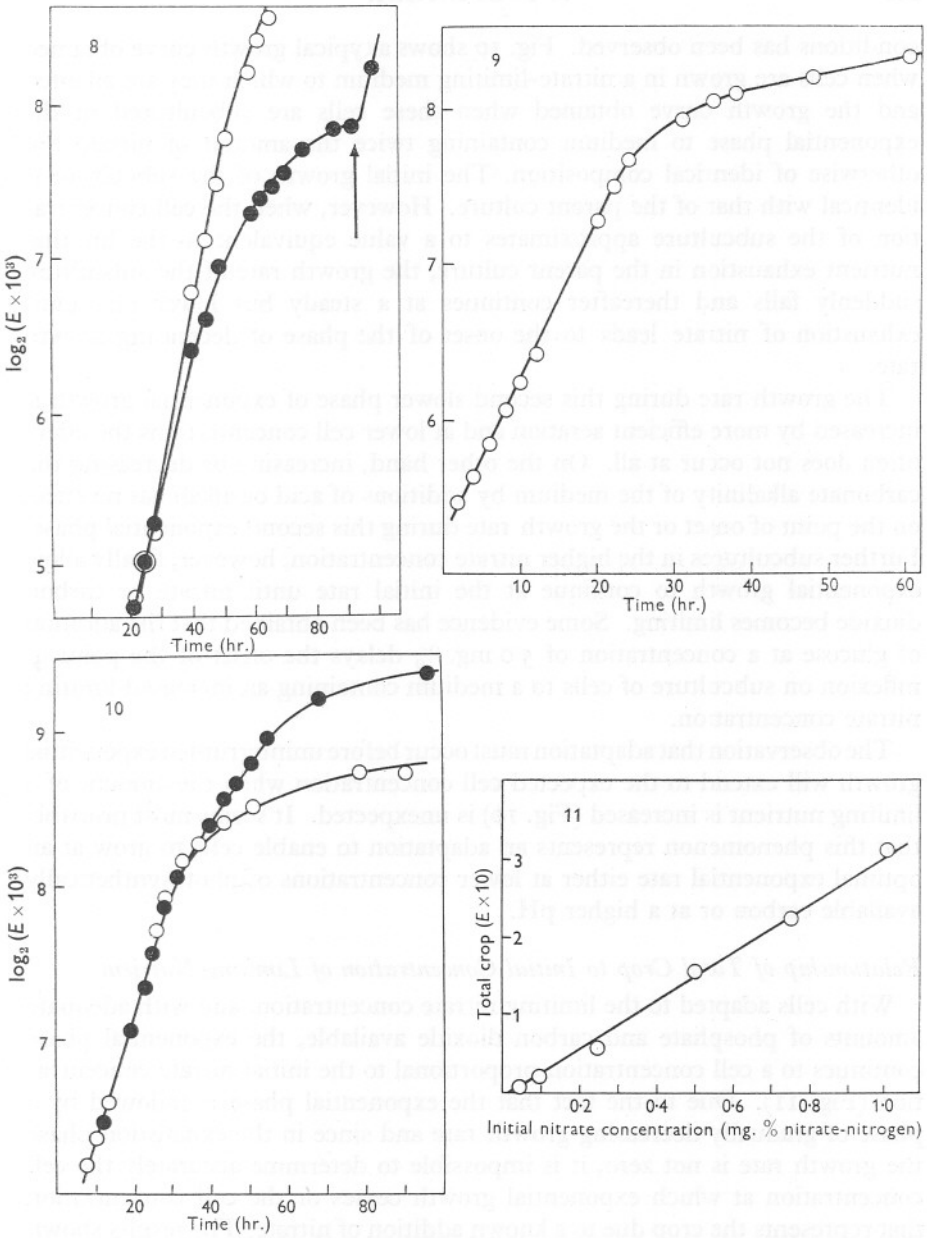


Fig. 8. Growth of *N. closterium* in the basic medium with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus with continuous illumination at 19° C. –○—○–, culture aerated; –●—●–, unaerated. Arrow indicates aeration restarted.

Fig. 9. Growth in basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus with continuous illumination and aeration at 19° C. showing the decrease in growth-rate caused by nitrate exhaustion.

Fig. 10. Growth in basic medium with additions of 0·3 mg. % phosphate-phosphorus with continuous illumination and aeration at 19° C., with varying amounts of nitrate as limiting nutrient. –○—○–, growth of parent culture with 0·6 mg. % nitrate-nitrogen as limiting nutrient. –●—●–, growth of subculture with 1·2 mg. % nitrate-nitrogen as limiting nutrient.

