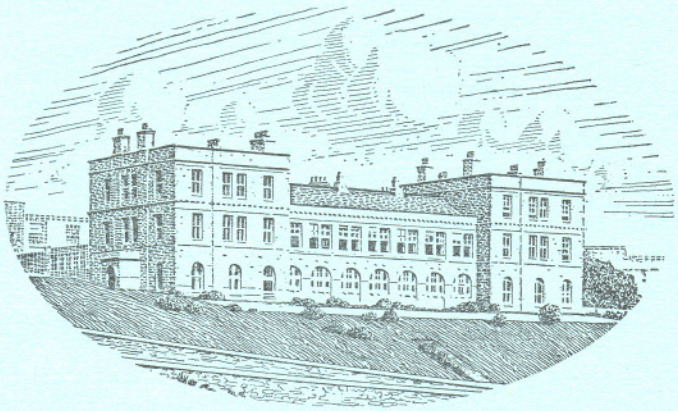
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



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PLANT PRODUCTION ON THE FLADEN GROUND

By John H. Steele
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(Text-figs. 1–19)

Introduction

The quantitative study of phytoplankton production may be pursued in many ways, but these ways can be divided into two general methods of approach. There is, first, the direct estimation of a production rate for a particular sample of the population; for example, the light-dark bottle technique for measuring oxygen production (Gaarder & Gran, 1927; Riley, 1939) and the new ^{14}C technique (Steeman Nielsen, 1952). These estimates are made under conditions which must be, to some extent, artificial. Secondly, there is the direct estimation of relevant variables in the sea (phosphate, oxygen, chlorophyll concentration, etc.) from which production is calculated on the basis of hypotheses about the behaviour of phytoplankton. These hypotheses are, of necessity, simplifications of a mass of laboratory experiments and of previous field work. Riley, Stommel & Bumpus (1949) give a full account of this approach and of the difficulties involved in it.

Since it is, in practice, impossible to use all methods at the same time, a choice of techniques must be made. The second approach was chosen in the investigations here described, for the following reasons: (*a*) the estimation of phosphate, oxygen and chlorophyll are, by now, routine techniques, and comparatively large numbers of samples can be analysed; (*b*) the mathematical techniques for working up the chemical results seemed particularly applicable to the Fladen ground, which is the main area studied; (*c*) from this method one can hope to obtain, not merely an estimate of production, but also some insight into causes of variations in that production.

Thus, by this approach one can provide quantitative data about standing crop and production which are not obtainable from the older methods of planktology. Alone, however, it provides no detailed insight into the species composition of the populations. Yet it is likely that purely quantitative results may be of most use ultimately in providing connexions between plankton and fishing theory. For example, the effects of variations in the food supply to larval fish and to the bottom fauna might be studied in this way. But before these problems can be understood it is necessary to obtain quantitative knowledge of the causal relations between the phytoplankton and its environment.

This is the aim of this paper, and it will be obvious that it depends on the work which Riley has done in organizing past data and developing this new approach.

The results given here cover 3 years, 1951–53. During the summer and autumn of 1951 general surveys of the Fladen ground were made as part of a herring research programme. Phosphate, temperature and salinity were sampled at 0, 20, 50, 100 m and near-bottom (approximately 140 m), at ten to eighteen stations on five occasions. Two stations were also worked during the first half of the year, one in April and one in June. The analyses of these data suggested that it would be of interest to obtain as often as possible from this area chemical samples with close vertical spacing. Therefore in 1952, at one station on the Fladen ground, phosphate, temperature and salinity were sampled at 10 m intervals on seven occasions between May and November.

Finally, in 1953, the twelve stations shown in Fig. 1 were visited nine times. At each of these stations samples were taken for phosphate, oxygen, salinity, temperature, chlorophyll and dry weight of net hauls. Also at stations 1, 7, 8, 10 and 11 phosphate samples were taken on five other occasions. On most occasions net hauls were taken for species analysis of the plankton.

The first part of this paper uses the phosphate data to provide estimates of production for the years 1951–53. In the second part the 1953 production on Fladen is linked with the chlorophyll and dry-weight data in an attempt to describe the causes of the variations in plant populations throughout the year.

It is a pleasure to thank Dr H. W. Harvey, F.R.S., and Dr G. A. Riley for reading and criticizing this work, although its final form is the author's responsibility.

Part I. Phosphate and Production

The General Hydrographic Conditions

In estimating the utilization of phosphate by the phytoplankton, it is necessary to estimate the changes in phosphate distribution due to water movement. The possible types of change in this area are large-scale lateral movements, i.e. currents, and small-scale movements, i.e. lateral and vertical turbulence. In this paper it is postulated that the only changes which need be taken into account directly in the calculations of production are those due to vertical turbulence. First, the extent to which this postulate holds for the Fladen ground will be considered.

Fladen is the south-west corner of the central area of the northern North Sea lying between the prime meridian and the Norwegian deeps and having a depth of 100–140 m (Fig. 1). During the winter and early spring there are large-scale hydrographic changes in this region due to the inflow of Atlantic water round the north of Shetland. (This can be seen from the surface salinity charts for the North Sea prepared by the Conseil Permanent International

pour l'Exploration de la Mer, 1933.) After March, however, the major changes take place in the uppermost layers only. This is shown by the fact that from April to September of any year the bottom temperatures, 6·0–7·5° C, within the dotted area (Fig. 1) remain approximately constant at a value lower than that of any of the surrounding water masses. Since temperature is also

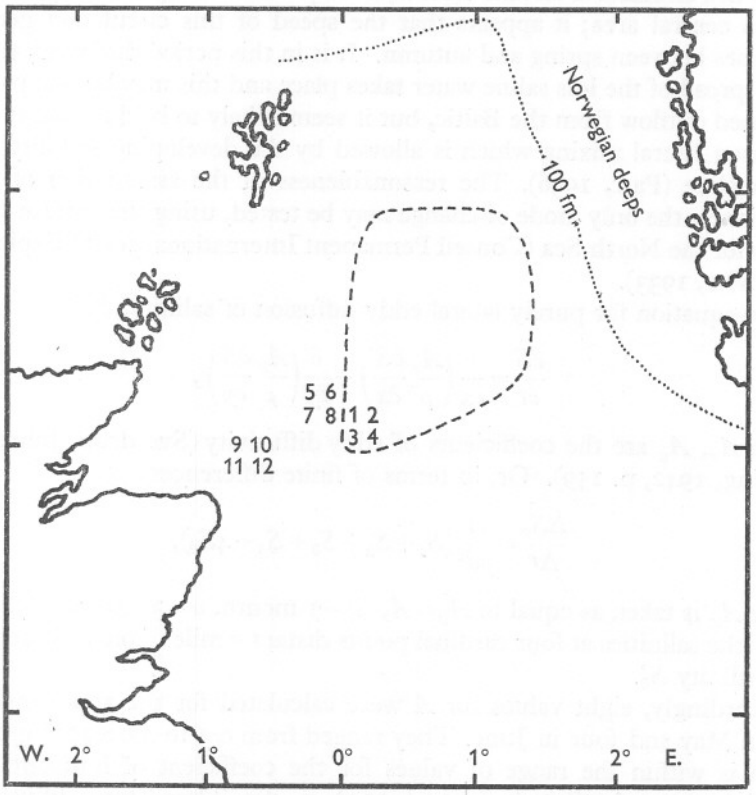

Fig. 1. Positions of stations worked in 1953 in the northern North Sea. 1–4: Fladen Group. 5–8: Northern Group. 9–12: Inshore Group. The dotted line indicates roughly the area inside which there is a nearly constant bottom temperature throughout the spring and summer.

constant from the foot of the thermocline (30–70 m) to the bottom, it follows that most if not all of the water below the thermocline which has this lower temperature during the summer must have been in this area since the spring. This water mass forms a cold, and therefore heavy core in the centre of the northern North Sea; the conditions on Fladen are typical in this respect. The possibility of changes within this core, either as circulation or as shifts of position, will be considered when the 3 years' results are discussed in detail.

In the surface layers above this cold core, however, large salinity changes occur during the summer and early autumn. These are produced by the westward spread of low salinity water from the current which issues from the Baltic and flows up the Norwegian coast. From the results of drift-bottle experiments, Tait (1937) has inferred that the surface waters of the northern North Sea circulate as an anti-clockwise eddy which occupies nearly the whole of this central area; it appears that the speed of this circulation generally decreases between spring and autumn. It is in this period, however, that the main spread of the less saline water takes place and this may be due partly to increased outflow from the Baltic, but it seems likely to be due largely to the increased lateral mixing which is allowed by the developing stability of the thermocline (Parr, 1936). The reasonableness of the assumption of lateral diffusion as the only mode of change may be tested, using the surface salinity charts for the North Sea (Conseil Permanent International pour l'Exploration de la Mer, 1933).

The equation for purely lateral eddy diffusion of salinity, S, is

$$\frac{\partial S}{\partial t}=\frac{\partial}{\partial x}\left(\frac{A_x}{\rho}\frac{\partial S}{\partial x}\right)+\frac{\partial}{\partial y}\left(\frac{A_y}{\rho}\frac{\partial S}{\partial y}\right),$$

where A_x, A_y are the coefficients of eddy diffusivity (Sverdrup, Johnston & Fleming, 1942, p. 159). Or, in terms of finite differences,

$$\frac{\Delta S_0}{\Delta t}=\frac{A}{\rho a^2}(S_1+S_2+S_3+S_4-4S_0),$$

where A_x is taken as equal to $A_y=A$, $\Delta t=1$ month, $a=30$ miles. S_1, S_2, S_3, S_4 are the salinities at four cardinal points distant a miles from a chosen point with salinity S_0.

Accordingly, eight values for A were calculated for the area considered, four in May and four in June. They ranged from 0·3 to $2{\cdot}0\times10^{+7}$ g/cm/sec, which is within the range of values for the coefficient of horizontal eddy diffusivity given by Sverdrup *et al.* 1942, p. 485. Thus lateral diffusion can be taken as a sufficient explanation of the changes in salinity. It is probable that the effects of this diffusion on the phosphate concentration on Fladen will be negligible since the diffusion has occurred across an area having the same characteristics as Fladen. This is shown in the charts of summer phosphate distribution (Johnston, 1953, fig. 5; and 1954, fig. 8).

Evaluation of 'Biological Change' from Phosphate Observations

By far the largest part of the data collected consists of inorganic phosphate observations. These will be used to provide the estimates of production. The profiles of temperature, phosphate and oxygen for a station on Fladen are shown in Fig. 2.

The calculations are based on two equations (Sverdrup *et al.* 1942, p. 159):

$$\frac{\partial p}{\partial t}=\frac{\partial}{\partial z}\left(\frac{A_p}{\rho}\frac{\partial p}{\partial z}\right)-\frac{\partial C'}{\partial t}+\frac{\partial R'}{\partial t},$$

$$\frac{\partial T}{\partial z}=\frac{\partial}{\partial z}\left(\frac{A_t}{\rho}\frac{\partial T}{\partial z}\right),$$

where p = phosphate concentration, C' = consumption of inorganic phosphate by plants, R' = return of inorganic phosphate due to regeneration from

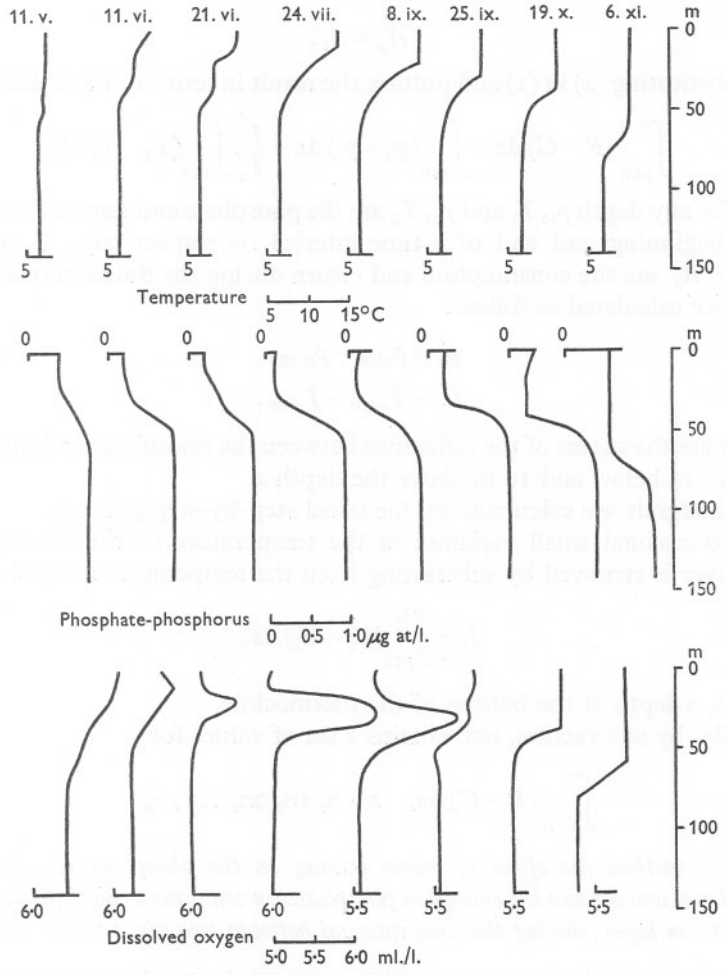

Fig. 2. The profiles of temperature, phosphate-phosphorus and dissolved oxygen at Station 1 during 1953.

organic compounds, T = temperature, z = depth, and A_p, A_t = coefficients of vertical diffusivity of phosphate and temperature respectively.

Integrating both equations from z to 140 m (bottom)

$$\frac{\partial}{\partial t}\int_{140}^{z}(p+C'-R')\,dz=\left[\frac{A_p}{\rho}\frac{\partial p}{\partial z}\right]_{140}^{z}, \tag{1}$$

$$\frac{\partial}{\partial t}\int_{140}^{z}T\,dz=\left[\frac{A_t}{\rho}\frac{\partial T}{\partial z}\right]_{140}^{z}. \tag{2}$$

Taking $$A_p\frac{\partial p}{\partial z}=A_t\frac{\partial T}{\partial z}=0 \text{ at the bottom}$$

and $$A_p=A_t,$$

then substituting (2) in (1) and putting the result in terms of finite differences,

$$\int_{140}^{z}(R-C)\,dz=\int_{140}^{z}(p_2-p_1)\,dz-\frac{p'_z}{T'_z}\int_{140}^{z}(T_2-T_1)\,dz \tag{3}$$

where for any depth p_1, T_1 and p_2, T_2 are the phosphate and temperature values at the beginning and end of a time interval Δt respectively; $C=C'_2-C'_1$, $R=R'_2-R'_1$ are the consumption and return during the time interval Δt, and p'_z, T'_z are calculated as follow:

$$p'_z=p_{z+10}-p_{z-10},$$
$$T'_z=T_{z+10}-T_{z-10},$$

i.e. they are the means of the difference between the phosphate or temperature values 10 m below and 10 m above the depth z.

The integrals are calculated by the usual step-by-step process.

The occasional small variation in the temperature of the homogeneous lower layer is removed by subtracting from the temperature integral

$$I_h=\int_{140}^{z_h}(T_2-T_1)\,dz,$$

where z_h = depth of the bottom of the thermocline.

Finally, by subtraction, one obtains a set of values for

$$\int_{z+10}^{z}(R-C)\,dz, \quad z=0, 10, 20, \ldots, z_h. \tag{4}$$

By this method the effect of water mixing on the phosphate distribution is removed and one obtains the change in phosphate content, due to biological activity, in each 10 m layer, during the time interval between two visits to the station.[1]

[1] Because equation (3) is an approximation, errors will be introduced whose magnitude will depend on the size of the changes in T, p, $\partial T/\partial z$ and $\partial p/\partial z$ between two stations. These are usually small so long as the time interval is not too large.

In practice, because of the low values of the gradients at 10 m, it is usually necessary to take the 0–10 and 10–20 m layers together. Also, of course, the layer from the depth z_h to the bottom cannot be subdivided.

A typical calculation is shown in Table I.

TABLE I. CALCULATION OF 'BIOLOGICAL CHANGE' IN 10 M LAYERS FOR 11–21 JUNE 1953 AT 58° 25′ N., 0° 20′ E.

m	$\int_{140}^{z}(T_2-T_1)\,dz$	p'_z	T'_z	$*\frac{pz'}{Tz'}\int_{140}^{z}(T_2-T_1)\,dz$	$\int_{140}^{z}(p_2-p_1)\,dz$	$\int_{140}^{z}(R-C)\,dz$	$\int_{z+10}^{z}(R-C)\,dz$
0	—	—	—	—	0·61	0·61	
20	8·4	−0·110	1·78	−0·05	0·58	0·63	−0·02
30	9·6	−0·285	1·07	−0·25	0·50	0·75	−0·12
40	5·4	−0·525	1·84	−0·15	0·54	0·69	+0·06
50	0·4	−0·295	0·90	−0·01	0·56	0·57	+0·12
60	0·2	−0·015	0·03	−0·01	0·48	0·49	+0·08
70	0·1	−0·020	0·04	0	0·41	0·41	+0·08
140	0	—	—	0	0	0	+0·41

* To convert to μg at/cm^2 the figures have been multiplied by 10^{-1}.

Analysis of Phosphate Observations

If the figures given by (4) are to be used to provide estimates of production, then it is necessary to make hypotheses about the ways in which consumption and return of inorganic phosphate occur in the sea.

As expected, the values of (4) show a decrease in the upper and an increase in the lower layers, with the change-over occurring at about 30–40 m. This depth of zero change will be called the 'zero level'.

Thus the simplest assumptions would be: (*a*) only production occurs above the zero level and only regeneration below; (*b*) the production in terms of carbon can be found by using a fixed carbon-phosphorus ratio (Sverdrup *et al.* 1942, p. 237).

The objections to these postulates must be considered; the main ones appear to be as follows:

(i) There will be some regeneration above the zero level. This regeneration may occur at a rate equal to the mean rate of regeneration below the zero level, or it may be much greater since there is more phytoplankton above the zero level than below it. The factors involved in regeneration are complex. Since the plants in the euphotic zone are assumed to be living and will not normally die until they sink below the euphotic zone it may be that regeneration from direct decay of plants will be very small. Whether or not regeneration from zooplankton metabolism will be equally spread throughout the water column is at present unknown. Thus no good estimate can be given for the effect of regeneration of phosphate above the zero level. In the conclusion to this part of the paper an estimate is made of the effect of assuming equal regeneration

at all depths, but in the body of the paper assumption (*a*) has been used as being the simplest method of stating the results.

(ii) There is evidence that plants can use organic, as well as inorganic, phosphorus compounds to meet their phosphate needs (Chu, 1946; Harvey, 1953). This is an unknown factor to the extent that this evidence comes from laboratory experiments and the process has not been demonstrated under natural conditions. (It has also been suggested (Lwoff, 1951) that some flagellates may be able to ingest particles of organic matter. This, however, does not affect estimates of basic production, which are in terms of the conversion of carbon from inorganic to organic form.)

(iii) The most serious objections to the second postulate comes from laboratory work on phosphate uptake of phytoplankton. Ketchum (1939) showed that in a reduced phosphate concentration ($< 0{\cdot}55$ μg at/l.) diatoms can continue to photosynthesize carbon without a corresponding uptake of phosphate. The phosphate concentration near the surface in the North Sea is less than $0{\cdot}55$ μg at/l. throughout most of the summer. Ketchum showed that cells in a deficient medium could double in number without the uptake of phosphate. To discover the possible effects of this, consider the position at the end of the spring outburst on Fladen. On 11 June 1953 the population in the euphotic zone, as estimated from the chlorophyll content, contained $2{\cdot}4$ g carbon/m^2. If this doubled itself without consuming phosphate then the increase in production would be $2{\cdot}4$ g carbon/m^2. But this is only 5% of the year's production. Further, before this deficient population can again divide, there must be replacement of the phosphate deficiency, and this will be shown by the phosphate changes. For these reasons, the effect of phosphate deficiency may not be appreciable.