Fig. 11. The variation in the total crop when grown in the basic medium with additions of 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration and with varying initial amounts of nitrate as the limiting nutrient.

conditions has been observed. Fig. 10 shows a typical growth curve obtained when cells are grown in a nitrate-limiting medium to which they are adapted and the growth curve obtained when these cells are subcultured in the exponential phase to medium containing twice the amount of nitrate but otherwise of identical composition. The initial growth of the subculture is identical with that of the parent culture. However, when the cell concentration of the subculture approximates to a value equivalent to the limiting nutrient exhaustion in the parent culture, the growth rate of the subculture suddenly falls and thereafter continues at a steady but lower rate until exhaustion of nitrate leads to the onset of the phase of decreasing growth rate.

The growth rate during this second slower phase of exponential growth is increased by more efficient aeration and at lower cell concentrations the effect often does not occur at all. On the other hand, increasing or decreasing the carbonate alkalinity of the medium by additions of acid or alkali has no effect on the point of onset or the growth rate during this second exponential phase. Further subcultures in the higher nitrate concentration, however, finally allow exponential growth to continue at the initial rate until nitrate or carbon dioxide becomes limiting. Some evidence has been obtained that the addition of glucose at a concentration of 5·0 mg. % delays the onset of the point of inflexion on subculture of cells to a medium containing an increased limiting nitrate concentration.

The observation that adaptation must occur before uninterrupted exponential growth will extend to the expected cell concentration when the amount of a limiting nutrient is increased (Fig. 10) is unexpected. It seems most probable that this phenomenon represents an adaptation to enable cells to grow at an optimal exponential rate either at lower concentrations of photosynthetically available carbon or at a higher pH.

Relationship of Total Crop to Initial Concentration of Limiting Nutrient

With cells adapted to the limiting nitrate concentration, and with adequate amounts of phosphate and carbon dioxide available, the exponential phase continues to a cell concentration proportional to the initial nitrate concentration (Fig. 11). Due to the fact that the exponential phase is followed by a phase of gradually decreasing growth rate and since in the exhaustion phase the growth rate is not zero, it is impossible to determine accurately the cell concentration at which exponential growth ceases or the cell concentration that represents the crop due to a known addition of nitrate. The results shown in Fig. 11 are expressed in terms of an arbitrary value for the total crop. This has been defined as the cell concentration at the intercept when the exponential growth and the exhaustion phase are extrapolated to meet. Corrections must also be applied for the amount of available nitrogen added with the inoculum and the amount in the sea water used for making up the media. Providing this

is done the crop is directly proportional to the initial concentration of the limiting nutrient. It is independent of the growth rate and of the inoculum size.

The results described above are for nitrate-limiting cultures. If the concentration of phosphate is made limiting analogous results are obtained with respect to the relationship between total crop and initial concentration of the limiting nutrient. In general, the total crop produced by a given limiting concentration of phosphate-phosphorus is equivalent to the total crop given by ten times this amount of nitrate-nitrogen.

The Initial Phases of Growth

Effect of Limiting Nutrient Exhaustion

Providing a sufficiently large inoculum is used, cells subcultured from the exponential phase into fresh medium of identical composition immediately continue growth at the same rate. In contrast, cells which are subcultured from the exhaustion phase do not grow immediately but exhibit an initial stationary phase. Fig. 12 shows the growth of inocula subcultured (*a*) from the exponential phase, (*b*) from the early exhaustion phase, and (*c*) after storage in the exhaustion phase for 4 days. The growth curves show an increasing lag time (as measured by the intercept of exponential growth on the time axis) related to the state of exhaustion of the parent culture. In addition, cells which have been stored in the exhaustion phase for several days show an initial phase of slow growth between the initial stationary phase and the onset of growth at the optimum exponential rate. Consequently, if lag time for such cultures is measured as above it includes a period of 'apparent lag' during which some cell division is occurring.

The variation of lag time with the condition of the cells of the inoculum is shown in Fig. 14. The upper part of the figure shows the growth curve of the parent culture which was inoculated with nitrate-exhausted cells. The lower part of the figure shows the lag times observed in daughter cultures in medium of the same composition inoculated at intervals with samples from the parent culture. The cells in each sample were sedimented by centrifuging and resuspended in fresh medium to avoid carrying over any old culture medium with the inocula. The size of each inoculum was adjusted so that each daughter culture initially had approximately equal cell concentrations.

Cells subcultured during the initial stationary phase of the parent culture show a decreasing lag, but this does not become zero until the cells of the parent culture have been growing for some hours. Throughout the exponential phase of growth cells show no lag on subculture, but the lag time rapidly increases as the cells of the inoculum pass into the phase of decreasing growth rate. The lag continues to increase rapidly for the first part of the exhaustion phase. The rate of increase in lag time then declines, and after about 30 hr. in the exhaustion phase its rate of increase reaches a steady value.