These objections must be borne in mind when the validity is considered of the following estimates as absolute measures of production. However, they should not seriously affect conclusions about variations in production from year to year or place to place, and such results are among the main aims of this paper.

Production on Fladen

For 1951, the temperature and phosphate values were obtained by choosing from extensive surveys those stations near the centre of the Fladen Ground that showed similar structure (the stations on the south and west edges of Fladen showed greater mixing and, often, salinity changes). From the average of the chosen stations the changes above and below 35 m were calculated, and the estimated production is given as a histogram (Fig. 3A). These values will not be particularly accurate due to the small number of depths at which chemical observations were made; thus it is not possible to study the data in more detail.

For 1952, the production is again given as a histogram (Fig. 4A), while the profiles for each interval are shown in Fig. 6.

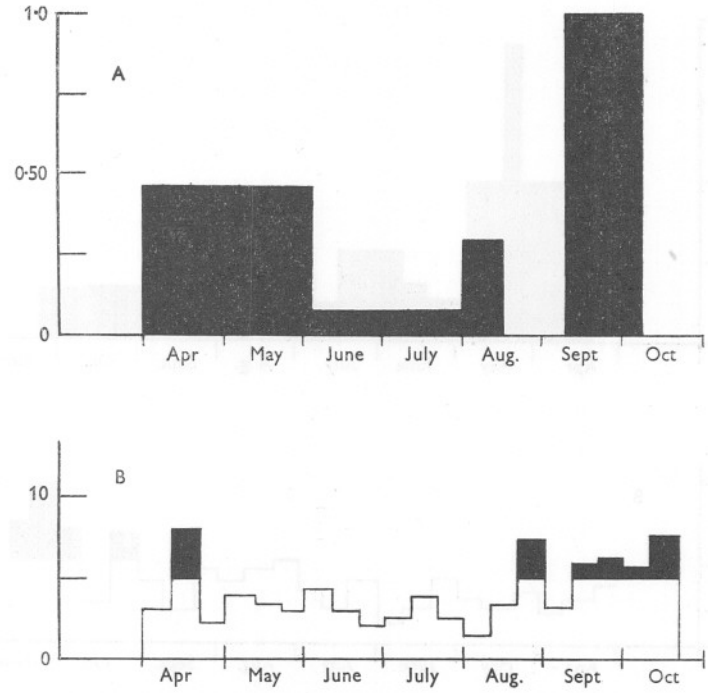

Fig. 3. 1951: A, production rate on Fladen, g carbon/m² day; B, wind velocity, m/sec.

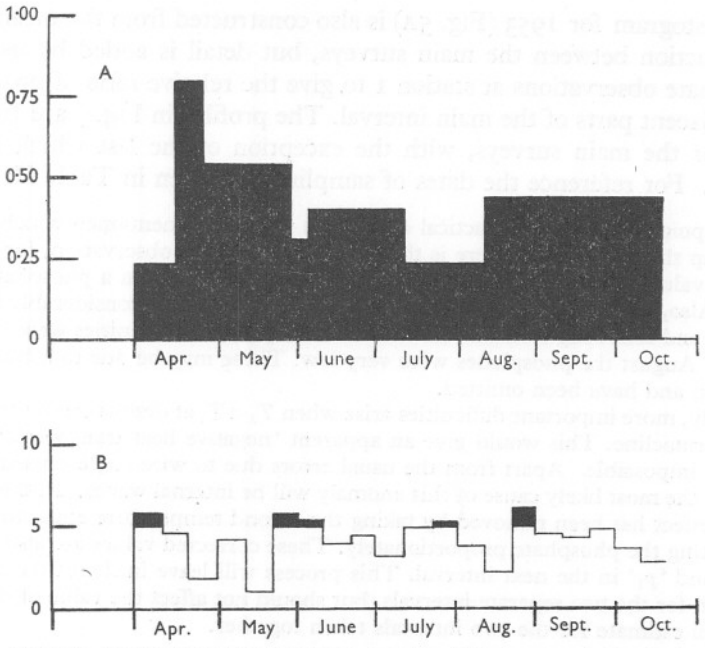

Fig. 4. 1952: A, production rate on Fladen, g carbon/m² day; B, wind velocity, m/sec.

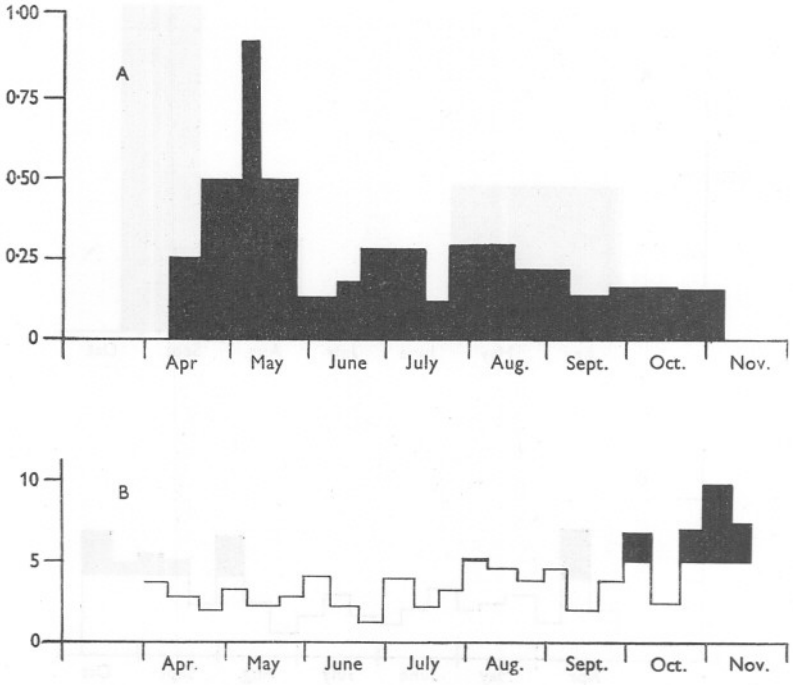

Fig. 5. 1953: A, production rate on Fladen, g carbon/m² day; B, wind velocity, m/sec.

The histogram for 1953 (Fig. 5A) is also constructed from the mean values for production between the main surveys, but detail is added by using the intermediate observations at station 1 to give the relative ratio of production in the adjacent parts of the main interval. The profiles in Fig. 7 are from the means for the main surveys, with the exception of the last which is from station 1. For reference the dates of sampling are given in Table II.

At this point some of the practical difficulties should be mentioned which arise in working up the data. First, there is the occasional aberrant observation, for which a corrected value is taken by interpolation from a graph (e.g. from a phosphate-depth profile). Also, on two occasions, all values at one station differed considerably from the preceding and following stations. At station 1 on 15 July the salinities were very low, and on 18 August the phosphates were very low. These may be due to a faulty standardization and have been omitted.

Secondly, more important difficulties arise when $T_2 < T_1$ at depths on the lower part of the thermocline. This would give an apparent 'negative heat transport', which is physically impossible. Apart from the usual errors due to wire angle caused by ship drift, etc., the most likely cause of this anomaly will be internal waves. In the calculations the effect has been removed by taking the second temperature equal to the first and adjusting the phosphate proportionately. These corrected values are also used for the 'T_1' and 'p_1' in the next interval. This process will leave in doubt the values of production for the two separate intervals, but should not affect the value of their sum as the total estimate for the two intervals taken together.

However, once such inaccuracies have been allowed in a few particular cases, it must be admitted that they may occur in others without their presence being noticed (i.e. without $T_2 < T_1$). Until detailed knowledge is available of the variations due to internal waves, the limits of these errors cannot be given.

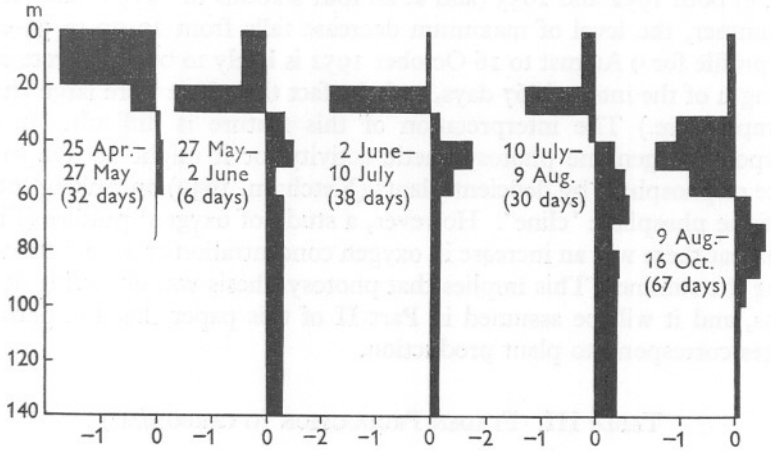

Fig. 6. 1952: profiles of 'biological change' in phosphate on Fladen in $10^2 \times \mu$g at./cm^2 day

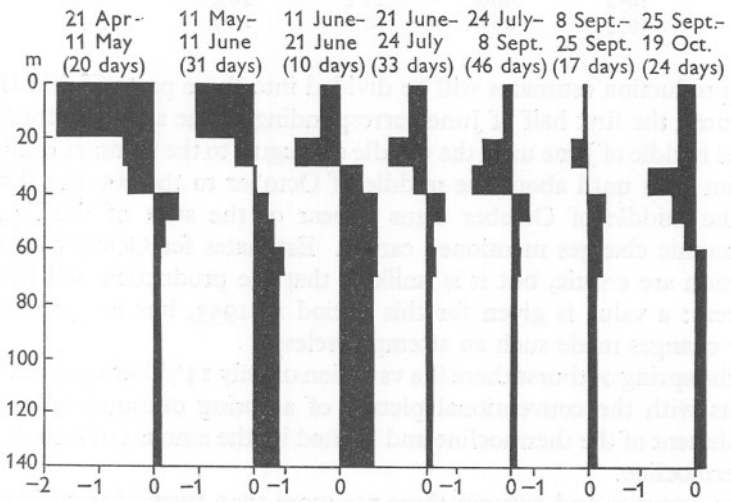

Fig. 7. 1953: profiles of 'biological change' in phosphate on Fladen in $10^2 \times \mu$g at./cm^2 day

TABLE II. DATES OF SAMPLING

1951	2. iv, 3. vi, 30. vii, 16. viii, 10. ix, 7. x
1952	15. iv, 25. iv, 27. v, 2. vi, 10. vii, 9. viii, 16. x
1953	5. iv, 21. iv*, 5. v, 11. v*, 26. v, 11. vi*, 21. vi*, 15. vii, 24. vii*, 18. viii, 8. ix*, 25. ix, 19. x, 6. xi

* Main surveys.

The profiles will be discussed first. Until the beginning of June they give the conventional picture of maximum production in the upper 20 m. From June onwards, maximum decrease in phosphate occurs below 20 m. This is found in both 1952 and 1953 (and at all four stations in 1953). Also, during the summer, the level of maximum decrease falls from 20–30 to 30–40 m. (The profile for 9 August to 16 October 1952 is likely to be inaccurate due to the length of the interval, 67 days, and the fact that there were large changes of temperature.) The interpretation of this feature is difficult. It could correspond to genuine photosynthetic activity, or it might be due to dark uptake of phosphate by deficient plants (Ketchum, 1939) on sinking into the top of the phosphate 'cline'. However, a study of oxygen[1] profiles (Fig. 2), shows that there was an increase in oxygen concentration at 20 and then 30 m during the summer. This implies that photosynthesis was occurring at these depths, and it will be assumed in Part II of this paper that the phosphate changes correspond to plant production.

TABLE III. FLADEN PRODUCTION (G CARBON/M^2)

	Spring	Summer	Autumn	Total
1951	28·0	8·9	28·0	64·9
1952	30·4	22·4	29·5	82·3
1953	26·1	17·6	13·7	57·4

The production estimates will be divided into three parts (Table III): the period until the first half of June corresponding to the spring outburst; that from the middle of June until the middle of August to the summer production; and from then until about the middle of October to the autumn flowering. After the middle of October signs appear of the start of the large-scale hydrographic changes mentioned earlier. Estimates for October–November production are erratic, but it is unlikely that the production will have been very great: a value is given for this period in 1953, but in 1952 the large salinity changes made such an attempt useless.

For the spring outburst there is a variation of only 14% between the 3 years. This fits with the conventional picture of a spring outburst following the establishment of the thermocline and limited by the amount of nutrient above this thermocline.

In the summer and autumn there are more than twofold variations in the production. The variations in the summer production will depend on three factors: (i) a general reduction in phosphate concentration in the euphotic zone from the June level of approximately 0·25 μg at/l. down to values below 0·10 μg at/l. (this may correspond with the ability of dinoflagellates to

[1] The analysis of the oxygen data involves discussion of several difficulties, and it is intended to use these data in conjunction with future and more detailed work in further attempts to understand these problems.

assimilate phosphate at lower concentrations than the spring forms—Barker, 1935; Braarud & Rossavik, 1951); (ii) an increase in the depth at which maximum consumption occurs; (iii) the consumption of any phosphate carried upwards by mixing.

To show that only the third of these factors varies considerably, the figures for phosphate decrease are separated into their components in Table IV. $\int p_2 - p_1$ = changes above the zero level due to (i) and (ii). I_p = upwards transport through the zero level corresponding to (iii). The net decrease is given by $\int (p_2 - p_1) - I_p$, in μg at /cm.^{2}.

TABLE IV. TRANSPORT THROUGH, AND CHANGE ABOVE, THE ZERO LEVEL

1952	$\int p_2 - p_1$	I_p	1953	$\int p_2 - p_1$	I_p
2. vi–10. vii	−0·71	0·44	11. vi–21. vi	0·07	0·15
10. vii–9. viii	−0·04	0·51	21. vi–24. vii	−0·67	0·03
9. viii–16. x	+0·12	1·92	24. vii–8. ix	0	0·73
			8. ix–25. ix	+0·18	0·27
			25. ix–19. x	−0·20	0·20
Total	−0·63	2·87		−0·62	1·38

Further, in 1951, when there was a very steep thermocline and apparently little transport, the summer decrease is 0·70 μg at/cm.^{2}. This value, with those of 0·75 and 0·60 for 1952 and 1953, shows that the variation in the summer due to the first two factors is comparatively small. This basic summer production is estimated at 8·7 g carbon/m^2 and has its effect at the end of June and the beginning of July. After that, production is solely dependent on the upwards transport of phosphate.

Thus, in the autumn, production depends on the breakdown of the thermocline. This occurred during September in 1951 and 1952, but in 1953 did not take place until after the middle of October, and it appears that owing to this delay the autumn production was less than half that of the previous year.

These variations in transport can best be shown in terms of the heat transported downwards through 40 m. Fig. 8 illustrates the differences between the three years. In a region such as Fladen, which is not subject to strong currents, vertical mixing must be almost entirely due to wind. Below each of the histograms of production is shown the corresponding wind data (Figs. 3B, 4B, 5B) for the northern North Sea (supplied by courtesy of the German Hydrographic Institute, Hamburg, through the Conseil Permanent International pour l'Exploration de la Mer). These are in the form of 10-day means calculated from atmospheric pressure gradients. The exact effect of strong winds on the thermocline is not known, especially in relation to the time factor. Thus it is not possible to say whether 10-day means should be a reliable guide

in comparing wind and thermocline changes. However, the main variations noted in mixing can be related to the wind histograms.

The greater amount of summer mixing in 1952 than in the other 2 years was due to the fact that the thermocline, which had half formed by the end of May, was partially broken down at the beginning of June with the result that, for the remainder of the year, the thermocline was not as steep as in 1951 and 1953. From the wind histograms it can be seen that only in 1952 were there mean winds in June exceeding 5 m/sec.

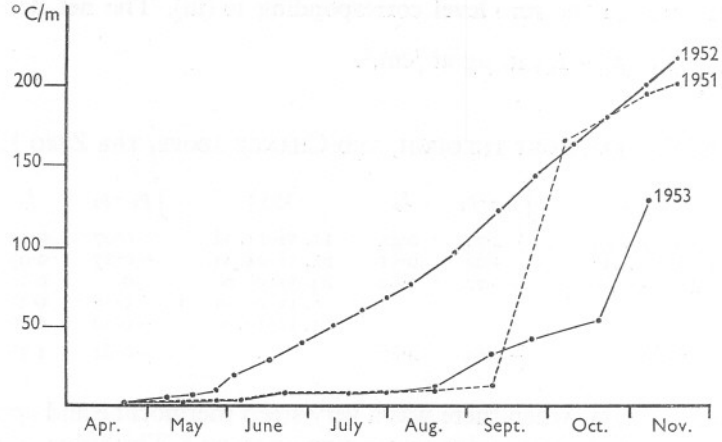

Fig. 8. Increase in the heat content below 40 m on Fladen.

Since it is reasonable to suppose that the strength of wind required to break down the thermocline is roughly inversely proportional to the steepness of the thermocline then, unless the thermocline is partly broken during early June, it is less likely to be broken throughout the remainder of the summer. For this reason, gales at the beginning of June should be critical in determining the excess of summer production over the basic amount previously mentioned.

During the first 10 days of August 1953 there were winds in excess of 5 m/sec which may have caused the transport noted for August. However, from then until October there are no signs of strong winds, and this feature corresponds to the delayed and consequently small production in the autumn, compared with 1951 and 1952 when there were winds greater than 5 m/sec.

Thus the expected chain of relations, strong winds—mixing—production, can be seen to operate on Fladen.

Phosphate Regeneration on Fladen

The estimates of regeneration (Figs. 9, 10) are extremely variable, both within years and between years. To bring any order into them requires a fair amount of 'inspired guessing' at the reasons for these variations, and so the conclusions reached are, to that extent, speculative.

First, it is necessary to consider the effect of known errors in phosphate observations for June 1953. Analyses of duplicate samples from station 1, 21 June 1953, were carried out at the Plymouth Marine Laboratory. These gave consistently lower values than those from the analyses in this laboratory; also there was an apparently greater scatter in our own results. Shortly afterwards the galvanometer in use in this laboratory broke down completely, and it is probable that the onset of the defect caused this difference in results.

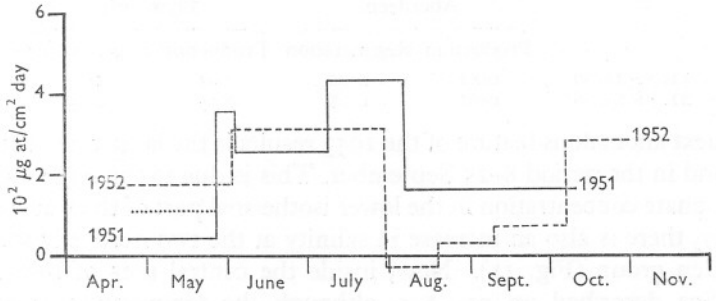

Fig. 9. Regeneration rate of inorganic phosphorus on Fladen in 1951 and 1952. ..., correction made by taking winter phosphate concentration in 1952 as 0·73 μg at/l. (see text). ($10^2 \times \mu$g at/cm² day.)

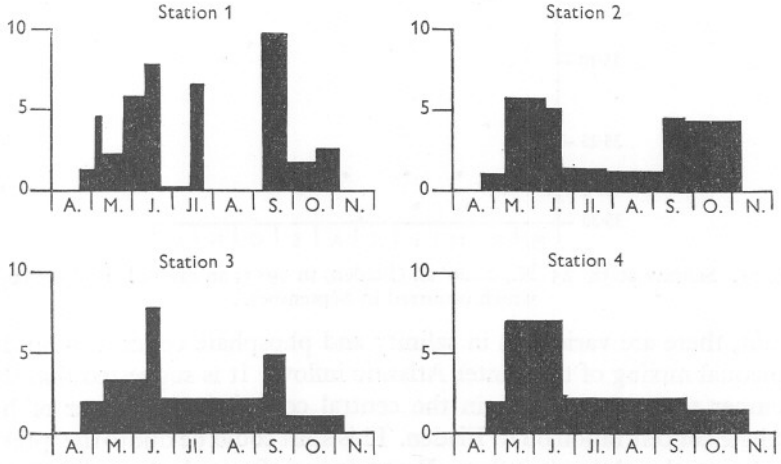

Fig. 10. Regeneration rate of inorganic phosphorus at the four Fladen stations in 1953 (in $10^2 \times \mu$g at/cm² day).

In Table V the effects of these differences are given. The effect on production is less than 10% and nearly balances over the two intervals. But, although regeneration also nearly balances, the internal differences are very large. This is due to the fact that regeneration is very sensitive to the absolute value of the phosphate concentration, whereas production depends more on the phosphate gradient. This example is of interest in showing the effect of inaccuracies on

the phosphate observations, and because of this, no alterations were made in the production estimates. The data have been used to correct both the 1953 estimates for station 1 and those for the other stations in the Fladen Group, and explains one of the differences between the histograms of regeneration for 1951, 1952 (Fig. 9) and 1953 (Fig. 10).

TABLE V. COMPARISON OF PHOSPHATE ESTIMATES (μg AT/CM^2)

	Aberdeen		Plymouth	
	Production	Regeneration	Production	Regeneration
11. v.–21. vi	0·82	2·02	0·91	1·22
21. vi–15. vii	0·62	0·01	0·55	0·83

The next anomalous feature of the 1953 results is the large rate of regeneration found in the period 8–25 September. This is due to the large increase in the phosphate concentration in the lower isothermal part of the water column. However, there is also an increase in salinity at the two northerly stations of the Fladen group (Fig. 11). Now, inside the central area of the northern North Sea described on pp. 2–4, although the temperature is relatively

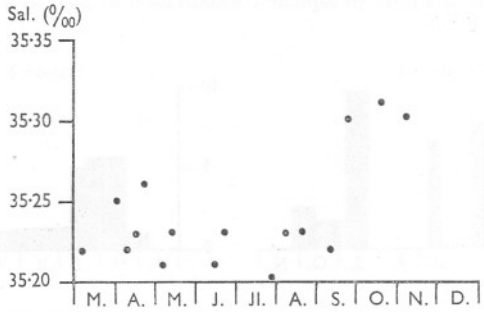

Fig. 11. Salinity at 58° 25′ N., 0° 20′ E. (Fladen) in 1953; an example of the changes which occurred in September.

constant, there are variations in salinity and phosphate concentration due to the unequal mixing of the winter Atlantic inflow. It is suggested that during September there was a shift in the central core, bringing water of higher phosphate concentration on to Fladen. This shift could be due to the 'pressure' begun by another Atlantic inflow. Fraser (1954), from plankton distributions, and Tait (1954) show that during the summer there was a surge of oceanic water round the north of Shetland, and that during the autumn this water penetrated into the North Sea till, by the end of the year, it covered the Fladen Ground. The plankton collections taken on Fladen agree with this general picture, which thus provides a reasonable explanation for the shifting water mass.

A further demonstration of the anomalous nature of this feature is shown in the graphs of integrated content of phosphate in the water column (Fig. 12),

where the graph for 1953 suddenly rises much above the content at the beginning of the season. This anomaly has been removed by using the mean rate of regeneration of the surrounding intervals.

Again, from Fig. 12, it is possible to notice another anomaly. In 1952 the spring regeneration was much lower than in the other 2 years. Now the winter phosphate concentration is obtained from three phosphate values, 0·73, 0·76, 0·79 μg at/l., in the lower part of the water column on 25 April; the mean 0·76 μg at/l. was chosen, but in Fig. 12 this is seen to put the winter level abnormally high compared with the remainder of the curve (even though there

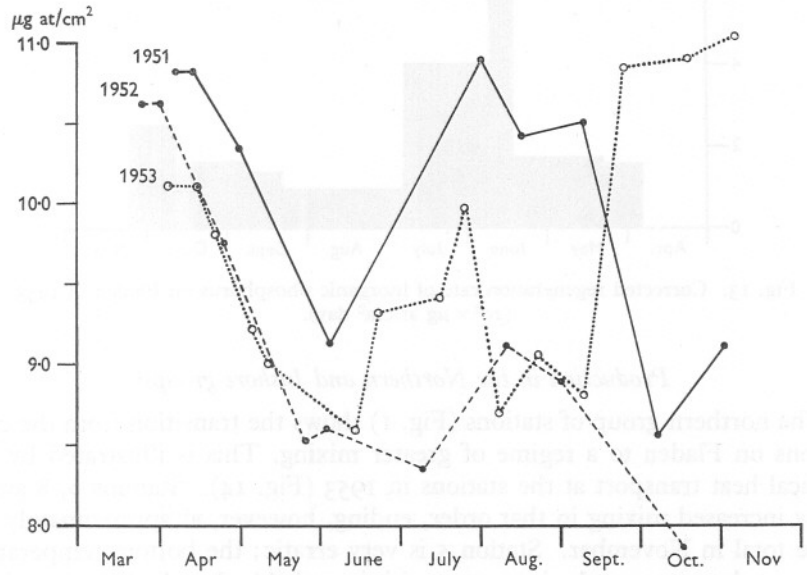

Fig. 12. Integrated content of inorganic phosphorus on Fladen in the years 1951–3 (μg at/cm^2).

was more production in this year than in the other two). A reduction in the winter level to 0·73 μg at/l. would raise the estimate of spring regeneration from 0·32 μg at/cm^2 to 0·56 μg at/l.; at the same time the reduction in the spring production would be 1·7 g carbon/m^2, bringing the total down to 28·7 g carbon/m^2, which is in fact nearer to the other two estimates.

The results of these various corrections are shown in Figs. 9 and 13, which are the histograms of regeneration for Fladen. They show that the main regeneration after the spring outburst occurs between the middle of June and end of July. Thus, the maximum rate of regeneration occurs about 2 months after the maximum rate of production, and this interval also corresponds to the interval between the end of the spring outburst (end of May) and the beginning (end of July) of the low rate of regeneration found in the summer of 1951 and 1953.

From this it appears that regeneration occurs about 2 months after production. Again, this effect can be seen from the graphs of Fig. 12, where the interval between the spring trough and summer peak is approximately 2 months, and the phosphate content at this summer peak in 1951 and 1953 is about the same as the winter level.

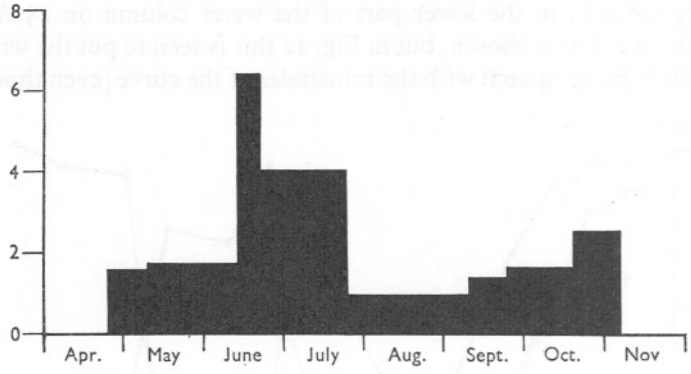

Fig. 13. Corrected regeneration rate of inorganic phosphorus on Fladen in 1953 ($10^2 \times \mu g$ at/cm^2 day).

Production in the Northern and Inshore groups

The northern group of stations (Fig. 1) shows the transition from the conditions on Fladen to a regime of greater mixing. This is illustrated by the vertical heat transport at the stations in 1953 (Fig. 14). Stations 6, 8 and 7 show increased mixing in that order, ending, however, at approximately the same total in November. Station 5 is very erratic; the bottom temperature does not show any regular increase, and it is probable that the lateral gradient in the amount of mixing is sufficiently steep for these changes to be due to small horizontal shifts in the water between times of sampling. The temperature profiles for station 7 are given in Fig. 15A.

Calculations for production were made in the same way as for Fladen, although the postulate of purely vertical water movement may not always be satisfactory.

During the summer of 1953, the greater mixing gave rise to an increased production which probably equals that of the summer production on Fladen in 1952 (the maximum on Fladen in the three years considered).

In the autumn of 1953, there is much larger production than on Fladen. An explanation of this may be found in Fig. 14. The heat transport by 6 November is much higher than on Fladen 1953 and is almost the same as that found on Fladen in 1951 and 1952. Thus it appears that the breakdown of the thermocline occurred earlier than on Fladen. This is confirmed by the production figures for station 7, where it is shown that the main part of the

autumn flowering occurred before 19 October. On Fladen (station 1) there was but little production before that date in 1953.

Thus the northern group, which is in an area of hydrographic change between the conditions on Fladen and those inshore, shows corresponding changes in the production.

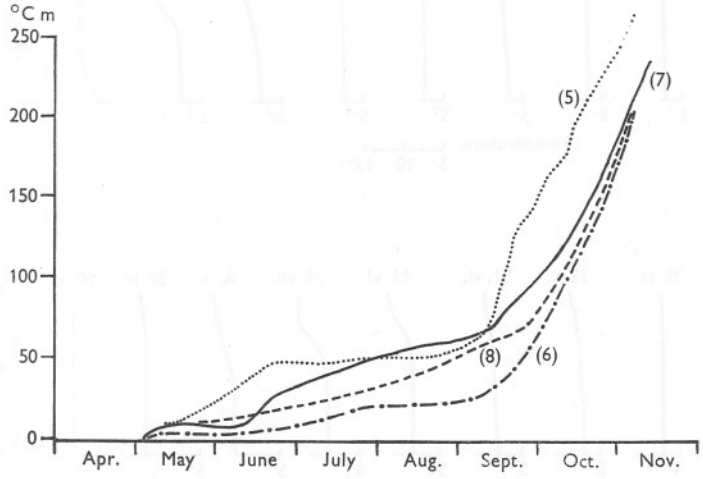

Fig. 14. Increase in heat content below 40 m at the Northern group of stations in 1953.

TABLE VI. 1953: PRODUCTION IN NORTHERN GROUP (G CARBON/M²)

Station no.	15. iv–11. vi	11. vi–8. ix	8. ix–25. ix	25. ix–19. x	19. x–6. xi	Total
5	24·2	20·5	8·3	27·0		80·0
6	23·2	18·7	5·7	20·9		68·5
7	23·7	36·5	19·0		7·1	86·3
8	28·9	25·7	4·3	11·6		70·5

The inshore group of stations lies in an area of confused water movement with large changes in salinity. From an examination of the salinities it seems likely that most of the time these changes are due to local wind drift which will produce variations in the seawards limit of the less saline coastal water. However, there will be occasions when the changes are not local, but will involve the intrusion of water from other regions. The temperature profiles for station 11 are given in Fig. 15B.

Thus it is obvious that the postulate of purely vertical water movement does not hold, yet, since these lateral changes may be of an oscillatory nature for much of the year, the total estimates of production, considered as a balance of these variations may give a reasonable estimate for the year.

The values of yearly production for the four stations were 103·9, 108·1, 108·4 and 127·2 g carbon/m².

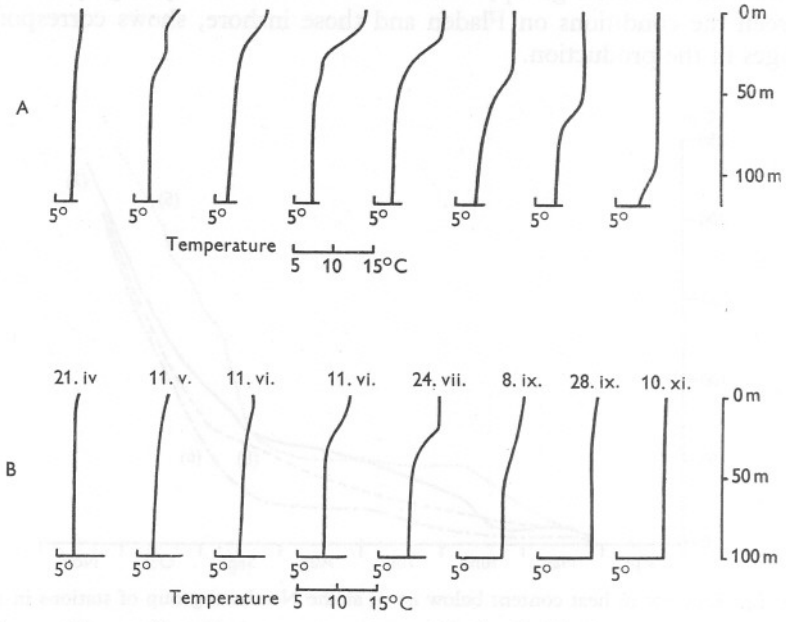

Fig. 15. The profiles of temperature, at A a station in the Northern group, and B a station in the Inshore group.

TABLE VII. SUMMARY OF THE ESTIMATES OF PRODUCTION (G CARBON/M²)

Group	1951	1952	1953			
			Station	1	2	3	4
Fladen	64·9	82·3		59·5	53·6	56·3	62·2
			Station	5	6	7	8
Northern	—	—		79·9	68·4	86·2	70·5
			Station	9	10	11	12
Inshore	—	—		127·2	103·9	108·1	108·4

Discussion

The values for yearly production given in Table VII show variations between years on Fladen and between groups in the year 1953. The main conclusion of this part is that the differences in the Fladen results can be explained by the variations in mixing, and these variations in turn can be linked with the wind data and show the critical effects of high winds near the beginning of June and at the middle of September.

Fladen was chosen as typical of those parts of the North Sea where least mixing occurs. The other groups were added to show the effects of increased

mixing and in their values for production they reflect this. Thus in 1953 the inshore group had a production nearly twice that of the Fladen group, while the northern stations had intermediate values.

This increased mixing will be largely due to the increase in the effect of tidal movement. On Fladen the wind effectively controls mixing, and it was possible to relate the two. Such relations would not be demonstrated in the more complex cases when wind and tide combine.

As was stated earlier (p. 7) the effect of regeneration above the zero level has been ignored. In Figs. 6 and 7 the depth distribution of regeneration below the zero level is shown. Although there are variations with depths, there is no sign of a consistent increase in the rate of regeneration just below the zero level. Thus the assumption that the regeneration rate is constant with depth may be satisfactory. On this basis the addition to the values of production already given would be about 17 g carbon/m^2 on the Fladen and Northern groups and about 31 g at the Inshore group. This is an increase of between a quarter and a third of the original estimates.

TABLE VIII. COMPARISON OF NORTHERN NORTH SEA, ENGLISH CHANNEL AND GEORGES BANK

	Winter phosphate (g at/l.)	Yearly production (g. carbon/m^2)	Maximum plant population (mg chlorophyll/m^2)	Maximum zooplankton (g carbon/m^2)
Fladen	0·7	54–82	100	5
Inshore	0·6	104–127	175	5
English Channel	0·35	55–91	210	5
Georges Bank	1·1	120–300	660	30

These estimates of production can be compared with those from other areas. It is pointless to compare production values by themselves, so three other relevant figures are given in Table VIII. It will be seen that the English Channel (station E. 1), although having a lower winter phosphate concentration than the northern North Sea, has a production which is probably between Fladen and Inshore values. This is due to the fact that there is at E. 1 vertical mixing similar to that occurring at the Inshore group. Georges Bank, which has both a large winter phosphate concentration and considerable mixing, gives a production which is certainly greater than any of the other regions, and these differences are reflected in the maximum plant and zooplankton populations.

Thus the estimates of production given in this paper fit reasonably into the pattern of previous results. Because of this, and because of the internal consistency of the Fladen results, it seems that the use of phosphate data can be considered as a comparatively satisfactory method of studying production.

Part II. Plant Population and Production

The first part of this paper was mainly concerned with the problem of estimating the production from the sea area typified by Fladen. The various assumptions required for these estimates were each more or less open to question, and thus the results are to that extent uncertain.

In this second part, these results will be linked with chlorophyll and dry-weight data, again on the basis of hypotheses whose validity is uncertain. The picture obtained, however, proves to be to some extent internally consistent and agrees reasonably with the results of other workers. To this extent the various methods of sampling and their attendant hypotheses can be held to be justified.

The attempts to describe quantitatively the causal relationships which control plant population and production began with the work of Fleming (1939) and were continued by Riley and his colleagues (Riley, 1946; Riley *et al.* 1949). The development of this approach is summarized by Riley (1953).

Any model of the plant population should take account of many factors, but there are three principal ones which must be considered: production, predation and water movement. Fleming (1939) and Riley (1946) did not deal with the effects of vertical mixing, and thus said nothing about the vertical distribution of the plants. Instead they concentrated on changes with time and obtained reasonable fits with the mean populations. The problem of explaining the distributions with depth was studied by Riley *et al.* (1949) but, in order to do this, areas and times of year were chosen in which the plankton was changing slowly and this was idealized by assuming a steady state. Again fairly good fits were obtained.

Thus the present position is that changes in mean population with time, and stationary distributions with depth, have been studied separately, but no attempts have yet been made to provide an example where these two factors occur together. The main obstacle to this is caused by the generally continuous lateral changes which occur in the sea. These ensure that the water sampled on a given date will frequently not be the same as that sampled previously. This is most serious when detailed vertical distributions are being studied.

However, it was shown in the first part of this paper that the Fladen area appears to approximate sufficiently to the required conditions. For this reason an attempt will be made to compare calculated and observed distributions of chlorophyll throughout 1953.

Ideally one should start from measurements of the physical environment alone and, by means of a set of simultaneous equations, build up models of the distribution of nutrients, plants, herbivores, etc. This will not be attempted here, and only the distribution of the plants as shown by chlorophyll concentration will be considered.

Also, there should be a genuine prediction which can be tested, but this is

impossible since, in the end, wind strength is unpredictable. Thus to explain the chlorophyll distribution at the end of a time interval, the information used is as follows: chlorophyll distribution at the beginning of the interval, phosphate, temperature and dry-weight of a net haul at both ends of the interval. In this way, for a series of intervals, a series of calculated chlorophyll distribution is obtained for comparison with the observed distributions.

Before giving the calculations, the chlorophyll and dry-weight observations will be described.

In 1953, when it was decided to supplement the chemical observations by estimates of the chlorophyll concentration, the following simple method was devised.

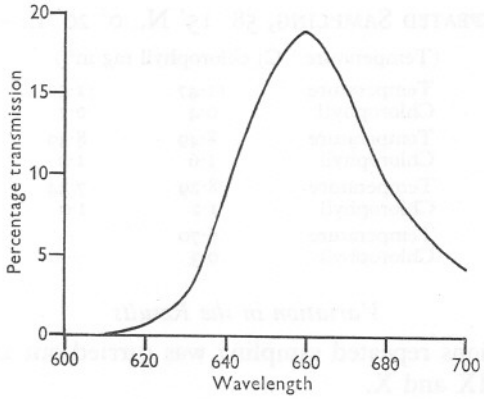

Fig. 16. Calculated spectral response of combination of photocell and 1608 filter.

The Chlorophyll Observations

Water samples were taken from 10, 30, 50 and 100 m. The quantity of water used for each estimation was either 1·6 or 2·4 l. This was filtered under suction through a no. 542 Whatman filter-paper and the paper preserved in 80% acetone. The sample was kept in the dark until it was taken to the laboratory where it was made up to 25 ml. after filtering and its light absorption measured. The measurement was made on the long cell model of the Hilger and Watts 'Spekker', using a 3 in. cell and Ilford 608 filters. The spectral response given by the combination of the filter and the cut-off of the photoelectric cell is shown in Fig. 16. Since the secondary chlorophyll maximum, which is used in this type of work, lies at about 655 mμ, the response curve used should be satisfactory. The main drawbacks are (*a*) less than half of the light beam is used, and (*b*) the maximum transmission of 19% is low.