The length of the lag period is clearly a function of the previous history of the cells and the long lags produced when the cells of the inoculum have been stored in the exhaustion phase for a long time must presumably be associated with a large number of reactions. Included in these must be the restoration of deficiencies caused by exhaustion of the limiting nutrient. There is considerable evidence to show that algal cells exhibit cell division after complete limiting nutrient utilization with the production of 'deficient' cells (Ketchum, 1939;

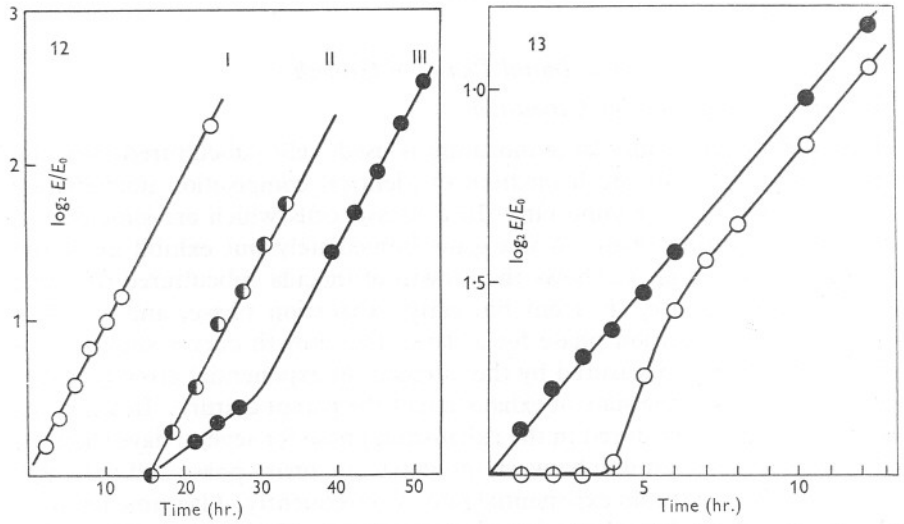


Fig. 12. Initial growth of *N. closterium* in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration. The cells of the inocula were adapted to these conditions by previous culture in the same media. The inoculum was of cells (I) from the exponential growth phase, (II) from the early exhaustion phase, (III) held with continuous illumination and aeration at 19° C. for 5 days in the exhaustion phase.

Fig. 13. Initial growth in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration on subculture to fresh medium. —●—●—, large inoculum. —○—○—, small inoculum. The cells of the inocula were adapted to the cultural conditions by previous subculture.

Harvey, 1953). Comparison of measurements of growth by $O.D._{(absorbed\ light)}$ and $O.D._{(absorbed\ light+scattered\ light)}$ in the early phases of growth correlate well with the assumption that the initial stationary period consists of an increase in cell density, but not cell concentration.

The initial stages of suboptimal growth obtained after long storage in the exhaustion phase (Fig. 12) might be caused by a proportion of the cells being capable of division at suboptimal rates. The magnitude of the effect tends to increase with increasing age of the inoculum as would be expected if this were so. The addition with the inoculum of a number of totally non-viable cells would not produce this type of growth.

Effect of Inoculum Size on Initial Growth

Cells subcultured during the exponential phase of growth show no lag if a sufficiently large inoculum is used. However, if smaller inocula are used a short lag is sometimes produced (Fig. 13). Close examination of the initial growth of subcultures using small inocula shows that the true division lag is greater than that obtained by extrapolating the normal exponential growth

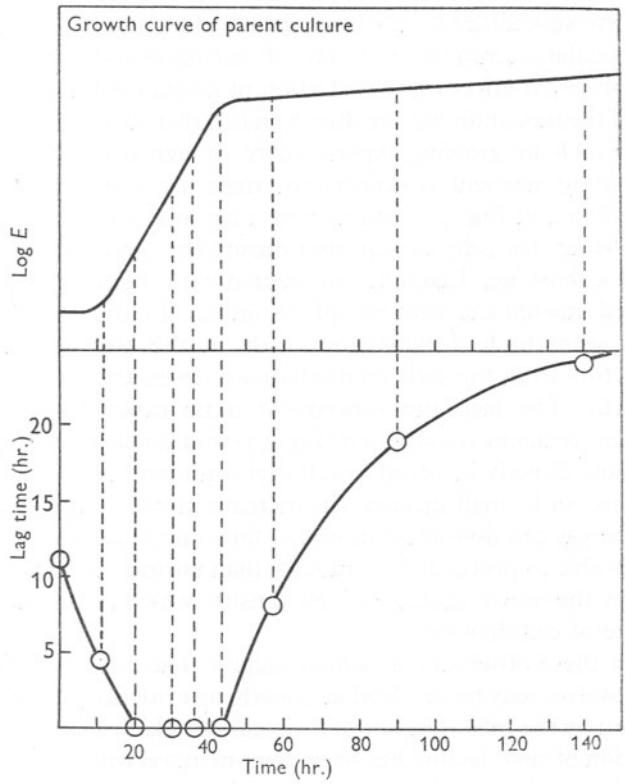


Fig. 14. Variation of the lag time (in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration) of cells of *N. closterium* with the age of the inoculum. The cells of all inocula were adapted to the cultural conditions by previous subculture.

to $E/E_0 = 1$. This is due to an initial period of more rapid growth, as shown in Fig. 13.

All the results on the determination of lag reported above were obtained by using inocula of cells separated from the old culture medium by centrifuging. The more normal method of subculture is to transfer a few ml. of the parent culture to the new media and some old culture medium is therefore added with the cells of the inoculum. Comparison of the lag times observed when old culture medium was or was not transferred with the cells of the inoculum has

shown no appreciable difference between the subsequent lags and growths produced when exhausted cells were used as an inoculum. If the subsequent lags and growths obtained using small inocula on subculturing cells from exponential growth to fresh medium and into re-enriched cell-free culture medium taken from an exponential culture are compared, somewhat irregular results are obtained. Nevertheless, these results do give a strong indication that under some conditions at least cells show less lag (of the type shown in Fig. 13) when subcultured into re-enriched old culture media than they exhibit on inoculation into new medium. It therefore seems possible that the lack of any observed effect on the addition of a small amount of old culture medium with the inoculum may be due to an insufficient amount being carried over. Cells which are growing exponentially, though at a reduced rate due to a higher limiting nutrient concentration than the one to which they are adapted (as shown in Fig. 10), often show a lag of 4–5 hr.

It is interesting that cells subcultured during the early part of exponential growth show a short lag. Taken in conjunction with the results obtained with different-sized inocula and with the effects on lag of old culture media, these observations seem to find explanation only in the assumption that some diffusable factors from the cells themselves are necessary for optimum exponential growth. The lags and subsequent more rapid initial growth rate observed when small inocula are used (Fig. 13) would make it appear that these factors are most directly involved in cell division than in the synthesis of cell material. Thus, with small inocula, the increase in cell density may continue normally, whereas cell division is inhibited for some time. When cell division can start, it is able to proceed more rapidly than normal for a short time until it is limited by the rate of increase of cell density, which presumably normally limits the rate of cell division.

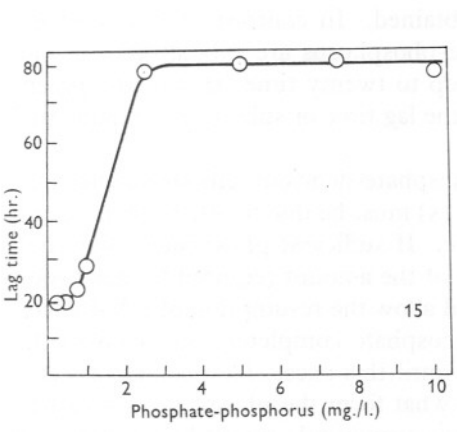
Apart from these observations, which suggest that diffusable factors from the cells themselves may be involved in growth, no evidence has been found for the production by the cells of inhibitory substances which tend to limit growth. The production of such factors has been demonstrated with *Chlorella* by Pratt (1940) and his associates and for a species of *Nitzschia* by Denffer (1948). It remains possible that the 'adaptation' of *Nitzschia* cells to an increased concentration of a limiting nutrient may involve an adaptation to a higher concentration of a diffusable inhibitory factor, produced by the cells themselves.

Lags Induced by Varying the Initial Phosphate Concentration

All the lags considered up to now are exhibited by cells adapted to the initial nutrient concentration of the medium. Subculture to widely different nitrate-nitrogen concentrations of nitrate-exhausted cells has no effect on the lag times produced. However, if exhausted cells which have been grown in phosphate-limiting media are subcultured to new medium with an increased phosphate concentration longer lag times are produced (Fig. 15). It will be

seen that the lag time increases with increasing phosphate concentration up to about a fourfold increase. Thereafter further increases in the initial phosphate concentration have little effect. Additions of phosphate in excess of 1·0 mg. % phosphate-phosphorus usually cause precipitation to occur. In some cases the initial growth in high phosphate concentrations is irregular.