The instrument was calibrated with a known chlorophyll concentration kindly provided by Dr de Kock of the Macaulay Institute for Soil Research, Aberdeen.

One source of error which should be mentioned is the possible loss of the smallest plants, which may pass through the filter-paper. Using a no. 2 Whatman paper, Riley (1941) concluded that he caught 90% of the phytoplankton, a membrane filter catching little more. Harvey (1950), however, found that a no. 2 paper caught just over half of what was retained on a Gradocol membrane. The no. 542 paper used here is probably more retentive than the no. 2, but the probability of the small flagellates escaping cannot be ignored.

Thus this arrangement cannot be expected to be very accurate. However, apart from inaccuracies in the method, one can expect significant and perhaps large errors to arise from sampling variations.

TABLE IX. REPEATED SAMPLING, 58° 15′ N., 0° 20′ E.—21 JUNE 1953

(Temperature ° C; chlorophyll mg/m³.)

10 m	Temperature	11·47	11·50	11·52
	Chlorophyll	0·4	0·2	0·4
30 m	Temperature	8·49	8·49	8·51
	Chlorophyll	1·6	1·0	1·4
50 m	Temperature	8·29	7·34	6·79
	Chlorophyll	1·2	1·0	0·4
100 m	Temperature	6·70	—	—
	Chlorophyll	0·3	—	—

Variation in the Results

On three occasions repeated sampling was carried out and the values are shown in Tables IX and X.

In Table IX are shown the results from a station of the Fladen Group. The temperature of each water sample was also taken. It will be seen that the largest variation in concentration occurs at 50 m where there are large temperature changes. These variations are related; the highest temperature at 50 m is near the 30 m temperature and the chlorophyll concentration lies within the range of the 30 m values; similarly, the lowest temperature at 50 m is near the temperature of the homogeneous lower layer and the corresponding concentrations do not differ significantly. Thus, at this particularly steep point of the thermocline, the variations in concentration depend upon the part of the thermocline from which the sample was taken.

Considering the further results from Table X, it may be said that, when the 50 m case in Table IX is omitted, the standard deviation is not more than 25% of the mean, except at such low concentration as 10 m on 21 June 1953.

The next form of variation to be considered is that between stations of the Fladen group at any given time. All the observations are given in Table XI.

This shows that on Fladen, although there are differences between the four stations on any occasion, the maximum concentrations nearly always occur at the same depth. That is to say, the samples from the four stations appear to be samples of populations having the same distribution with depth. Thus it is

reasonable to take the mean values of the four stations, as shown in Fig. 18, as representing the chlorophyll distribution on Fladen.

Since the chlorophyll observations are to be used in conjunction with phosphate and dry-weight data, some common quantity is required and, as in the phosphate results, carbon content will be used. However, the initial question is whether it is possible to have a single conversion factor from chlorophyll to carbon content. It has been shown that the concentration of

TABLE X. REPEATED SAMPLING FROM THE INSHORE GROUP

57° 55′ N., 1° 00′ W., 25. vii. 53	10 m	0·6	—	0·7	—
	30 m	2·1	2·0	2·6	—
58° 05′ N., 1° 00′ W., 8. ix. 53	10 m	1·8	1·5	1·6	1·9

TABLE XI. CHLOROPHYLL CONCENTRATION IN MG/M³ ON FLADEN IN 1953

	21. iv				11. v				11. vi				21. vi			
Station no. ...	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
10 m	0·6	—	1·0	0	2·9	1·9	—	2·9	0·8	0·4	1·2	1·1	0·4	0·4	0·4	1·0
30 m	0	—	0·8	0	1·6	0·7	—	—	1·5	1·3	1·4	1·2	1·4	1·2	1·6	1·7
50 m	0	—	0·6	0	0·2	0·3	—	0·4	0·4	0·4	0·6	1·4	0·8	0·4	1·1	0·7
100 m	0	—	0·2	0	0	0·3	—	0·5	0·2	0·6	0·4	0·6	0·4	0·4	0·3	0·4

	24. vii				8. ix				25. ix			
Station no. ...	1	2	3	4	1	2	3	4	1	2	3	4
10 m	0·2	0·2	0·2	0·1	0·4	0·4	—	0·4	0·7	0·3	0·8	0·9
30 m	0·3	0·4	0·5	0·4	0·4	0·8	—	0·6	0·3	1·0	0·7	1·2
50 m	1·0	1·0	0·6	1·7	0·8	0·8	—	0·7	0	0·2	0·3	0·6
100 m	0·2	0·3	0·6	0·5	0·4	0·4	—	0·1	0·2	0·2	0·2	0·2

pigments in plant cells varies with the light intensity in which they are grown (Rabinowitch, 1945). Similar variations have been found in the sea and appear to include differences with season and with depth. This may explain the large variations which are to be found in the figures quoted by workers in this field. Both Harvey and Riley have made several estimates of the relevant quantities. Riley (1949, 1952) decides on a factor

$$1 \text{ mg chlorophyll} \equiv 54 \text{ mg carbon.}$$

Harvey's value (1950) is in terms of units of plant pigments which on conversion gives

$$1 \text{ mg chlorophyll} \equiv 27 \text{ mg carbon.}$$

Harvey says of his own figure—'This estimated ratio is no more than a rough approximation. It is unlikely to be less than half or more than double the true mean ratio.'

The ratio given by Riley will be used since one purpose of this paper is to compare certain results obtained here with those of Riley.

Dry Weights of Zooplankton

Since knowledge is required of predation on the plants, some figures must be found that will represent the herbivore population. It is probable that the dry weight of a net haul is the best compromise between simplicity and accuracy.

A Hensen egg-net was used with no. 3 silk (60 meshes per inch). The net was hauled vertically from bottom to surface. The collections were preserved in ethyl alcohol and, on return to the laboratory, dried for 12–18 h at 90° C and then weighed. The values used for the subsequent calculations are the means of the four stations as shown in Fig. 17, with the figure for 21 June found by interpolation.

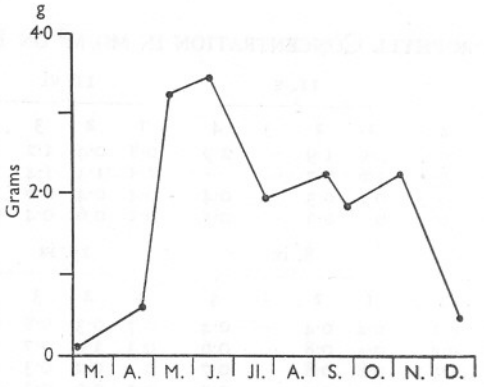

Fig. 17. Mean dry weights of net hauls on Fladen in 1953.

As with chlorophyll data, these results are converted into carbon content. Since the opening of the net has an area of approximately one-third of a square metre, then, assuming 100% filtration and taking the carbon content to be half the dry-weight, the values in Fig. 17 should be multiplied by 1·5 to give carbon content below 1 m^2.

These results are not only very variable, but they are also very much minimum values, since a no. 3 silk catches but a small proportion of the smaller animals. This effect is important when considering the assumptions necessary for using these data in the subsequent calculations.

These data are intended to provide information about the grazing on the plants, and the simplest assumptions which would be required for the calculations are that (*a*) the animals are filter feeders and filter a constant quantity of water per unit of dry weight in a net haul, and (*b*) the zooplankton population spends equal time at all depths.

The most serious objection to (*a*) has been pointed out by Raymont & Gauld (1951) and Gauld & Raymont (1953), who drew attention to the fact that, whereas weight is approximately proportional to the cube of the length,

respiration is approximately proportional to the square. Thus the food requirements of the zooplankton will depend on the size frequency-distribution of the population, with the smaller animals having much higher requirements per unit of weight.

Concerning the second assumption, Nicholls (1933) showed that copepodite stages I–III of *Calanus* in Loch Fyne were always most abundant above 30 m during 1932. If this result was regarded as generally applicable, which is not obviously the case, then these two facts together could thus upset the postulates suggested to describe the grazing effects. However, these postulates are not merely the simplest but probably provide the only available approach for linking predation to the plant population.

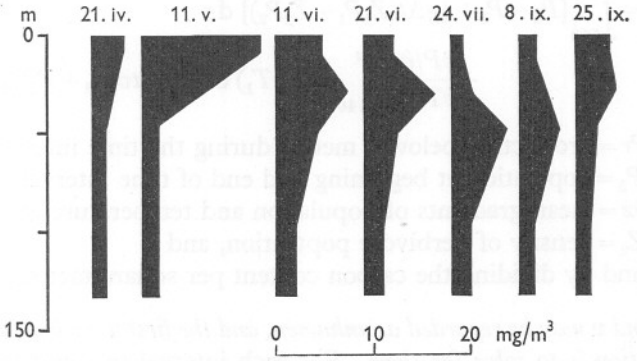

Fig. 18. Profiles of chlorophyll concentration (mg/m³) on Fladen in 1953 constructed from the mean values at 10, 30, 50 and 100 m.

Sinking Rate and Filtering Rate

The value of the constant which converts dry weight to filtering rate will be one of the two unknowns which must first be evaluated. The other initially unknown quantity is the rate at which the plants sink through the water. This and the filtering rate must be evaluated to give a quantitative picture of the reasons for the changing distribution of the plants.

Qualitatively, this sequence of events can be seen in Fig. 18. After the spring outburst, production does not occur at a high rate and, during the summer, the gradual sinking of the population and its grazing down is shown by the successively greater depths at which maximum concentration occurs and by the decreasing total chlorophyll content.

The basic mathematical form which will express these various effects is as follows (see Riley *et al.* 1949):

$$\frac{\partial P}{\partial t} = (p_h - r - gZ)P + \frac{\partial}{\partial z}\left(\frac{A}{\rho}\frac{\partial P}{\partial z}\right) - v\frac{\partial P}{\partial z}, \qquad (1)$$

where P = density of the plant population, p_h = photosynthetic rate, r = respiration rate, g = filtering rate, Z = density of herbivore population, A = coeffi-

cient of vertical diffusivity, v = sinking rate, z = depth. The units used here are grams, metres, days. The term $(p_h - r)\,P$ can be replaced here by the estimates of production rate derived from the phosphate results.

Riley *et al.* (1949) also made the further assumption throughout their work that $\partial P/\partial t = 0$, which allowed them to study each station separately. It is obvious from the profiles shown in Fig. 18 that the phytoplankton on Fladen cannot be considered as existing in a steady state. Thus the methods used by Riley could not be applied here. It is necessary to consider the changes between two stations and, as with the phosphate, this involves making several approximations. The integrated approximate form of (1) is

$$Pr = \int_{140}^{z} [P_2 - P_1 + \tfrac{1}{2} g \Delta t (Z_1 P_1 + Z_2 P_2)]\, dz - \frac{\partial P/\partial z}{\partial T/\partial z} \int_{140}^{z} (T_2 - T_1)\, dz - \tfrac{1}{2} \Delta t v [P_1 + P_2]_{140}^{z}, \qquad (2)$$

where Pr = production below z metres during the time interval Δt,
P_1, P_2 = population at beginning and end of time interval Δt,
$\partial P/\partial z$, $\partial T/\partial z$ = mean gradients of population and temperature at depth z,
Z_1 and Z_2 = density of herbivore population, and
Z_r is found by dividing the carbon content per square metre by 140.

In (2) g and v may be regarded as unknown, and the first use which will be made of this equation is to solve for them. For each interval two equations can be found, corresponding to the depth intervals $0 - z_0$ metres and $z_0 - 140$ m, where z_0 is the zero level. These equations can be solved simultaneously to give values for g and v.

Of the results shown in Table XII, those for the first interval appear to be abnormally high; the reasons for this will be discussed later. Excluding this interval the others show a comparatively small range of variation with mean values of 3·0 m/day and 1·34 m³/day/g zooplankton carbon.

The sinking rates proposed by Riley were dependent on temperature, but such a variation is small compared with the overall variation of the results and has not been included here. Over the temperature range found here Riley's choice of sinking rate varies between 3·5 and 4·3 m/day. The values given here cover this range but have a lower mean value. However, when one remembers the great range of values for sinking rate which has been found previously (Riley *et al.* 1949, pp. 84–5), the agreement between the figures given here and

TABLE XII. SINKING RATES AND FILTERING RATES

	21. iv.–11. v.	11. v.–11. vi.	11. vi.–21. vi.	21. vi.–24. vii.	24. vii.–8. ix.	8. ix.–25. ix.
Sinking rate (m/day)	13·0	2·4	2·1	4·2	4·9	1·4
Filtering rate (m³/day/g carbon)	2·9	1·0	1·2	1·7	1·4	1·4

those of Riley can be taken as very satisfactory. Similarly, the mean filtering rate does not differ greatly from Riley's estimate of 1·26 m^3/day/g C.

With values for filtering and sinking rates available, the detailed shape of the profiles can now be considered. Equation (2) can be put in the form

$$(1+\tfrac{1}{2}g\Delta tZ_2)\int_{140}^{z} P_2\,dz-\tfrac{1}{2}\Delta tvP_2=Pr+(1-\tfrac{1}{2}g\Delta tZ_1)\int_{140}^{z} P_1\,dz$$
$$+\frac{\partial P/\partial z}{\partial T/\partial z}\int_{140}^{z}(T_2-T_1)\,dz+\tfrac{1}{2}\Delta tv\,(P_1-[P_1+P_2]_{140}^{z}), \qquad (3)$$

where
$$\frac{\partial P}{\partial z}=\tfrac{1}{40}[P_1+P_2]_{z+10}^{z-10}. \qquad (4)$$

The left-hand side of (3) contains P_2 alone, but the right-hand side, beside terms in P_1, involves P_2 in the term $\partial P/\partial z$, as shown in (4). The third term on the right-hand side is, however, found to be much smaller than the others and *thus* (3) *can be used to predict* P_2 *by successive approximation.* In fact, because of the low degree of accuracy, no second approximation is necessary.

As a first step, a mean value is found for the 0–20 m layer; then this value is used to find the 30 m value; and so on to the top of the homogeneous bottom layer. In this way a profile is obtained from surface to about 50–70 m. This profile can be compared with the three observed values at 10, 30 and 50 m. With the exception of the first time interval, the computations were first made with the values for sinking and filtering rates given in Table XII. Then, for the first interval and some of the subsequent ones, other values were used in search of a better fit. The results of these calculations are shown in Fig. 19.

It is in the first four cases that the observed points show the greatest changes with depth, so that these cases are of the most interest from the point of view of predicting results.

These four profiles show the same type of distribution with depth as the observed points. The last two profiles also show quite large changes with depth, but these are not borne out by the observed points.

The two profiles which have been drawn for the first interval show that a high filtering rate and low sinking rate are required to give a reasonably shaped profile with approximately the observed quantity of plankton above 50 m. Similarly, increasing the filtering rate in the second and third cases brings the profiles into better agreement with the observed points. In the fourth interval the initial profile is as good a fit as can be obtained. The fifth profile does show an increase with depth which is also true of the observed points; decreasing the sinking rate will give a rather better fit, but because of the small number of observed points there is no confirmation of the predicted maximum at 40 m. In the last case it was not found possible to obtain a reasonable fit.

Returning to the first interval, in both sets of calculations it gives results which are very different from the succeeding intervals. This interval covers

the spring outburst and so it is likely that the population will consist largely of juvenile stages of the copepods. This would provide the conditions which, as suggested on p. 27, would not fulfil the postulates on which the grazing effects were based. During this period it is possible that grazing, dominated by the juveniles with their proportionately higher metabolic rate, was confined to the water above 50 m which, in turn, provides an explanation of the anomalous results of the first calculation and of the high filtering rates given by the second. Such a hypothesis might well be necessary since the juveniles, with their proportionally greater food requirements, would perhaps be unable

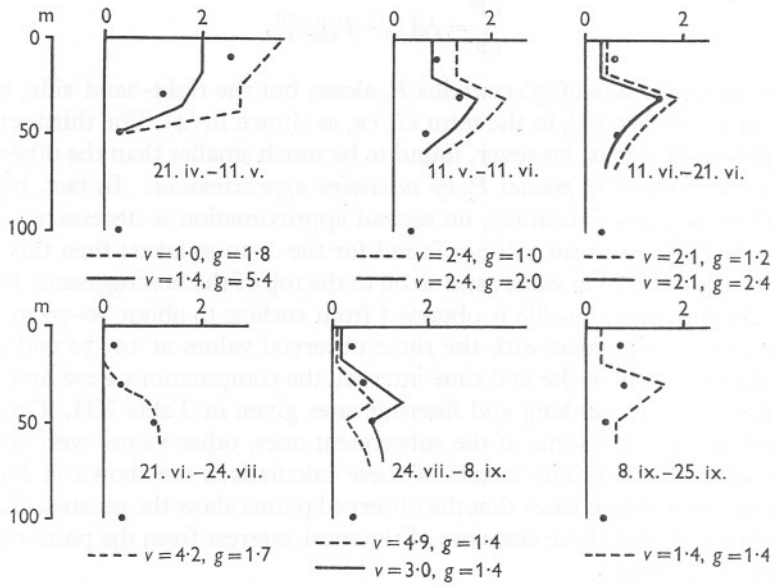

Fig. 19. Comparison of calculated and observed chlorophyll concentrations (mg/m³).

to obtain sufficient food if they spent only a small part of their time in the zone of high plant concentration. Later, when they are adult and when the maximum concentration of phytoplankton may occur at widely varying depths, vertical migration could be expected to be more extensive, and the postulate of equal depth concentration over long time intervals becomes more acceptable.

The need for a greater filtering rate is still apparent in May, but by June the differences between observed and calculated values are so small that a greater filtering rate does not provide a significantly better fit. Thus the anomalies between the two calculations decrease from April onwards, perhaps because of the progressive domination of the population by the later stages of copepods.

Regarding the interval 8–25 September, it will be remembered that the phosphate results for this interval were somewhat erratic (p. 16), and it was suggested (p. 16) that there was some shift in the water during this period.

Possibly this could also explain the poor fit for the chlorophyll data on the 25th.

It also seems possible that there are significant changes in the sinking rate. The values deduced here start at about 1·4 m/day, rise to above 4 m/day at the end of July and then in September return to 1·4 m/day. Gross & Zeuthen (1948) have suggested that under suitable conditions diatoms do not sink, since their specific gravity equals that of the surrounding sea water. But to maintain this buoyancy requires the expenditure of energy and so sinking occurs when these energy requirements cannot be met. This situation is most likely to occur in a senescent population. Thus it is possible that the variations in the calculated sinking rate may correspond to the gradual change from a new plant population in April and May, living in a suitable environment, to a senescent population in July, with a return in the autumn to a population of comparatively recent origin.

From this discussion it would appear that neither the sinking rate, v, nor the filtering rate, g, will be constant throughout the year but will depend on the age composition of the phytoplankton and zooplankton respectively.

Finally, it has not proved possible to start with given constant rates of sinking and filtering and from them to predict the cycle of plant distribution throughout the year. Yet it does seem that by varying the sinking and filtering rates from one interval to the next the changes in plant distribution can be largely explained in terms of the factors listed at the outset; further, these variations in the rates of sinking and filtering are not unreasonable.

Conclusions

The aim of the first part of this paper was to develop a method of estimating the basic plant production. The second part was designed to measure the factors which control the distribution of the plants with time and with depth. In each there has been some measure of success, but most of the results are open to question and, further, in this paper the methods have been applied in detail to one area only.