A preliminary investigation of the phosphate exchange occurring during the long lag phase produced if phosphate deficient cells are subcultured into high phosphate concentrations shows that a complicated situation exists. The



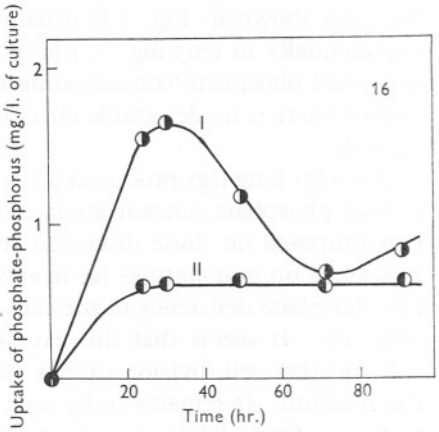


Fig. 15. Variation in the lag time of cells of *N. closterium* when grown in a medium containing 1·2 mg. % nitrate-nitrogen and 0·05 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration and subcultured from the exhaustion phase under the same conditions of lighting, temperature and aeration into medium containing identical amounts of nitrate and varying amounts of phosphate.

Fig. 16. Uptake of phosphate by two cultures of equal cell concentration inoculated with cells from the exhaustion phase of a phosphorus-limiting culture grown in basic medium containing 1·20 mg. % nitrate-nitrogen and 0·06 mg. % phosphate-phosphorus. Curve I, basic medium with additions of 1·20 mg. % nitrate-nitrogen and 0·24 mg. % phosphate-phosphorus. Curve II, basic medium with the same addition of nitrate and 0·062 mg. % phosphate-phosphorus. Culture held at 19° C. with continuous illumination and aeration. Phosphorus estimations conducted on suitable samples after removal of the cells by centrifuging.

inorganic phosphate in the medium was estimated by Harvey's method (1948). The culture containing 0·062 mg. % phosphate-phosphorus took up practically the whole of this during the lag phase. A slight increase of the phosphate in the medium occurred after about 160 hr. when the culture was in the exhaustion phase. This is presumably due to autolysis. The cells subcultured in 0·24 mg. % phosphate-phosphorus took up 0·16 mg./100 ml. of culture in the first 30 hr. Growth did not start in this culture until after nearly 90 hr., during which time about 0·105 mg./100 ml. of the phosphorus originally taken up by the cells reappeared in the medium. When growth finally started in this culture

the phosphorus content of the cells was therefore approximately the same as at the end of the lag phase in the cells subcultured to a lower phosphate concentration. Phosphate uptake recommenced in the culture in high phosphate as soon as growth started and continued until at 160 hr. the whole of the phosphate had been taken up.

The prolonged lag produced on subculture to high phosphate concentrations is restricted to exhausted cells grown in a limiting amount of phosphate. If the exhausted cells have been grown in a limiting amount of nitrate and subcultured to high phosphate concentration, irregular initial growth of the type shown in Fig. 3 is usually obtained. In contrast, if cells growing exponentially in 0·05 mg. % phosphate-phosphorus are subcultured into an increased phosphate concentration of up to twenty times that of the parent culture there is no detectable effect on the lag time or subsequent exponential growth.

The very long lags produced when phosphate-deficient cells are subcultured to high phosphate concentrations (Fig. 15) must be due to other factors being superimposed on those discussed above. If sufficient phosphate is supplied it is taken up in quantities far in excess of the amount required to make good the phosphate deficiency of the cells and allow the resumption of cell division (Fig. 16). It seems that this excess phosphate completely disorganizes the cell and that cell division cannot start until this excess has been returned to the medium. It remains to be seen in what form the phosphate is returned to the medium; labile organic phosphate compounds would be estimated as inorganic phosphate by the methods used.

Discussion

The techniques developed during the present studies have made possible quantitative measurements of the growth of cultures of *Nitzschia closterium* forma *minutissima*. Regular exponential growth can be obtained which within certain limits is independent of the initial concentrations of the main nutrients and which varies with temperature according to the Arrhenius Law. The total crop produced is directly proportional to the initial concentration of a limiting nutrient and is independent of the growth rate. Control of experimental conditions has made possible a high degree of quantitative repeatability from culture to culture.

The fact that growth can be obtained which obeys a simple law suggests that under the defined conditions the basic medium used is suitable for quantitative growth studies. It is possible to predict accurately and control the rate of exponential growth under a given set of conditions and to control its extent by varying the initial concentration of a nutrient known to be limiting. The lag exhibited by an inoculum may be predicted from a knowledge of the previous history of the cells.