This type of work requires chemical and biological data collected over many years to provide information on the possible kinds of variation that may occur. The results from the northern North Sea cover, at most, three years, with only one year's data for the areas outside Fladen. Thus the problems tackled so far have been concerned with the basic regularities and the more important variations. For this purpose Fladen is very suitable since it appears to give a good approximation to the case of purely vertical mixing.

In the areas outside Fladen the difficulties which arise are due mainly to the effects of lateral water movements. The measurement of these would be required before the changes at, say, the Inshore group could be properly understood. Similarly, features in the Fladen results such as the apparent

production between 20 and 30 m in the summer, or the anomalous results in the chlorophyll prediction, probably require knowledge of the metabolism of plants and animals which is not at present available. But these are problems which face any method of studying production.

SUMMARY

In the first part of the paper estimates of the plant production are given for the years 1951–53 in one area, Fladen, of the northern North Sea. These estimates, based on the changes in inorganic phosphate, give values for the yearly production of 55–81 g carbon under each square metre of surface—a range which is nearly the same as that in the English Channel; its variation can be related to the varying occurrence of high winds in the different years. It appears that the zone of phosphate utilization sinks progressively during spring and summer to a depth around 30–40 m.

In the second part these estimates are then used, together with chlorophyll and zooplankton data, (*a*) to study the changes in the vertical distribution of the plants, and (*b*) by comparing calculated and observed vertical chlorophyll profiles, to discover to what extent a few simple postulates can explain the changing vertical distribution of plants throughout one year.

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PORPHYRIN PIGMENTS IN THE TECTIBRANCH MOLLUSC *AKERA BULLATA* O. F. MÜLLER

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The sudden appearance of *Akera bullata* in the static water tanks of H.M. Dockyard, Devonport, provided specimens for the investigation of pigments. Morton & Holme (1955) have given an account of certain aspects of the biology of this interesting tectibranch mollusc.

Fifteen fresh specimens were minced in the Waring blender, and the resulting material was treated in the following ways.

Method 1

A portion of the minced material was extracted with absolute methanol, and the extract filtered. The green solution was intensely red-fluorescent in ultra-violet light, and was submitted to long-paper chromatography (Kennedy, 1953). The solvent phases were 2:6-lutidine (5 parts) and water (3 parts), and the chromatograms were run at 23° C in an atmosphere of ammonia.

When the chromatograms were viewed by ultra-violet light, they revealed a spot, red-fluorescent, with R_F value 0·95, indicating a monocarboxyl porphyrin compound such as phaeophorbide. There was another spot with R_F value 1·0 (i.e. travelling with the solvent), which was red, and had a blue fluorescence. This was most probably carotenoid with fat.

The main purpose of this preliminary experiment was to determine the presence or absence of unchanged chlorophyll in the animal. The fact that no red-fluorescent spot with an R_F value 1·0 was found, indicated the absence of this pigment.

Method 2

A portion was extracted with a mixture of methanol and concentrated sulphuric acid (19:1) and allowed to stand overnight in the ice-chest. It was then diluted with an equal volume of water, and the pigmented material was extracted with Analar chloroform, giving an extract which was intensely fluorescent when viewed in ultra-violet light. This extract was roughly dried by passing it through chloroform-soaked paper, and evaporated to dryness *in vacuo*. The residue was redissolved in dried Analar chloroform, and chromatographed on magnesium oxide grade III (Nicholas, 1951). A deep

red, red-fluorescent, band appeared on development with chloroform which passed slowly down the column and was collected as Fraction A (Kennedy, 1953). A deep green, non-fluorescent, band remained on the column and could not be eluted with graded chloroform/methanol mixtures.

Fraction A was evaporated to dryness *in vacuo*. A portion of the residue was dissolved in Analar pyridine, and the remainder in Analar chloroform. Spectrophotometric examination of the pyridine solution gave the following absorption maxima:

I	II	III	IV	Soret band
626	572	536	502	405·5 mμ

A specimen of uroporphyrin I, in pyridine, gave the following maxima:

627	571	536	501	406 mμ

Fraction A was, therefore, considered to be uroporphyrin. Examination of this fraction by the ascending paper chromatography methods of Chu, Green & Chu (1951) and of Falk & Benson (1953) showed that the uroporphyrin was present as isomer I.

The deep-green non-fluorescent material remaining on the column was eluted with ethyl acetate, which was later removed *in vacuo*. The residue was re-dissolved in dry chlorofrom, and chromatographed on alumina (B.D.H. chromatographic grade). Two bands were obtained, the top one green, and the lower one yellow. The green band was entirely non-fluorescent. The yellow band was slightly yellow-fluorescent, suggesting carotenoids, and gradually passed through the column to give a solution with an indeterminate spectrum.

On further development with chloroform the whole column became green-blue and the washings were colourless. After many attempts the green-blue pigment was finally eluted with a mixture of *n*-butanol and formic acid (50:0·5). The formic acid was removed from the eluate with water, using the smallest volumes possible, and adding compensatory volumes of *n*-butanol to avoid losing pigment. The butanol was then removed *in vacuo*. The pigment residue could then be dissolved in chloroform, and was flocculated with light petroleum (b.p. 40–60° C), giving a dark-green product. This was dried *in vacuo*, and a portion redissolved in chloroform gave the following maxima (in the Hartridge reversion spectroscope):

I	I	III	IV	
655·3	604·0	554·2	465·5 downwards	mμ

A sample dissolved in toluene and examined in the Unicam spectrophotometer gave the following maxima:

660	559	427	mμ

This pigment gave none of the typical bile pigment reactions. The absorption curve, together with the absence of fluorescence, suggested that the pigment might be a metal complex of a chlorophyll derivative.

Metals may frequently be removed from metalloporphyrins by treatment with acids or with hydrazine, and therefore the green pigment was warmed with concentrated hydrochloric acid on a water-bath, and driven into ether by adding saturated potassium acetate. The pigment was unchanged, which suggests the probable absence of zinc, divalent tin and silver. Treatment with concentrated sulphuric acid produced some red fluorescence which rapidly disappeared, due undoubtedly to the destruction of the pigment. Concentrated sulphuric acid is known to remove copper, divalent iron, nickel and cobalt. It is therefore possible that one of these metals could be present.

On dissolving the green pigment in propionic acid and boiling with hydrazine for several minutes, the mixture gradually acquired a fine red fluorescence (when viewed with ultra-violet light) and gave an absorption spectrum (Hartridge reversion spectroscope) consisting of one very strong band at 662 mμ and two weak bands at 540 and 509 mμ respectively. This indicates that the pigment could be a metal derivative of phaeophorbide *a*.

A chemical test for metals was kindly carried out by Mr F. A. J. Armstrong. The pigment gave 30% residue after ignition, and a very strong reaction for copper. There was a trace of iron, but no nickel.

A sample of phaeophorbide *a*, prepared from chlorophyll *a*, was dissolved in boiling ethanol, and while boiling, freshly precipitated copper powder was added, the whole operation being conducted under ultra-violet light. The intense red-fluorescence of the phaeophorbide disappeared almost at once, and, after boiling for a further 60 sec, and examination in chloroform in the Hartridge Reversion Spectroscope, the spectrum was found to be:

I	II	III	IV	
656·0	604·0	554·0	465	mμ

This agrees well with the spectrum of the non-fluorescent pigment in chloroform.

A sample of the ethanolic solution was evaporated to dryness and dissolved in toluene, and the spectrum examined in the Hartridge Spectroscope:

661 560 427 mμ

Thus it is reasonable to deduce that the two pigments—natural and synthetic —are identical, that is, the non-fluorescent green pigment of *Akera* is a copper co-ordination complex of phaeophorbide *a*.

Method 3

The second portion of the original fresh minced material was extracted with a mixture of ether and acetic acid (5:1), and gave a green solution which was intensely red fluorescent. The acetic acid was washed out with water containing 0·05% of Teepol detergent; this was used to avoid emulsification (A. S. C. Lawrence, personal communication, 1953). The remaining ether

solution was extracted successively with 1, 8 and 15%, w/v, hydrochloric acid. The 1 and 8% acids extracted no fluorescent material, thus indicating the absence of coproporphyrin and protoporphyrin. The 15% hydrochloric acid extract was, however, deep green-blue, and intensely red-fluorescent, suggesting the presence of free phaeophorbides, which have HCl numbers of 15 (phaeophorbide *a*) and 19·5 (phaeophorbide *b*) (Willstätter & Stoll, 1913). The whole ether solution, therefore, was completely extracted with 15% hydrochloric acid (uroporphyrin is not extracted by the ether/acetic acid method, and therefore remains in the original minced material).

The 15% hydrochloric acid extract was repeatedly shaken with chloroform, and the deep-blue hypophases pooled. The chloroform solution was washed with water and roughly dried by passing through chloroform-soaked paper, and evaporated to dryness *in vacuo*. An iridescent blue-black residue was obtained, which was re-dissolved in dry peroxide-free ether and fractionated with 0·2% aqueous sodium bicarbonate. Phaeophorbide *b*, being slightly more acidic than phaeophorbide *a*, is extracted quantitatively from a mixture of the two by 0·2% aqueous sodium bicarbonate (Willstätter & Stoll, 1913). No fluorescent hypophases, however, were obtained, indicating the presence of phaeophorbide *a* only. The ether solution was evaporated *in vacuo* and the residue redissolved in Analar chloroform. Examined in the Hartridge reversion spectroscope this solution gave the following absorption maxima:

I	II	III	IV	
666·6	606·7	539·2	507·3	mμ

This is clearly phaeophorbide *a*.

Discussion

Kennedy & Vevers (1954) found uroporphyrin I in the integument of *Aplysia punctata*, and it is noteworthy, though not surprising, that the same porphyrin was isolated from *Akera*, a related form.

It is curious that only phaeophorbide *a* was detected, in view of the fact that *Akera*—like *Aplysia*—grazes on *Ulva* (Morton & Holme, 1955) which contains chlorophyll *a* and *b*. Dales & Kennedy (1954) reported a similar result from *Nereis diversicolor*. Both chlorophylls *a* and *b* must be ingested by the animals, and it is very difficult to account for the absence of the *b* degradation component. The reason may lie, as far as *Akera* is concerned, in the fact that phaeophorbide *b* is difficultly soluble in ether, and, if present originally in very small amounts, as is most likely (the ratio of chlorophyll *a* to chlorophyll *b* is often as high as 3:1), the pigment may have been washed out in the process of removal of the acetic acid. This explanation is not really satisfactory, and in some further work the full reason will be sought.

The presence of a copper complex of phaeophorbide *a* is of great interest. Some molluscs are known to concentrate copper from the sea, and of course

the respiratory pigment haemocyanin, which is a copper-polypeptide, occurs in the cephalopod and gastropod molluscs, and in the arthropods. However, there is no doubt that the green non-fluorescent pigment isolated from *Akera*, is a copper phaeophorbide. That this is not an artifact, caused by the co-ordination of copper ions with phaeophorbide in the course of extraction, is supported by the fact that free phaeophorbide also occurs, whereas the affinity of copper for porphyrin pigments is so great that if free copper were present in the animal, *all* the phaeophorbide would have become changed to the metal complex. The reagents used were, of course, beyond reproach, but the same thing would have been true had they contained copper salts or impurities—*all* the phaeophorbide would have formed the copper derivative. In some further work on the pigments of *Aplysia*, to be completed in the near future, J. E. Morton and G. Y. Kennedy will show that a similar pigment occurs in that animal.

SUMMARY

Some pigments of *Akera bullata* (O. F. Müller) are described with methods for their isolation. The pigment of the integument is uroporphyrin I, and from the viscera phaeophorbide *a* together with its non-fluorescent copper complex were isolated. Some points of interest concerning these pigments are discussed.

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VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

IV. MOLLUSCA: LORICATA, LAMELLIBRANCHIATA, AND GASTROPODA

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Introduction

Apart from a general account (Kon, 1954) of our work on vitamin A and carotenoids in invertebrates, our publications so far (Kon & Thompson, 1949*a*, *b*; Batham, Fisher, Henry, Kon & Thompson, 1951; Fisher, Kon & Thompson, 1951, 1952, 1953, 1954, 1955) have been concerned only with marine Crustacea. We have also studied, during our investigation of the metabolism of vitamin A and its possible precursors in the sea, numerous species from most other phyla of marine invertebrates. Except for the nematode worm, *Anisakis physeteris* Baylis, taken from the stomach of a sperm whale, the only two phyla of invertebrate animals in which we have so far found vitamin A are the Arthropoda and the Mollusca. The vitamin was present in at least some species from each of the molluscan classes, Loricata, Gastropoda, Lamellibranchiata and Cephalopoda, but we have no information yet about the Solenogastres or the Scaphopoda of which we have analysed no representatives. So far as our studies were concerned, the cephalopods differed considerably from the other molluscs examined, and the relatively large amount of information they have provided will be more conveniently presented in a subsequent paper. The account which follows, therefore, deals only with species from the first three classes just listed.

To the best of our knowledge, Jameson, Drummond & Coward (1922) were the first to report vitamin A activity in molluscs, but they did not name the species they had tested. Since that date all the published work on vitamin A known to us has been concerned with the food value of clams (Jones, Nelson, Murphy & Devine, 1928) and oysters, which were analysed in the United States by Jones & Murphy (1926), Jones, Murphy & Nelson (1928) and Whipple (1935), in New Zealand by Malcolm (1926, 1927, 1928) and in Malaya by Leong (1939). All these reports gave values for vitamin A activity rather than for vitamin A itself, since they were based on biological tests of whole specimens without previous separation of the vitamin from its precursors. The presence of vitamin A in Loricata and in Gastropoda has not previously been reported.

The history of the investigation of molluscan carotenoids began with the work of Merejkowsky (1881, 1883) and MacMunn (1883*a*, *b*) who extracted a red fat-soluble pigment they called tetronerythrin from many marine invertebrates, including several species of molluscs. Tetronerythrin had the properties of carotenoids, and probably represented the sum total of these pigments in extracts. No further work on the carotenoids of molluscs was reported until the appearance of Lönnberg's papers (Lönnberg, 1931, 1934*a*, *b*; Lönnberg & Hellström, 1932), in which were given the absorption spectra of pigments extracted from many species of marine invertebrates including several molluscs. More specialized studies of carotenoid metabolism in molluscs have been made by Scheer (1940) on mussels, Brooks & Paulais (1939) on oysters, and Goodwin (1950) and Goodwin & Taha (1950, 1951) on limpets. Goodwin (1953) has also investigated the carotenoids of the freshwater mussel, *Anodonta cygnea*.

Our own work has been concerned primarily with the detection and measurement of vitamin A in molluscs, and carotenoids have only been identified and measured when they were separated in our normal technique of analysis.

Material Collected

Eighteen species of Gastropoda, nine species of Lamellibranchiata and one species of Loricata were collected, many of them by ourselves when visiting the shores of different parts of Great Britain, and during a visit by one of us (S. K. K.) to the Scripps Institution of Oceanography, at La Jolla, California, in May 1953. The staff of various marine biological and fisheries laboratories have kindly collected much material for us or allowed us facilities to do our own collecting.

From La Jolla we had the following species: *Cypraea spadicea* Swainson, *Haliotis fulgens* Philippi, *Megathura crenulata* (Sowerby), *Astrea undosa* (Wood) and *Stenoplax conspicua* (Carpenter). We obtained *Lima hians* (Gmelin) and *Pecten maximus* (Linnaeus) at Millport, *Mya arenaria* Linnaeus and *Cardium edule* Linnaeus from Fairlie Sands, and *Aporrhais pes-pelecani* (Linnaeus) and *Chlamys septemradiatus* Müller from Loch Fyne. More species were collected on various parts of the Essex coast; these included *Scrobicularia plana* (da Costa) taken from mud flats at Leigh-on-Sea, *Ostrea edulis* Linnaeus, *Gryphea angulata* Lamarck and *Crepidula fornicata* (Linnaeus) from the rivers Crouch and Roach near Burnham, and *Littorina littoralis* (Linnaeus), *L. littorea* (Linnaeus), *L. rudis* (Maton), *Patella vulgata* Linnaeus and *Mytilus edulis* Linnaeus from the breakwater at Blackman's Point, Harwich. Groups of *Buccinum undatum* Linnaeus were collected at both Millport and Burnham-on-Crouch. *Osilinus lineatus* (da Costa) was collected for us at Plymouth. *Aplysia depilans* Linnaeus and *Murex trunculus* Linnaeus were obtained from collections of marine animals received by the London Zoological Society's aquarium from Madeira. The specimens of

Clione limacina Phipps were taken during a cruise of F.R.S. *Scotia* to Iceland in August 1951, and those of *Limacina retroversa* (Fleming) from R.V. *Ernest Holt* in the Barents Sea in January 1955. *Helix aspersa* Linnaeus was collected from a garden in Shinfield and *Planorbis corneus* (Linnaeus) var. *rubra* Oldham was purchased from an aquarist in Reading. These pulmonates, although not marine, were included for comparative purposes. The red variety of *P. corneus* seemed to be especially interesting in view of its apparent carotenoid colouring.

Methods of Preservation

Apart from the two planktonic species, *Clione limacina* and *Limacina retroversa*, which were preserved in the same way as Crustacea, by immersion in boiling sea-water for 2 min. (Fisher *et al.*, 1952), all the material was preserved either whole, or various organs separately, in absolute alcohol. On receipt at the laboratory the boiled specimens were weighed, preserved in alcohol and stored in the deep-freeze. Where possible, specimens or organs preserved in alcohol were weighed first. If not, a known volume of absolute alcohol was added so that the weight of the animals could be determined by difference, that of the alcohol being calculated from its specific gravity. Where specimens were sent to us in an unknown volume of alcohol the net weight was roughly determined by draining off the alcohol and weighing the specimens. Weights given in the results and tables were determined after removal of the shells, except for *Stenoplax conspicua* and *Limacina retroversa*.

Analytical Methods

Basically the analytical technique was that outlined by Fisher *et al.* (1952). Some difficulty was experienced in the Carr-Price reaction owing to interference by xanthophylls which were eluted with vitamin A alcohol in the chromatography following saponification. Recently we have found that some of this pigment may be removed at an early stage by passing the extract in *n*-hexane solution through an alumina column weakened with 8% ethanol in *n*-hexane before the chromatography on a column not so treated as used in method (*b*) of Fisher *et al.* (1952). A large proportion of the other carotenoid pigments is eluted with the vitamin A ester and β-carotene, leaving vitamin A alcohol and a reduced amount of pigments on the column. These are eluted with 8% ethanol in *n*-hexane. The first fraction is then re-chromatographed on an untreated alumina column. Vitamin A ester and β-carotene are eluted by 2% acetone in *n*-hexane, leaving the remaining pigments adsorbed on to the column.

So far in our work vitamin A has been measured by the blue colour produced in the antimony-trichloride reaction and the values determined as described by Thompson (1949). Since pure crystalline vitamin A is now readily available the photoelectric spectrophotometer has been recalibrated with solutions in chloroform of the alcohol, acetate and palmitate forms of vitamin A.

We found that these pure preparations gave 20% less blue colour per unit of vitamin A than the oils originally used as standards. The probable presence in these original oils of about 25% of neo-vitamin A (Dalvi & Morton, 1951) may account in part, at least, for the difference. Pure vitamin A alcohol now gives, under our conditions, a value for $E\,{}^{1\%}_{1\,cm.}$ (620 mμ) of 5000, in sensible agreement with that of 5070 given by Cama, Collins & Morton (1951).

Since the method of measuring vitamin A is purely chemical it seems logical to express results in terms of micrograms rather than international units. This will be done in the present and future papers of this series except where a measurement is the result of a biological assay, when we shall continue to use international units.

To compensate for the recalibration of the spectrophotometer and to convert international units to micrograms the factor 0·39 should be used to multiply all values resulting from chemical measurements of vitamin A given in our previous papers (Thompson, Ganguly & Kon, 1949; Kon & Thompson, 1949*a*, *b*; Batham *et al.*, 1951; Kon, 1954; Fisher *et al.* 1951, 1952, 1953, 1954, 1955).