On the other hand, in sea-water medium, the limits within which such studies are possible are rather narrow. Due mainly to difficulties in the supply of photosynthetically available carbon, the culture used must not increase above a rather low cell concentration. Even so, in cultures aerated with air the pH rises rapidly during optimal exponential growth. This is not ideal and some way of controlling the pH would be advantageous. This is especially important since for many purposes it is desirable to work at higher cell concentrations. The use of a higher partial pressure of carbon dioxide in the aerating gas may in any case be necessary with higher cell concentrations, and an attempt should also be made to develop a medium, suitable for marine algae, that is buffered by a component not utilized during growth. This will necessitate changes in the major ionic constituents since the high concentration of calcium makes sea water a very inflexible basis for media.

Similar factors may produce troublesome precipitation during the growth of cultures. This precipitation is not obvious to the naked eye, but shows itself by anomalous optical-density readings and often in sudden fluctuations in the pH of the culture. No way has been found of controlling this type of precipitation, and its occurrence has led to many cultures having to be rejected. Similarly, sea water is often difficult to sterilize by autoclaving due to precipitation occurring. Filtration techniques for sterilization have not been widely investigated, but it seems probable that, owing to the very low concentration of many ions in sea water, such techniques may lead to difficulties resulting from exchange phenomena and adsorption. The procedure used in the present studies, however, of diluting the sea water before autoclaving, mitigates the precipitation. A preliminary survey (Miss D. Ballantine, private communication) shows that many marine unicellular algae will tolerate this decreased salinity and the technique may be generally applicable.

It is difficult to obtain uniformity from one batch of sea water to the next. The 'ageing' process assists qualitative uniformity but it may produce widely different quantitative compositions, due, for example, to the release of available nitrogen by the bacterial decomposition of organic matter. When working at low cell concentrations these increases are large compared with the small amounts of nutrient added.

This work was performed at the Plymouth Laboratory of the Marine Biological Association. I should like to express my thanks to the Director, Mr F. S. Russell, F.R.S., for making freely available all the facilities of the laboratory; to all the staff for their helpfulness, and especially to Dr Mary Parke and Mr F. A. J. Armstrong, and to the latter for carrying out the phosphate estimations. Finally, it is a great pleasure to thank Dr H. W. Harvey, F.R.S., for introducing me to the field, for advice in the construction of the absorptiometer, and for his continued interest and many stimulating discussions.

SUMMARY

Techniques are described for the culture of a bacteria-free strain of *Nitzschia closterium* forma *minutissima* under carefully controlled conditions. An investigation has been made of the absorption and scattering of light by cultures of the organism and a technique developed which enables an absorptiometric method to be used for measuring the growth of cultures.

Using the techniques described, the nature of the growth of the diatom has been studied in media made up by the addition to sea water of nutrient salts and trace elements. These studies have defined the limits within which such media may be used for quantitative growth studies and have indicated the limitations imposed on growth studies by media of this type.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

Über die Seewasserresistenz von Meerstrandpflanzen [The Resistance of Sea-shore Plants to Sea Water]

By Richard Biebl

Photogr. u. Forsch., Bd. 5, 1953, pp. 174–80

Some observations are described on the cell physiology of land halophytes from the rocky shore of Wembury, South Devon. Photographs of some of these plants are given. The observations on the osmotic value, permeability, and resistance to salt were carried out in different concentrations of sea water. Sea water is the best naturally balanced salt solution. Shore plants have a higher osmotic value and a greater resistance to sea water than glycophytes like plants of meadows and gardens. Also the permeability to sea water is different. R.B.

Further Work on Pagurids and their Commensals

By L. R. Brightwell

Proc. zool. Soc. Lond., Vol. 123, 1953, pp. 61–4

In September 1951 efforts were made at the laboratory to investigate reports from London Zoo Aquarium that *Nereis fucata* would eject the Vestlet Anemone from its tube, and even *Eupagurus bernhardus* from its borrowed shell. Anemones were not available, but by means of glass Buccinum shells it was established that two worms may force entry to the same shell, rendering it untenable for the crustacean. It was further noted that the Lesser Spotted Dog-fish and *Nereis fucata* between them may do much to provide homes for the common hermit. The fish bites off the whelk's head and foot, whilst the worm disposes of the visceral mass. The worm will also eat the abdominal portion of a dead hermit after the cephalothorax and legs have been eaten by almost any bottom-feeding fish.

Observations were made on the largely planktonic diet of *Eupagurus bernhardus*. When a *Calliactis* emitted a stream of ova, for over 2 hr. without pause, a hermit sat beside the anemone, and by its inhalatory respiratory current, very deliberately diverted large numbers of ova to its own interior.