Our earlier work (Fisher *et al.*, 1952) on euphausiids showed a discrepancy between the vitamin A potencies measured by physico-chemical and by biological methods. We now know that this discrepancy is due to the presence of a vitamin A isomer (Kon, 1954). No biological tests have been done on vitamin A from the molluscs analysed, so that no information is available regarding the nature of the vitamin in them.

Results

Class Loricata

Stenoplax conspicua (Carpenter)

Ten specimens were collected on the shore at La Jolla on 20 May 1953. The shells were not removed before analysis and the results were as follows:

Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene
		μg/spec.	μg/g	μg/spec.	μg/g	μg/g
17	1·2	0·73	0·044	85	5·1	0·41

The vitamin A was all in the ester form. Their chromatographic behaviour indicated that the carotenoid pigments included both carotenes and xanthophylls and possibly astaxanthin or its esters. Chlorophyll was also present.

Class Lamellibranchiata

Order Filibranchiata

Mytilus edulis Linnaeus

Three groups of mussels were collected near the breakwater at Blackman's Point, Harwich. The analytical results are shown in Table 1. In the whole

animals only vitamin A ester was measured, since the presence of much pigment in the fraction that would have contained vitamin A alcohol prevented a satisfactory reading in the Carr-Price reaction in the first two groups of specimens.

The pigmented edge of the mantle is believed to be light-sensitive in some molluscs and, because of our finding that vitamin A is concentrated in the eyes of Crustacea (Fisher *et al.*, 1951), this mantle edge was separated from the rest of the mussel before analysis of the second group. It contained no vitamin A, and the concentration of fat and total carotenoids differed little from those of the rest of the mussel, which, however, contained vitamin A ester.

TABLE I. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *MYTILUS EDULIS*

(All specimens collected at Harwich.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene
					μg/spec.	μg/g	μg/spec.	μg/g	(μg/g)
11. ix. 50	Whole animal	29	1·3	1·6	0·66	0·51	49	37	1·3
29. i. 51	Mantle edge	40	0·1	1·1	0	0	4	34	0
	Visceral mass	40	1·4	1·5	0·25	0·18	43	32	0·77
	Total	40	1·5	1·5	0·25	0·16	47	32	0·70
23. iv. 51	Digestive gland	36	0·45	1·2	0·58	1·29	91	203	14
	Mantle, with eggs	36	0·55	1·1	0·04	0·08	16	29	0
	Foot	36	0·04	3·2	0	0	2·6	74	0
	Ctenidia, muscle and rest of visceral mass	36	0·77	0·9	0·04	0·05	27	34	1·1
	Total	36	1·81	1·1	0·66	0·37	137	76	4·2

The mussels of the third group were dissected into several parts before analysis and, as Table I shows, vitamin A was concentrated mainly in the digestive gland where it was in the alcohol form. The small amounts of vitamin A in other parts of the animal were present entirely as the ester. Apart from β-carotene, the carotenoids were xanthophylls, with no evidence of astaxanthin. There was a trace of chlorophyll in the digestive gland.

Pecten maximus (Linnaeus)

The analytical results for various parts of a group of seven specimens are shown in Table II. The carotenoids were mainly xanthophylls and no astaxanthin was detected.

Chlamys septemradiatus Müller

Table II shows results for a group of whole animals of this species. The only carotenoids present were xanthophylls, and considerable amounts of chlorophyll were also observed.

Lima hians (Gmelin)

Two groups were analysed, the first as whole animals and the second after dissection. The results are shown in Table III. Vitamin A was present in the first group, all in the ester form, but not in the second. The carotenoids were all xanthophyllic, apart from traces of a carotene in the digestive gland.

TABLE II. DISTRIBUTION OF OIL AND CAROTENOIDS IN TWO SPECIES OF PECTINIDAE

(Both species collected in Clyde Sea area.)

Date	Species and tissue	No. of specimens	Average weight (g)	Oil (%)	Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	
13. ii. 51	*Pecten maximus*	7	—	—	—	—	—
	Muscle		56	0·3	28	0·5	Trace
	Mantle edge		19	0·3	9·2	0·5	Trace
	Visceral mass		9·5	4·2	364	39	0·58
	Gonad and foot		13	1·9	186	14	Trace
	Ctenidia and mantle		7·4	0·5	62	8·4	0
	Total		105	0·9	649	6·2	0·05
13. iii. 52	*Chlamys septem-radiatus*	31	1·5	0·6	3·6	2·4	0

Vitamin A absent from both species.

TABLE III. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *LIMA HIANS*

(All specimens collected near Millport.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Carotenoids	
					μg/spec.	μg/g	μg/spec.	μg/g
21. x. 53	Whole animal	12	7·5	1·7	0·20	0·022	188	25
7. xii. 53	Foot	27	0·1	0·5	0	0	2·8	29
	Digestive gland	27	0·7	6·1	0	0	31	44
	Visceral mass	27	1·7	1·1	0	0	5·8	3·4
	Mantle	27	3·6	0·6	0	0	64	18
	Total	27	6·1	1·4	0	0	104	17

β-carotene absent from all specimens.

Ostrea edulis Linnaeus

The analytical results for three groups of edible oysters are given in Table IV. Only vitamin A ester was measured in the whole animals, owing to interference of carotenoids with the measurement of any vitamin A alcohol that might have been present. In the dissected groups satisfactory readings were obtained for all organs. The visceral masses contained vitamin A ester but no alcohol, and the carotenoids were mainly xanthophylls with small amounts of β-carotene. The carotenoids of the remainder of the body were all xanthophylls. An unidentified olive-green pigment was strongly adsorbed at the top of the alumina column in the extracts of whole oysters and visceral masses.

TABLE IV. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *OSTREA EDULIS*

(All specimens collected near Burnham-on-Crouch.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	μg/spec.	μg/g	
10. x. 50	Whole animal	4	9·2	2·3	2·4	0·26	181	20	1·7
11. xii. 50	Visceral mass	16	2·6	1·2	0·35	0·14	12	46	0·05
	Mantle edge	16	0·8	0·5	0	0	1·0	1·2	0
	Muscle	16	1·8	0·5	0	0	1·2	0·7	0
	Ctenidia	16	1·3	0·2	0	0	0·8	0·6	0
	Total	16	6·5	0·7	0·35	0·05	15	2·3	0·02
25. iii. 52	Digestive gland	16	0·5	1·7	0	0	4·8	9·8	0
	Mantle & ctenidia	16	2·5	0·7	0	0	2·5	1·2	0
	Visceral mass	16	2·2	1·8	0·43	0·20	27	12	0·02
	Muscle	16	1·1	0·5	0	0	0	0	0
	Total	16	6·3	1·1	0·43	0·07	34	5·4	0·007

TABLE V. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *GRYPHAEA ANGULATA*

(All specimens collected near Burnham-on-Crouch.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	μg/spec.	μg/g	
10. x. 50	Whole animal	2	12	2·4	1·9	0·16	180	15	1·1
11. xii. 50	Visceral mass	7	15	2·3	5·4	0·35	270	18	0·36
	Mantle edge	7	2·0	0·9	0	0	1·8	0·9	0
	Muscle	7	5·1	0·5	0	0	2·0	0·4	0
	Ctenidia & mantle	7	7·2	1·1	0	0	6·2	0·9	0
	Total	7	29	1·6	5·4	0·18	280	9·4	0·19
27. ii. 51	Labial palps	30	0·7	3·6	0·20	0·30	1·3	1·9	Trace
	Digestive gland	30	1·7	2·2	1·4	0·83	229	134	3·1
	Visceral mass	30	4·5	2·0	0·64	0·14	16	3·5	0·07
	Muscle, mantle & ctenidia	30	9·6	0·7	0·29	0·03	4·3	0·5	0
	Total	30	17	1·4	2·6	0·15	251	15	0·34
25. iii. 52	Mantle & ctenidia	15	2·5	1·0	0·14	0·06	4·4	1·8	0
	Visceral mass	15	3·2	2·0	2·1	0·66	68	21	0·11
	Muscle	15	1·0	0·4	0	0	0	0	0
	Digestive gland	15	0·78	2·6	0·82	1·1	39	51	0·49
	Total	15	7·5	1·5	3·1	0·41	111	15	0·06

Gryphaea angulata Lamarck

Table V shows the analytical results for four groups of Portuguese oysters. In the analysis of the whole specimens the chromatographic behaviour of the pigments was very similar to that in *Ostrea* and the concentrations of fat, vitamin A, total carotenoids and β-carotene were of the same order in both kinds of oyster. The green pigment found in *Ostrea* was also present in *Gryphaea*.

The results for the second group were very similar to those obtained for the group of *Ostrea edulis* collected at the same time and dissected in the same way, except that the vitamin A of the visceral mass comprised about 6 parts alcohol to 1 part ester, whereas in *Ostrea* it was all in the ester form. Analysis of various organs in the third group showed that this species differed from *Ostrea* in having most of the vitamin A in the digestive gland, where the ester-alcohol ratio was 1:5. In the rest of the visceral mass the ratio was 2:1, and in other parts of the body containing it the vitamin was entirely in the ester form. The relative amounts of the two forms of vitamin A in the various parts of the last group were similar to those in the first three, except that in the visceral mass there was over twice as much vitamin A alcohol as ester. Xanthophylls and carotenes were present in all the organs from all the groups, but no astaxanthin.

TABLE VI. DISTRIBUTION OF OIL AND CAROTENOIDS IN *SCROBICULARIA PLANA*

(All specimens collected at Leigh-on-Sea.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	
16. x. 50	Whole animals	46	1·55	0·9	38	22	1·0
29. i. 51	Visceral mass	32	1·22	1·3	14	12	0·42
	Siphon	32	0·37	1·4	3·5	9·6	0
	Mantle edge	32	0·23	1·1	0·4	1·6	0
	Total	32	1·82	1·3	18	9·9	0·28

Vitamin A absent.

Order Eulamellibranchiata

Scrobicularia plana (da Costa)

As shown in Table VI two groups of this species were analysed. Carotenoids were mainly xanthophyllic with some carotenes in the visceral mass. The green pigment noticed in the oysters also appeared in this species.

Cardium edule Linnaeus

Of five groups of cockles analysed, only the first, as Table VII shows, contained any vitamin A. As in the previous species, the carotenoids were mostly xanthophylls. The gonads, separated in the second group, contained a large proportion of the total carotenoids.

Mya arenaria Linnaeus

Four groups of clams were analysed and the results are given in Table VIII. In the first group, analysed whole, only vitamin A ester was measured, because of interference of carotenoids in the determination of the alcohol fraction, but in the subsequent groups vitamin A alcohol was absent from all the organs.

TABLE VII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *CARDIUM EDULE*

(All specimens collected from Fairlie Sands.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	μg/spec.	μg/g	
16. v. 51	Whole animal	20	4·0	0·8	0·66	0·16	47	12	0·34
4. ix. 51	Foot	22	0·25	0·8	0	0	1·8	7·5	0
	Gonad	22	1·86	1·2	0	0	15	7·9	0
	Mantle, muscles and ctenidia	22	1·71	0·2	0	0	3·0	1·8	0
	Visceral mass	22	0·36	0·5	0	0	4·7	13	0·38
	Total	22	4·2	0·7	0	0	24	5·8	0·03
16. x. 51	Mantle, muscles and ctenidia	30	2·12	0·8	0	0	6·9	3·3	0
	Digestive gland	30	0·40	1·1	0	0	9·4	23	0·70
	Visceral mass and foot	30	2·49	0·5	0	0	8·4	3·4	0
	Total	30	5·0	0·7	0	0	25	4·9	0·06
24. iv. 52	Crystalline style	25	0·02	0·5	0	0	0	0	0
	Digestive gland	25	0·48	0·9	0	0	5·4	11	0·22
	Visceral mass and foot	25	2·22	0·4	0	0	4·6	2·1	0
	Mantle, muscles, ctenidia, siphons	25	1·36	0·8	0	0	4·1	3·0	0
	Total	25	4·1	0·6	0	0	14	3·5	0·03
24. iv. 52	Whole animal	25	4·8	0·4	0	0	14	2·9	0·04

TABLE VIII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *MYA ARENARIA*

(All specimens collected from Fairlie Sands.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	μg/spec.	μg/g	
16. v. 51	Whole animal	6	34	0·7	0·80	0·02	527	16	0·39
4. ix. 51	Visceral mass	12	5·9	1·5	0·81	0·14	36	6·0	0·12
	Siphons	12	14	0·4	0·18	0·01	27	1·9	0·03
	Mantle, muscle and ctenidia	12	14	0·6	0·35	0·03	88	6·4	0·25
	Digestive gland	12	1·7	1·7	0·15	0·09	130	75	9·5
	Foot	12	0·4	0·8	0	0	2·3	5·8	0
	Total	12	36	0·5	1·5	0·04	283	7·8	0·58
16. x. 51	Visceral mass and foot	9	2·5	1·4	0·74	0·11	86	13	0·18
	Siphons	9	14	0·2	0·16	0·01	25	1·8	Trace
	Mantle, muscle and ctenidia	9	11	0·4	0·19	0·02	26	2·3	0
	Digestive gland	9	6·8	2·0	0	0	161	64	2·5
	Total	9	34	0·7	1·1	0·03	298	8·8	0·22
12. ii. 52	Crystalline style	18	0·18	0·01	0	0	0	0	0
	Visceral mass	18	5·9	1·2	0·34	0·06	13	2·2	Trace
	Mantle, muscle, ctenidia, siphons	18	18	0·4	0·13	0·007	22	1·2	Trace
	Digestive gland	18	1·3	1·2	0	0	1·8	1·4	0
	Foot	18	0·33	1·0	0	0	1·1	3·2	0
	Total	18	26	0·6	0·47	0·02	38	1·5	Trace

Vitamin A was present in the digestive gland of the second group, but absent from it in the last two groups. The carotenoids were much as in other lamellibranchs, with xanthophylls predominating. Variations in concentration between different organs are shown in the table.

Class Gastropoda

Subclass Prosobranchiata

Order Diotocardia

Haliotis fulgens Philippi

Two specimens were analysed singly, with the results given in Table IX. No vitamin A ester was detected. The presence of much xanthophyllic pigment in the alcohol fractions interfered with the measurement of vitamin A alcohol. The fractions were therefore passed through a column of calcium carbonate, using light petroleum (b.p. 40–60° C) as a solvent and eluent in

TABLE IX. OIL, VITAMIN A AND CAROTENOIDS IN SOME PROSOBRANCHS

(First three species collected at La Jolla on 20. v. 53 and *Osilinus lineatus* at Plymouth on 9. vi. 52.)

Species	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
				μg/spec.	μg/g	μg/spec.	μg/g	
Haliotis fulgens	1	268	0·6	11	0·04	1066	4·0	0·84
H. fulgens	1	122	0·7	4·6	0·04	1034	8·5	1·7
Megathura crenulata	1	179	0·4	9·2	0·05	1863	10	3·0
Astrea undosa	1	8·7	1·0	0	0	26	3·0	Trace
Osilinus lineatus	50	1·1	1·1	0·12	0·10	58	52	6·1

an attempt to remove the pigment. Some of it, however, was still eluted and the fractions were dissolved in *n*-hexane and their absorption spectra determined in the Beckman spectrophotometer. Both fractions showed maxima at about 327 mμ, indicating the presence of vitamin A alcohol. The values for vitamin A were calculated after appropriate corrections for irrelevant absorption by the procedure of Cama *et al.* (1951). The pigments were carotenes and xanthophylls, astaxanthin being absent. A noticeable feature was the high proportion (*ca.* 20%) of β-carotene in the total carotenoids.

Megathura crenulata (Sowerby)

One specimen was analysed with the results shown in Table IX. Vitamin A was all in the alcohol form which was measured spectrophotometrically, because of the interference by carotenoids with the antimony-trichloride reaction. The pigments were similar to those of *Haliotis*, but the percentage (30) of β-carotene was even higher in this species.

Astrea undosa (Wood)

The analytical results for a single specimen are given in Table IX. The pigments were mainly xanthophyllic.

Osilinus lineatus (da Costa)

A group of fifty whole specimens was analysed with the results shown in Table IX. Vitamin A was in the ester form and the carotenoids were xanthophylls and carotenes.

Patella vulgata Linnaeus

The analytical results for all the limpets examined are shown in Table X. Analysis of the first group established the presence of vitamin A, although only the ester was measured. Nearly a third of the total carotenoids in this group was β-carotene.

The second group was analysed with mantle edge and tentacles, as possible photoreceptors, separated from the rest of the animals, but all the vitamin was in the main part of the body.

The tentacles and mantle edge had lower concentrations of carotenoids than the rest of the animal and β-carotene was absent from the tentacles.

The distribution of vitamin A and carotenoids in the limpet was further investigated in the third group. These animals were dissected as shown in Table X. The highest concentration and largest amount of vitamin A were in the digestive gland and excretory organ and the ratio of ester to alcohol was 1:9. In the intestine the ratio was about 1:5.

Vitamin A in the gonads was all in the ester form, and the content and concentration in the testis were two or three times higher than in the ovary. The carotenoid concentration was also much higher in the testis than in the ovary despite the more darkly pigmented appearance of the latter. More than half of the ovarian carotenoids consisted of β-carotene, but in the testis this pigment accounted for little more than a quarter of the total.

By far the highest concentrations of carotenoids were in the digestive gland, excretory organ and intestine. Xanthophylls and carotenes were present in all the extracts.

The ratios of vitamin A alcohol to ester and the proportions of different carotenoids were very similar in the group collected on 24 March 1952 to those of the preceding group. Before the two groups obtained on 12 May 1952 were analysed they were kept alive for 24 hr. and the faeces were collected. Scrapings were also taken of the algae encrusting the rocks on which the limpets were attached and feeding. Neither faeces nor scrapings contained vitamin A and the concentrations of carotenoids were almost the same in both but that of β-carotene was higher in the scrapings than in the faeces.

The limpets from Hoy were still alive on arrival and were treated in the same way as the previous group, the faeces being analysed separately. The ovaries of the dissected animals contained no vitamin A and very little carotenoid pigment. The carotenoid concentrations of the rock-scrapings and faeces were very similar, and no conclusion can be drawn regarding possible preferential absorption of carotenoids by the limpet.

TABLE X. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *PATELLA VULGATA* AND ITS FOOD

(All Patella from Harwich, except those of 4. viii. 52 from near Berry Head, Hoy, Orkneys.)