It was found that quite frequently *Eupagurus bernhardus* will accept almost any refuge, even a straight glass tube, rather than leave its abdomen open to attack. This lends colour to Sinel's suggestion that the pagurids may have evolved from one of the so-called burrowing prawns, such as *Callianasa*.

L.R.B.

The Larva and Adult of *Polycitor crystallinus* Renier (Ascidiacea, Polycitoridae)

By **D. B. Carlisle**

Proc. zool. Soc. Lond., Vol. 123, 1953, pp. 259–65.

The anatomy of the adult and larva of this species are described and the differences from *Polycitor vitreus* (Sars) discussed in detail. A synonymy of the species is given, together with a summary of its known distribution, which is confined to the Mediterranean area. Its relationships with the Polyclinidae are discussed, and the suggestion is made that the Polycitorinae should be raised to full familial rank and separated from the Clavelinidae. D.B.C.

The X-organ of Crustacea

By **D. B. Carlisle** and **L. M. Passano**

Nature, Lond., Vol. 171, 1953, pp. 1070–1.

The X-organ consists of three types of cells at least. In Brachyura, where the sensory pore is poorly developed or wanting these three different types are commonly found together, usually at the proximal ventral corner of the medulla terminalis, but in Stomatopoda, Anomura, Natantia and *Dromia* the X-organ may be separated into two parts connected by a nerve. The three-cell types are large neurosecretory cells, smaller isodiametric cells, often containing each a single large inclusion, and oval or irregular 'concretions of concentric structure' which show no nucleus and may be products of secretion rather than cells. In most Brachyura all three types are associated into one localized organ, while in the other groups examined the first cell type forms a single group in the medulla terminalis, whereas the other two types of cells are located in a separate organ near the sensory pore. This organ is named the *pars distalis X-organi*, and that in the medulla terminalis is named the *pars ganglionaris X-organi*; the nerve connecting the two parts is the X-organ connective. These findings may serve to explain why X-organ extirpation has different results in different groups of Crustacea. D.B.C.

On a Sporozoon in the Coelomic Corpuscles of *Phascolosoma minutum* Keferstein (Sipunculoidea)

By D. Etherington

Parasitology, Vol. 43, 1953, pp. 160–9.

A description is given of a sporozoon infecting the coelomic corpuscles of *Phascolosoma minutum*. The parasite undergoes schizogony in the haematids of the coelomic fluid; the schizonts are enclosed in tough envelopes. Two types of merozoites are produced; micromerozoites repeat the schizogonic cycle, while macromerozoites invade haematids, but develop into encysted bodies when the haematids are ingested by amoebocytes. In the discussion of the affinities of the parasite, it is suggested that the encysted bodies are gamonts, and that the parasite is related to the Haemogregarinidea. D.E.

New Forms of Visual Purple from the Retinas of certain Marine Fishes: A Re-examination

By E. M. Kampa

J. Physiol., Vol. 119, 1953, pp. 400–9.

Dark adapted retinas of three marine fishes (plaice, *Pleuronectes platessa*; pollack, *Gadus pollachius*; and gurnard, *Trigla hirundo*), previously reported to possess unique photopigments (Bayliss, Lythgoe & Tansley, *Proc. Roy. Soc.* B, Vol. 120, 1936, pp. 95–113), have been shown to contain pigments with absorption spectra indistinguishable from that of frog rhodopsin.

The dark-adapted retina of the freshwater tench, *Tinca vulgaris*, has been shown to contain a pigment with an absorption spectrum characteristic of porphyropsin. The absorption spectrum of an extract of dark-adapted trout (*Salmo trutta*) retinas indicates that this anadromous teleost may utilize a mixture of rhodopsin and porphyropsin.

Differences between the methods used in the present work and those employed by Bayliss *et al.* are discussed. None of these seems sufficient to explain the differences between the two sets of results. E.M.K.

The Effect of Enucleation on the Development of Sea-Urchin Eggs. 1. Enucleation of one Cell at the 2-, 4- or 8-Cell Stage

By I. J. Lorch, J. F. Danielli and S. Hörstadius

Exp. Cell Res., Vol. 4, 1953, pp. 253–62.

Enucleated blastomeres left in contact with one, three or seven normal blastomeres did not play any part in the development of the larva, except in so far as purely mechanical distortion was produced.

After an initial delay, during which asters and incomplete division furrows appeared and disappeared again, the enucleated cells divided rapidly into a number of spheroidal cells of various sizes. The final diameter of these cells ranged from 3 to 50 μ. The cells remained optically similar to nucleated cells of the same size and retained their semi-permeability for 3 days or more.