Date	Tissue	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	μg/spec.	μg/g	
11. ix. 50	Whole animal	12	7·8	4·7	1·5	0·19	399	51	16
29. i. 51	Visceral mass	22	5·6	2·9	2·3	0·41	208	37	12
	Mantle edge	22	0·2	2·1	0	0	3·9	22	5·9
	Tentacles	22	0·01	5·3	0	0	0·2	21	0
	Total	22	5·8	2·8	2·3	0·40	212	37	11
23. iv. 51	Muscle, foot and mantle	36	2·8	1·2	0·99	0·35	64	23	2·4
	Head	36	0·2	0·9	0	0	3·8	17	4·3
	Digestive gland and kidney	36	1·1	5·7	2·5	2·4	239	226	35
	Intestine	36	0·2	4·9	0·40	2·1	28	142	24
	Ovary	19	1·4	8·5	0·14	0·11	27	19	9·7
	Testis	17	0·8	3·8	0·56	0·67	45	112	30
	Total	36	5·3	3·4	4·2	0·79	398	74	13
24. iii. 52	Head and foot	40	5·9	0·4	0·25	0·04	56	9·6	2·9
	Intestine	40	0·4	2·6	0·28	0·67	21	52	5·4
	Visceral mass	40	1·2	6·2	2·6	2·1	238	192	40
	Testis	18	1·3	0·7	0·40	0·32	76	62	21
	Ovary	22	1·7	4·4	0·11	0·07	25	15	5·0
	Total	40	9·0	1·7	3·4	0·37	364	41	9·5
12. v. 52	Whole animal	13	8·4	2·2	5·2	0·62	479	57	8·8
12. v. 52	Digestive gland	12	1·8	9·6	5·1	2·8	439	239	43
	Intestine	12	0·2	3·9	0·37	1·5	29	121	15
	Rest of body	12	6·3	0·4	0	0	63	10	0·24
	Total	12	8·3	2·5	5·5	0·66	531	64	10
12. v. 52	Faeces	—	2·4	0·7	—	0	—	84	1·9
12. v. 52	Scrapings from rocks	—	7·3	0·3	—	0	—	83	4·8
4. viii. 52	Intestine	20	0·2	6·0	0·06	0·35	25	139	35
	Digestive gland	20	1·0	14	2·5	2·6	272	278	73
	Ovary	10	0·4	3·8	0	0	2·3	5·7	0
	Testis	10	1·0	2·6	0·70	0·74	79	83	12
	Rest of body	20	4·5	0·6	0·45	0·10	59	14	2·3
	Total	20	6·3	3·1	3·1	0·50	395	63	15
4. viii. 52	Whole animal ♀	10	5·4	2·8	2·2	0·40	216	40	7·1
4. viii. 52	Whole animal ♂	10	6·7	2·4	4·4	0·66	383	58	10
4. viii. 52	Faeces	—	0·17	2·8	—	0	—	113	3·5
4. viii. 52	Scrapings from rocks	—	0·2	20	—	0	—	132	Trace

Order Monotocardia

Buccinum undatum Linnaeus

As Table XI shows, two groups of whelks and a group of eggs were analysed. These molluscs were devoid of vitamin A and poor in carotenoids.

Murex trunculus Linnaeus

A singel specimen was analysed with results shown in Table XI. The carotenoids were mainly xanthophylls, although there were traces of β-carotene and possibly astaxanthin.

TABLE XI. OIL AND CAROTENOIDS IN *BUCCINUM UNDATUM* AND *MUREX TRUNCULUS*

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Total carotenoids μg/spec.	Total carotenoids μg/g	β-carotene (μg/g)
10. x. 50	*Buccinum undatum*	Burnham-on-Crouch	19	5·9	1·0	34	5·8	0·80
11. xii. 50	*B. undatum* (eggs)	Burnham-on-Crouch	—	3·81*	0·6	—	2·4	0
9. vii. 52	*B. undatum*	Loch Fyne	31	18	0·3	14	0·79	0·07
13. v. 54	*Murex trunculus*	Madeira	1	11	2·2	147	13	Trace

Vitamin A absent from all specimens.

* Total weight.

TABLE XII. OIL AND CAROTENOIDS IN *LITTORINA* SPP.

(All specimens collected at Harwich on 11. ix. 50.)

Species	No.	Average weight (mg)	Oil (%)	Total carotenoids μg/spec.	Total carotenoids μg/g	β-carotene (μg/g)
Littorina littoralis	72	210	1·7	9·2	44	14
L. littorea	98	810	4·2	59	73	19
L. rudis	23	61	12	6·1	100	13

Vitamin A absent from all species.

Littorina littoralis (Linnaeus)
Littorina littorea (Linnaeus)
Littorina rudis (Maton)

Results given in Table XII show that these three species all lacked vitamin A. The pigments were xanthophylls and carotenes, with some chlorophyll. In *L. littoralis* β-carotene formed nearly one-third of the total carotenoids. *L. rudis* was richer in fat and carotenoids than its two congeners.

Aporrhais pes-pelecani (Linnaeus)

Table XIII shows the analytical results. The carotenoids included both carotenes and xanthophylls.

Crepidula fornicata (Linnaeus)

Two groups were analysed as shown in Table XIII. They contained good concentrations of carotenoids, both carotenes and xanthophylls.

Cypraea spadicea Swainson

Results for this specimen appear in Table XIII. The carotenoids were mainly xanthophylls.

TABLE XIII. OIL AND CAROTENOIDS IN *APORRHAIS*, *CREPIDULA* AND *CYPRAEA*

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Total carotenoids μg/spec.	Total carotenoids μg/g	β-carotene (μg/g)
13. iii. 52	*Aporrhais pes-pelecani*	Loch Fyne	24	1·13	1·4	2·6	2·3	0
10. x. 50	*Crepidula fornicata*	Burnham-on-Crouch	80	1·42	0·7	24	17	1·3
11. xii. 50	*C. fornicata*	Burnham-on-Crouch	60	1·21	0·7	36	30	1·8
20. v. 53	*Cypraea spadicea*	La Jolla	1	6·1	1·6	49	8·0	0·37

Vitamin A absent from all species.

TABLE XIV. OIL, VITAMIN A AND CAROTENOIDS IN SOME TECTIBRANCHS

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Vitamin A μg/spec.	Vitamin A μg/g	Total carotenoids μg/spec.	Total carotenoids μg/g	β-carotene (μg/g)
13. v. 54	*Aplysia depilans*	Madeira	2	64	0·3	7·0	0·11	170	2·7	0·32
29. viii. 51	*Clione limacina*	63° 18′ N. 18° 39′ W.	99	0·034	7·6	0	0	3·4	102	1·8
30. i. 55	*Limacina retroversa*	Barents Sea	405	0·00087	1·3	0·008	9·2	0·005	6·0	0

Sub-class Opisthobranchia

Order Tectibranchia

Aplysia depilans Linnaeus

Results for a group of two specimens are given in Table XIV. The ratio of vitamin A ester to alcohol was about 1:5. Both carotene and xanthophylls were detected in the small concentration of carotenoids present.

Clione limacina Phipps

The results for a group of this species appear in Table XIV. The high concentration of carotenoids included both carotene and xanthophylls.

Limacina retroversa (Fleming)

Table XIV shows that this species of pteropod, which was analysed with its shell, was relatively rich in vitamin A, all in the ester form. What little there was of carotenoid pigment appeared from its chromatographic behaviour to be either xanthophyll or possibly astaxanthin ester.

Subclass Pulmonata

Order Stylommatophora

Helix aspersa Linnaeus

Table XV shows the results for a group of garden snails. The pigments were mainly xanthophylls.

Order Basommatophora

Planorbis corneus (Linnaeus) var. *rubra* Oldham.

Results for a dozen of these bright orange snails show (Table XV) that vitamin A was absent and that the concentration of carotenoids was not especially high, the pigment being mainly xanthophyllic, with only traces of carotene.

TABLE XV. OIL AND CAROTENOIDS IN SOME PULMONATES

Date	Species	Locality	No.	Average weight (g)	Oil (%)	Total carotenoids μg/spec.	Total carotenoids μg/g	β-carotene (μg/g)
11. x. 51	*Helix aspersa*	Garden at Shinfield	60	3·9	0·4	6·0	1·5	0·04
25. x. 54	*Planorbis corneus* var. *rubra*	Aquarium dealer	12	2·1	0·3	30	14	Trace

Vitamin A absent from both species.

DISCUSSION

In the lamellibranch species we have examined, vitamin A was found in the mussel, *Mytilus edulis*, the clam, *Mya arenaria*, and the oysters, *Ostrea edulis* and *Gryphaea angulata*, but it was absent from the scallops, *Pecten maximus* and *Chlamys septemradiatus*, and the deposit-feeding bivalve, *Scrobicularia plana*. It was occasionally present in the cockle, *Cardium edule*, and the file-shell, *Lima hians*. Thus in both the lamellibranch orders, the Filibranchiata and the Eulamellibranchiata, there were some representatives with and others without vitamin A and there was likewise no apparent relationship between the carotenoid constituents of the various species and their affinities. All species except *Mytilus edulis* and *Lima hians*, which were richer, had carotenoid concentrations between 1·5 and 20 μg/g, and all species except *Chlamys septemradiatus* contained measurable amounts of β-carotene.

Analyses of the photoreceptive tissues of some of the species showed that vitamin A was absent and so does not appear to be concerned with vision as it may be in Crustacea (Wald, 1941; Fisher *et al.*, 1952). In this connexion it is not possible to determine whether vitamin A in the chiton, *Stenoplax conspicua*, had any visual role, since the animals of this species were analysed whole.

The vitamin A and carotenoid contents of the lamellibranch species do not appear to be associated with the food or feeding mechanism since some ciliary and deposit feeders contained vitamin A and others did not. In those species with vitamin A, *Gryphaea angulata* and *Mytilus edulis* had their richest concentrations in the digestive gland rather than in the rest of the visceral mass, whereas in *Mya arenaria* vitamin A was not invariably present in the digestive gland, and was in all instances absent from that of *Ostrea edulis*. In *Mya* the concentrations of vitamin A were always higher in the rest of the visceral mass

than in the digestive gland. The gonads would be included with the rest of the visceral mass in these species and so may have contained the vitamin. This lack of uniformity in the distribution of vitamin A between species indicates no very obvious function for it in the metabolism of these molluscs, in which the vitamin is more probably a chance by-product of metabolism of carotenoids derived from the algae of the food and appearing in those species with suitable enzyme systems. A similar explanation probably applies to the distribution and nature of the carotenoids present.

It is difficult to determine, from their distribution in those lamellibranchs studied, any special functions of the carotenoids present. Scheer (1940) was able, by feeding experiments, to shed some light on the metabolism and role of carotenoids in *Mytilus californianus*. He found that this mussel selected from its food xanthophylls in preference to carotenes and that the gonads acted as storage organs for carotenoids but not for lipids. Our own finding of higher concentrations of carotenoids in the gonads of *Cardium edule* indicates a similar tendency and also a possible reproductive role. In support of his suggestion that carotenoids play a part in the metabolism of the mussel and are not just stored, Scheer observed a reduction in the reserves of carotenoid pigments following feeding on a carotenoid-free diet. It is surprising that he does not mention vitamin A in *Mytilus californianus* since we have repeatedly found it in *M. edulis*. In *M. edulis*, the vitamin A, like the carotenoids, was mainly in the digestive gland, but we do not know whether it is derived unchanged from the food or formed from a precursor. From Scheer's work, conversion seems to be more likely, although vitamin A may not be present in *M. californianus* or essential to its existence, since ZoBell & Landon (1937) have shown that this species can be maintained exclusively on a diet of bacteria and state that Kincaid had found the same to be true for oysters.

Brooks & Paulais (1939) studied the distribution of carotenoids in the oysters, *Ostrea edulis* and *Gryphaea angulata*, and found little difference in carotenoid concentration between the 'green' and 'white' varieties of each species. They showed that the visceral mass in both species contained about twenty times as much pigment as the gills and mantle. We obtained ratios of a similar order and found that most of the pigment was in the digestive gland.

Among the gastropods analysed, vitamin A was present in *Haliotis fulgens*, *Megathura crenulata*, *Osilinus lineatus*, *Patella vulgata*, *Aplysia depilans* and *Limacina retroversa*, but absent from *Astrea undosa*, *Buccinum undatum*, *Murex trunculus*, *Littorina littoralis*, *L. littorea*, *L. rudis*, *Aporrhais pespelecani*, *Crepidula fornicata*, *Cypraea spadicea*, *Clione limacina*, *Helix aspersa* and *Planorbis corneus* var. *rubra*. Of those gasteropods with vitamin A, *Haliotis*, *Megathura*, *Osilinus* and *Patella* are all diotocardian prosobranchs, and *Aplysia* and *Limacina* are opisthobranchs of the order Tectibranchia. *Buccinum*, *Murex*, *Littorina* spp., *Aporrhais*, *Crepidula* and *Cypraea* which all lacked vitamin A are monotocardian prosobranchs. Of the other species

without the vitamin, *Astrea* is a diotocardian, *Clione* a tectibranch, *Helix* a stylommatophorous pulmonate and *Planorbis* a basommatophorous pulmonate. Apart from the absence of vitamin A from the single specimen of *Astrea undosa*, all the Diotocardia analysed contained the vitamin and all the Monotocardia lacked it. The Monotocardia are regarded as being less primitive than the Diotocardia, and it may be that in their evolution they have developed an enzyme system capable of breaking down the carotenoids of the food beyond the stage of vitamin A.

The presence or absence of the vitamin does not appear to be related to the food in the prosobranchs since those with vitamin A are all algal feeders, whereas those without it vary from ciliary feeders like *Crepidula*, through algal feeders like the *Littorinas*, to carnivorous predators like *Buccinum* and *Murex*. *Crepidula* has a similar feeding mechanism to that of the oysters and competes with them for the same food in the same environment, and yet the bivalves contain vitamin A. Such a difference would best be explained by the presence of diverse enzyme systems. Two planktonic tectibranchs, the pteropods *Limacina retroversa* and *Clione limacina*, were respectively possessed and devoid of vitamin A, the former being as rich in the vitamin as many of the Euphausiacea, richer in vitamin A than any other invertebrates so far analysed (Fisher *et al.*, 1955). *Limacina* contributes to the diet of plankton-feeding fish and so may be important to them as a source of preformed vitamin A. *Clione*, on the other hand, contained more carotenoids (102 μg/g) than *Limacina* (6 μg/g). Further investigations of the vitamin A content of these and other species of pteropods will be made when material is available to us.

In addition to *Clione*, some species of gastropod, namely, *Osilinus*, *Patella*, *Littorina* spp. and *Crepidula* also had high concentrations of carotenoids, whereas the other species studied had much smaller reserves. Whether these pigments have any positive function cannot be determined from comparison of the different species. We have, however, studied more fully the distribution of both vitamin A and carotenoids in the limpet, *Patella vulgata*. In this species most of the vitamin A (as well as the carotenoids) was in the digestive gland and there was always an appreciable concentration in the intestine, indicating its food origin. Vitamin A was absent from the food, so that *Patella* appears capable of converting a precursor to the vitamin. Comparison of the β-carotene concentrations in the rock-scrapings and in the faeces for the specimens collected on 12 May 1952 (Table X) indicates a possible preferential absorption of this pigment, but the evidence from similar specimens taken on 4 August 1952 is inconclusive or even contradictory, and so judgement must be withheld.

The gonads of *Patella* were of particular interest. They have already been the subject of an intensive study in *P. vulgata* and *P. depressa* by Goodwin (1950) and Goodwin & Taha (1950). These workers found in both testis and ovary β-carotene, echinenone, cryptoxanthin and zeaxanthin in the ratio

5:2:2:2. We found a similar ratio of β-carotene to total carotenoids. Goodwin & Taha (1951) have shown that echinenone and myxoxanthin, an algal carotenoid, are probably identical. They found echinenone in the gonads of *Patella* but we did not observe this pigment in our chromatographs of *Patella* extracts, nor did we see myxoxanthin in the extracts from the rock-scrapings, so that the question of whether echinenone in limpets was obtained by direct passage of myxoxanthin from algae or by conversion was not answered. The much higher concentrations of vitamin A and carotenoids in the testis of *P. vulgata* than in the ovary indicate a difference in the metabolism of carotenoids in the two sexes and possibly a function of the vitamin or pigments in reproduction, although in both ovary and testis the vitamin A was all in the ester (storage) form.

The form of the vitamin A in those molluscs containing it was extremely variable. In *Stenoplax*, *Lima*, *Ostrea*, *Mya*, *Osilinus* and *Limacina* only vitamin A ester was detected, but in *Haliotis* and *Megathura* only the alcohol form was found. In *Mytilus*, *Gryphaea*, *Patella* and *Aplysia* both ester and alcohol were present, but the alcohol form was always in excess. The alcohol is the active form of vitamin A and, in contrast to the condition in Mollusca, vertebrates and Crustacea almost invariably contain an excess of vitamin A ester in the storage organs, although vitamin A alcohol may predominate in the circulatory system of vertebrates.

There seems to be no obvious explanation of the extreme variability in this respect that we have found in the molluscs in relation to their food, ecology or affinities.

We have shown that vitamin A is present in a species of Loricata, some Lamellibranchiata and some Gastropoda, and absent from other species of the second and third classes. It had not previously been reported in either Amphineura or Gastropoda. Those species used as human food contain little or no vitamin A.

We are thus faced with the puzzling finding that some molluscs appear to manage quite well in the absence of vitamin A and its precursors, and it seems difficult to postulate for these substances a function as fundamental in molluscs as in vertebrates. The evidence we present in the paper that follows convinces us that vitamin A is essential for cephalopods in which it may well function in a way similar to that in vertebrates. When it comes to other molluscs, all we can say now is that the presence or absence of vitamin A and its precursors may possibly indicate in them nothing more than the peculiarities of their enzyme equipment.

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Summary

1. Vitamin A, total carotenoids and β-carotene were measured, where present, in one species of Loricata, nine species of Lamellibranchiata and eighteen species of Gastropoda.

2. Vitamin A was found in *Stenoplax conspicua*, *Gryphaea angulata*, *Mya arenaria*, *Mytilus edulis*, *Ostrea edulis*, *Aplysia depilans*, *Haliotis fulgens*, *Megathura crenulata*, *Osilinus lineatus*, *Patella vulgata* and *Limacina retroversa*, but not in *Chlamys septemradiatus*, *Pecten maximus*, *Scrobicularia plana*, *Astrea undosa*, *Buccinum undatum*, *Clione limacina*, *Crepidula fornicata*, *Cypraea spadicea*, *Helix aspersa*, *Littorina littoralis*, *L. littorea*, *L. rudis*, *Murex trunculus*, *Planorbis corneus* var. *rubra* or *Aporrhais pes-pelecani*. The vitamin was found in one of five groups of *Cardium edule* and one of two groups of *Lima hians*. Vitamin A has not previously been reported in Loricata or Gastropoda.

3. In those species containing it, vitamin A was concentrated mainly in the digestive gland or in the visceral mass.

4. With *Patella vulgata* there was evidence that the vitamin A reserves are derived by conversion of carotenoid precursors in the algal diet. In this species the testis contained much higher concentrations of both vitamin A and carotenoids than the ovary.

5. Except in the gonads of *Patella*, there was little evidence that vitamin A has any special function in the molluscs studied. The concentrations of vitamin A were low, except in *Limacina retroversa*, and there was no consistency as between species in the ratios of active to stored forms of the vitamin. The vitamin A and carotenoid contents of any species may possibly reflect its enzymic capacity to break down the carotenoids of the food.

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VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

V. MOLLUSCA: CEPHALOPODA

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(Text-fig. 1)

Introduction

In our previous paper (Fisher, Kon & Thompson, 1956) on Mollusca we pointed out that the Cephalopoda were so different from the other classes in vitamin A and carotenoid relationships that they would be more satisfactorily considered separately. We have so far analysed ten species of cephalopods and found vitamin A in all of them. The studies of previous investigators of vitamin A in these molluscs were confined to its function in the visual cycle and to its contribution to the vitamin A reserves of sperm whales feeding on cephalopods. The carotenoids of cephalopods have been given even less attention than vitamin A.

MacMunn (1883*a*, *b*) was probably the earliest worker to study the pigments of a cephalopod. He examined the alcohol- and ether-soluble pigments of *Octopus vulgaris* and reported that the chromatophores and eyes contained 'tetronerythrin', a substance which probably represented the sum total of carotenoids in the tissues. We have found no further record of similar work on cephalopods until that of Lönnberg (1935) on the carotenoids of *Sepiola scandica*, *Rossia macrosoma* and *Eledone cirrosa*. Extracts from the eyes of all the species contained a xanthophyll and gave a blue colour with antimony trichloride. An extract from the eggs of *Rossia* contained no pigment but gave a strongly blue reaction with antimony trichloride. The remaining tissues of *Sepiola* and *Rossia* contained very little pigment and so were not further investigated. The liver of *Eledone* contained large quantities of carotenoids and gave a greenish blue antimony-trichloride reaction.