Enucleated cells were never seen to differentiate or participate in the formation of tissues or organs. The presence or absence of asters in the early stages made no difference to the final fate of the enucleated cells.

Thus it seems that the nucleus is essential for differentiation, though not for cell-division. The proximity of normally differentiating cells does not induce similar changes in the enucleated cells. I.J.L.

Further Studies on Ionic Regulation in Marine Invertebrates

By James D. Robertson

J. exp. Biol., Vol. 30, 1953, pp. 277–96.

In a study of ionic regulation in the blood or coelomic fluid of sixteen marine invertebrates, differences from a passive equilibrium with the external medium were found in all. This active regulation is most pronounced in *Sepia* and members of the decapod Crustacea, all of which have excretory organs capable of selective ionic excretion. Little regulation is shown by holothuroids or lamellibranchs. In a group of sixteen crustaceans, it was found that the magnesium of the plasma varied between 14 and 101% of the value in the surrounding sea water. Those with high levels (e.g. *Maia*, *Dromia*, *Lithodes*) are slow-moving and inactive, those with low levels (e.g. *Squilla*, *Homarus*, *Pachygrapsus*) are capable of swift movement and are generally more active. J.D.R.

New Turbellaria Parasites in Echinoderms

By Einar Westblad

Ark. Zool., Ser. 2, Bd. 5, 1953, pp. 269–88.

Three turbellarian parasites are described, found in intestines of echinoderms at Plymouth. In all six turbellarian parasites were found in echinoderms:

Acoela:

Gen. *Avagina* Leiper (1902, 1904).
A. incola Leiper: in *Spatangus purpureus* (common).
A. glandulifera Westblad, 1953: in *Spatangus purpureus*.

RHABDOCOELA:

Gen. *Umagilla* Wahl, 1910.
U. forskalensis Wahl: in *Holothuria forskali* (common)
Gen. *Anoplodium* Ant. Schneider, 1858.
A. tubiferum Westblad, 1953; in *Holothuria forskali.*
Gen. *Marcusella* Westblad, 1953.
M. atriovillosa Westblad, 1953: in *Spatangus purpureus.*
Gen. *Syndesmis* W. Silliman, 1881.
S. echinorum François: in *Echinus esculentus* (common).

In addition, *Fecampia erythrocephala* Giard 1886 was found in small specimens of *Carcinus maenes* from Rum Bay.

BOREOHYDRA SIMPLEX WESTBLAD, A 'BIPOLAR' HYDROID

By Einar Westblad

Ark. Zool., Ser. 2, Bd. 4, 1953, pp. 351–4.

MARINE MACROSTOMIDA (TURBELLARIA) FROM SCANDINAVIA AND ENGLAND

By Einar Westblad

Ark. Zool., Ser. 2, Bd. 4, 1953, pp. 391–401.

In these papers the following species have been recorded in the Plymouth area:

Boreohydra: at Plymouth (Hamoaze at a depth of *c.* 16 m., 26 July 1949).

Gen. *Macrostomum* O. Schmidt, 1848.
M. appendiculatum (O. Fabr, 1826) Graff, 1905: Salcombe estuary, in July 1949.
M. pusillum Ax, 1951: Yealm estuary, common in mud, in July 1949.
Gen. *Microstomum* O. Schmidt, 1848.
M. hamatum Westblad, 1953: Plymouth, the harbour in black mud at *c.* 40 m. depth, in July 1949
M. rubromaculatum Graff, 1882: Wembury, in tide pools, in July 1949

BOOK REVIEW

THE GENUS *EUGLENA*

By Mary Gojdics

Madison, University of Wisconsin Press, pp. viii + 268, 1953.

This is an admirable work, and although it is primarily taxonomic the first forty pages or so are devoted to a clear description of the organism's general biology and physiology. This part of the work is well done, and it is clear that the numerous papers connected with it have been carefully studied by the author.

The bibliography comprises some 300 references.

There are numerous illustrations including thirty-nine plates with very clear line figures.

In addition to the 'keys' the environment of each species is described. The importance of studying cultures grown from a single individual, as advocated by S. P. Chu, is advocated but not described.

Since *Euglena* is the standard type of an autotrophic flagellate it should loom largely in the teaching of general biology; and for teaching purposes as well as for those engaged in physiology alone a short description of actual culture technique would add further value to the work.

A. G. LOWNDES

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. XXVII (p. 761) and Vol. XXXI (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

TERMS OF MEMBERSHIP

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

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

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 32 EAST 57TH STREET, 22
CANADA AND INDIA: MACMILLAN

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)