In the following year, Verrier & Pannier (1936) first reported the presence of vitamin A in a cephalopod. They stated that they had extracted the vitamin from the visual pigment in the retinas of the eyes of *Eledone moschata*. Escher-Desrivières, Lédérer & Verrier (1938) subsequently examined the pigment from the edges of the pigmented epithelia of the retinas of *E. moschata*, *Sepia officinalis* and *Octopus vulgaris*, and found that it closely resembled vertebrate retinal purples, vitamins A_1 and A_2 as such being absent. Wald (1941) made a quantitative study of the retinas of the squid, *Loligo pealii*, in

which he found 1–2 μg of vitamin A_1 and three times this amount of retinene$_1$ (vitamin A aldehyde) per retina. He found no carotenoids in other tissues of this squid. The amount of vitamin A in the retinas was the same in light and darkness, indicating that the vitamin did not participate directly in the visual process. Wald suggested that squid visual purple was reversibly changed by light into retinene plus protein. Bliss (1948) obtained squid visual purple in an almost pure state, and showed that its properties were very similar to those of vertebrate visual purples.

Further observations on cephalopod carotenoids were reported by Wagner & Vermeulen (1939) and Leong (1939) who detected no carotenoids in cuttlefish, and by Fox & Crane (1942) who studied *Paroctopus bimaculatus* and *Loligo opalescens*. They found only traces of pigment in any of the squid organs but, in the octopus, the liver contained 3·5 mg of carotenoids per 100 g moist tissue and the ink 0·55–0·7 mg/100 g.

Cephalopods had long been considered the most likely source of the rich stores of vitamin A in the liver of the sperm whale, but experimental evidence was first produced by Brachi (1953) who analysed the liver from a 3 ft. long female specimen of *Moroteuthis ingens* taken from the stomach of a sperm whale during the 1951–52 antarctic whaling season. He experienced great difficulty in obtaining a squid free from the nematodes infesting the stomach of the whale, since they immediately attack the liver of any squid eaten. We have found vitamin A in these nematodes, *Anisakis physeteris* Baylis, taken from the stomach of a sperm whale being flensed at the whaling station of Scottish Whalers Ltd in the island of Harris, Outer Hebrides, in 1951. They are the only representatives so far of an invertebrate phylum other than the Arthropoda and Mollusca we have found to contain vitamin A. Brachi also analysed the unsaponifiable matter from squid, *Loligo forbesi*, and cuttlefish, *Sepia officinalis*, caught near Plymouth, but, by his technique, found no vitamin A in them.

Material and Methods of Preservation

Ten species of cephalopods were collected, some by ourselves and others by marine biologists on research cruises from Plymouth or Millport. They comprised four species of squid, five of cuttlefish and one octopus.

Alloteuthis media (Linnaeus), *Eledone cirrosa* (Lamarck), *Sepia officinalis* (Linnaeus), *Parasepia elegans* (d'Orbigny), *Todaropsis eblanae* (Ball) and some groups of *Loligo forbesi* Steenstrup were collected for us from one of the research ships of the Plymouth laboratory and brought back there alive where they were immediately dissected and the organs weighed and preserved in absolute alcohol.

Several groups of *Loligo forbesi* were collected by a research ship of the Torry Research Station at Aberdeen and dissected by the staff of that station. This material was also preserved in absolute alcohol.

Rossia macrosoma (Delle Chiaje) and *Sepietta oweniana* (d'Orbigny) were taken on various cruises in the Clyde Sea area by the M.V. *Calanus* from the Millport Laboratory. These were dissected and preserved, or preserved whole, after weighing, in absolute alcohol. A single specimen of *S. oweniana* was taken on the surface of Loch Hourn, Inverness-shire, in the bay at Arnisdale, having been attracted by a light lure. This squid was preserved in solid carbon dioxide until arrival at the laboratory at Shinfield, when it was dissected, the organs being weighed and preserved in alcohol.

A single specimen of *Sepiola* sp. was collected near Monaco on 2 February 1952.

Ommastrephes pteropus Steenstrup was caught with a hand-line at two stations of a north Atlantic cruise of the R.R.S. *Discovery II* in November 1954. Only the nidamental gland, eyes and livers were preserved, by immersion in boiling sea water followed by cold-storage. These specimens were weighed when they arrived at Shinfield, and preserved in alcohol.

All the alcohol-preserved specimens were kept in the deep-freeze at $-20°$ C until they were analysed.

Chemical and Physical Tests

Methods

The method of analysis for carotenoids and vitamin A was that described in the first paper of this series (Fisher, Kon & Thompson, 1952). Most cephalopod tissues were much richer in sterols than any material we had previously studied, and in the analyses of extracts from large quantities of tissue these sterols interfered with the Carr-Price reaction for vitamin A. It was necessary, therefore, to remove them. The separation was achieved by extracting the tissues with light petroleum, removing the solvent and dissolving the solids in methanol. The methanol solution was placed for an hour in the deep-freeze at $-20°$ C when most of the sterols precipitated. They were filtered off and washed several times with methanol at $-20°$ C. The methanol was evaporated off from the filtrate and the lipids dissolved in *n*-hexane for the first chromatography.

Since it is broken down during saponification, retinene (vitamin A aldehyde) must be separated from extracts of cephalopod eyes by an additional chromatography. The first chromatography was done as previously (Fisher *et al.*, 1952), but the alumina was weakened with 8% ethanol in *n*-hexane as for the second chromatography. This procedure separated vitamin A ester, which was eluted by 2% acetone in *n*-hexane, from vitamin A alcohol which was eluted by 8% ethanol in *n*-hexane. Retinene was eluted with the ester fraction. This fraction was evaporated down and the residue dissolved in light petroleum (b.p. 40–60° C) for the next chromatography done by the method of Ball, Goodwin & Morton (1948) on a column of Peter Spence activated alumina

type 'O' previously weakened by shaking with 10% (w/w) of distilled water. Vitamin A ester was eluted with light petroleum and then saponified and subjected to further chromatography by our usual technique. Elution of the column with a solution of 10% diethyl ether in light petroleum removed the retinene. This fraction was evaporated down, dissolved in *n*-hexane and the absorption spectrum examined in the Beckman quartz photoelectric spectrophotometer.

A sample of pure crystalline retinene, kindly supplied by Prof. R. A. Morton, F.R.S., of Liverpool University, gave in *n*-hexane $E^{1\%}_{1\,cm}$ 370 mμ = 1610, and this value was used for the calculation of the concentration of retinene from the absorption maximum, after the curves had been corrected for irrelevant absorption by the method of Morton & Stubbs (1946).

Subsequently the retinene solution was again evaporated and dissolved in chloroform for the Carr-Price reaction. The colour in this reaction was read at 664 mμ, 180 sec after addition of the antimony-trichloride reagent. The Thompson (1949) direct reading photoelectric spectrophotometer was also calibrated with the pure crystalline retinene. $E^{1\%}_{1\,cm}$ 664 mμ for this sample was 4150.

In our earlier analyses retinene was not separated from eye extracts, and where only vitamin A alcohol is reported in these extracts retinene might well have been present initially, but would have been destroyed during saponification.

Results

Order Decapoda

Alloteuthis media (Linnaeus)

A group of six specimens including five females with ripe ovaries, was dissected into the parts shown in Table I. Carotenoid pigments were not present in measurable amounts and vitamin A occurred only in the eyes and ovaries.

TABLE I. DISTRIBUTION OF OIL AND VITAMIN A IN SIX SPECIMENS OF *ALLOTEUTHIS MEDIA*

(Collected near Plymouth on 10. vi. 1952.)

Organ	Average weight (g)	Oil (%)	Vitamin A	
			μg/spec.	μg/g
Eyes (pairs)	0·38	0·9	0·25	0·61
Ovary*	0·44	0·7	0·31	0·71
Liver	0·15	2·3	0	0
Rest of body	7·95	1·2	0	0
Total	8·85	1·2	0·51	0·06

Carotenoids not present in measurable amounts.

* Average values for five animals, see text above.

Loligo forbesi Steenstrup

Five groups of squid were analysed, with the results shown in Table II. In the first group the ratio of vitamin A ester to alcohol in the liver and rest of the body was nearly 3:1, and in the eyes it was 1:11. In those parts with carotenoids, both carotenes and xanthophylls were observed, but no astaxanthin.

In the second group, which was much poorer in vitamin A than the first, the ester:alcohol ratios were, in the eyes, about 1:3, in the liver, about 1:4, and in the rest of the body 6:1. The small amounts of carotenoid pigment in

TABLE II. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *LOLIGO FORBESI*

Date	Locality	Organ	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
						μg/spec.	μg/g	μg/spec.	μg/g	
7. vi. 51	Plymouth	Eyes (pairs)	9	7·8	0·9	3·3	0·43	4·1	0·5	0·033
		Liver	9	5·6	11	88	16	34	6·1	0·078
		Ink sac	9	0·6	1·9	0	0	0	0	0
		Rest of body	9	163	1·4	1·3	0·01	41	0·3	0·001
		Total	9	177	1·7	93	0·52	79	0·4	0·005
6. viii. 51	Aberdeen	Eyes (pairs)	30	5·0	1·2	0·37	0·07	5·0	0·5	Trace
		Liver	30	4·3	1·8	4·8	1·1	3·1	0·7	0
		Rest of body	30	107	2·0	4·9	0·05	17	0·2	0
		Total	30	116	1·9	10	0·09	25	0·2	Trace
27. xi. 51	Aberdeen	Eyes (pairs)	547	7·5	0·53	1·8	0·24	—	—	—
		Livers	547	7·3	1·1*	7·0	0·96	0·25	0·04	0
9. vi. 52	Plymouth	Ink sac	1	3·6	2·6	0	0	0	0	0
		Eyes (pairs)	1	17	1·3	1·0	0·06	20	1·1	0
		Testis	1	3·4	1·8	0	0	0	0	0
		Gills and hearts	1	20	2·0	0	0	0	0	0
		Alimentary canal	1	13	2·1	2·4	0·18	0	0	0
		Male reproductive ducts	1	16	0·7	0	0	0	0	0
		Liver	1	33	9·0	17	0·52	46	1·4	0
		Caecum	1	47	3·9	11	0·24	74	1·6	0
		Muscle, skin and tentacles	1	830	0·5	5·8	0·01	0	0	0
		Total	1	983	1·0	37	0·04	140	0·14	0
9. vi. 52	Plymouth	Ink sac	14	0·36	2·4	0	0	0	0	0
		Eyes (pairs)	14	5·3	1·1	0·76	0·14	3·5	0·66	0
		Alimentary and reproductive systems	14	3·1	2·7	0·15	0·05	0	0	0
		Caecum	14	1·4	2·8	0·19	0·12	0	0	0
		Gills and hearts	14	3·2	1·8	0	0	0	0	0
		Liver	14	3·6	2·8	0·94	0·26	0	0	0
		Muscle, skin and tentacles	14	100	1·3	0·50	0·005	0	0	0
		Ovaries†	2	31	3·3	13	0·42	0	0	0
		Testis†	1	0·88	0·7	0	0	0	0	0
		Nidamental glands†	2	20	2·4	0	0	9·9	0·50	Trace
		Total	14	117	1·4	4·4	0·04	3·5	0·03	Trace

* Including 1·03% precipitated as sterols.

† Only three animals with recognizable gonads.

the eyes included both carotenes and xanthophylls, but in the liver and rest of the body only xanthophylls and possibly traces of astaxanthin were observed.

The eyes and livers were dissected from the third group at Aberdeen, preserved separately in abolute alcohol and despatched to us. Portions of the extracts were analysed for vitamin A by physical and chemical methods. Carotenoids were measured in the liver extract, but, inadvertently, not in the eye extract. The bulks of both extracts were retained for biological assay of vitamin A, dealt with later in this paper. The results for vitamin A given in Table II are, as usual, those calculated from the Carr-Price reaction. The value for the eyes was intermediate between those for the two earlier groups and that for the livers was similar to that for the previous lot from Aberdeen. The liver carotenoids were lower in content and concentration than before.

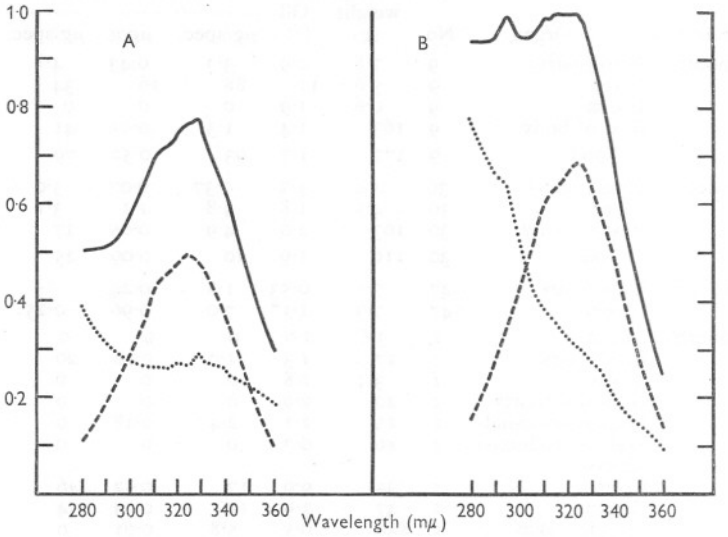

Fig. 1. Absorption spectra of fractions containing vitamin A from non-saponifiable residues of oils extracted from (A) eyes and (B) liver of *Loligo forbesi* (solvent, *n*-hexane). ——, *E* observed; – – – –, *E* corrected; . . . ., irrelevant absorption.

Vitamin A in these two extracts was also examined spectrophotometrically and absorption spectra for vitamin A in the eyes and livers are shown in Fig. 1, with curves corrected for irrelevant absorption by the method of Cama, Collins & Morton (1951). The values calculated from the absorption maximum in (spectroscopic) *n*-hexane at 324 mμ taking $E^{1\%}_{1\,cm}$ for pure vitamin A alcohol as 1800 were, after applying the correction procedure for irrelevant absorption, for the eyes 2·2 μg per pair or 0·30 μg per g, and, for the livers 5·1 μg per organ or 0·69 μg per g. The large irrelevant absorption, especially at the lower wavelengths, indicates that, despite efforts to remove sterols, a large amount of extraneous materials was still present in the final extracts.

Of the squid collected near Plymouth in June 1952, one was a large male, which weighed nearly 1 kg. It was dissected into the parts shown in Table II. Vitamin A was concentrated mainly in the liver, caecum and alimentary canal, but the amounts were smaller than those found in earlier specimens. The ester:alcohol ratio for the liver vitamin A was 4:1, and for that in the muscle-skin-tentacles extract 1:3. In the caecum the vitamin A was entirely in the ester form. The two components were not separated in the other organs containing the vitamin. The sparse amounts of carotenoids were mainly xanthophylls, although there may also have been a trace of astaxanthin in the liver.

TABLE III. VITAMIN A ESTER, ALCOHOL AND ALDEHYDE IN THE EYES OF *LOLIGO FORBESI* AND *OMMASTREPHES PTEROPUS*

						Vitamin A					
						Ester		Alcohol		Aldehyde	
Date	Locality	Species	No. of eyes	Average weight (g)	Oil (%)	μg/spec.	μg/g	μg/spec.	μg/g	μg/spec.	μg/g
14. xi. 53	Aberdeen	*L. forbesi*	100	3·1	1·6*	0·026	0·0086	0·088	0·029	0·77 (0·91)	0·25 (0·30)
10. xii. 53	Plymouth	*L. forbesi*	67	3·8	1·1†	0·019	0·0050	0·038	0·010	0·96 (0·93)	0·25 (0·25)
16. xi. 54 / 25. xi. 54	North Atlantic	*O. pteropus*	3	21	0·42	0·57	0·027	0·85	0·040	5·0 (4·1)	0·24 (0·19)

* Including 0·79 % subsequently precipitated as sterols.
† Including 0·52 % subsequently precipitated as sterols.
Figures in parentheses are values obtained from spectrophotometric measurements.

In the last group, the ovaries were dissected from two females and the testis from one male. The remaining animals were either immature or spent so that the gonads were not recognizable. Other organs were dissected and grouped for analysis in the way shown in Table II. The distribution of vitamin A was as in the male specimen, with concentrations again rather low; carotenoids were almost entirely absent. In the livers the ester:alcohol ratio for vitamin A was 2:9 and in the eyes it was 1:6. In the extracts from the alimentary and reproductive systems and from the muscle, skin and tentacles the vitamin A was all in the alcohol form, but in the caecum it was present only as the ester. The most striking feature of this group of analyses was the large amount of vitamin A, all as the alcohol, in the mature ova.

The possible presence of retinene in squid eyes was more fully investigated in two groups. The results in Table III show that, whereas the vitamin A ester and alcohol concentrations were higher in the eyes of the Aberdeen specimens than in the Plymouth ones, retinene was present in much greater amounts than either of the other two vitamin A components and the concentrations were the same in both groups.

Ommastrephes pteropus Steenstrup

Two specimens of this large squid, taken during a cruise of R.R.S. *Discovery II* in November 1954, were dissected and some organs preserved for us on the ship. The parts we received were the liver, one eye and the nidamental gland from one specimen, and both eyes and the liver from the other. For analytical purposes the two livers were treated separately, but the three eyes were extracted together. The results are shown in Table IV. The livers contained good concentrations of both vitamin A, of which about 75% was in the ester form, and carotenoids, which were mainly xanthophyllic although traces of astaxanthin may also have been present.

TABLE IV. OIL, VITAMIN A AND CAROTENOIDS IN SOME ORGANS OF *OMMASTREPHES PTEROPUS*

Date	Organ	No.	Average weight (g)	Oil (%)	Vitamin A		Carotenoids	
					μg/spec.	μg/g	μg/spec.	μg/g
16. xi. 54	Nidamental gland	1	15	2·0	0	0	21	1·4
16. xi. 54	Liver	1	20	9·4	134	6·6	273	13
25. xi. 54	Liver	1	32	11	474	15	1340	42
16. xi. 54 / 25. xi. 54	Eyes	3	21	0·42	1·4	0·07	31	1·4

β-carotene absent from all organs.

The concentration of vitamin A in the eyes was similar to that found in *Loligo forbesi*. Retinene was also separated from the extract, and the values for the various vitamin A components are shown in Table III. The close similarity in concentration of retinene in the eyes of *Ommastrephes* and in those of the two groups of *Loligo* is very striking.

Todaropsis eblanae (Ball)

Analytical results in Table V show that vitamin A was present only in the liver where the concentration was high; ester and alcohol were not separated in this analysis. The liver was also richest in carotenoids, which were xanthophylls with possibly a trace of astaxanthin.

Parasepia elegans (d'Orbigny)

Results in Table VI show that, as in the previous species, vitamin A was found, entirely in the ester form, only in the liver, but in *Parasepia* the concentration was much lower. Carotenoids were much more widespread in this species and were mainly xanthophyllic. No vitamin A was found in the eyes. It may have been present only as retinene and so destroyed during saponification by our routine analytical technique.

Rossia macrosoma (Della Chiaje)

A single specimen of *Rossia* was dissected into the parts shown in Table VII. The liver was richest in both vitamin A, which was not separated into ester and alcohol, and carotenoids. Small amounts of carotenoids were also found in all other parts.

TABLE V. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *TODAROPSIS EBLANAE*

(Specimen caught near Plymouth on 10. vi. 1952.)

Organ	Weight (g)	Oil (%)	Vitamin A		Carotenoids	
			μg/spec.	μg/g	μg/spec.	μg/g
Ink sac	0·29	2·5	0	0	0	0
Eyes (pair)	9·6	0·9	0	0	8·6	0·9
Alimentary canal	3·7	2·1	0	0	0	0
Male reproductive system	11	1·8	0	0	0	0
Gills and hearts	4·1	1·1	0	0	4·5	1·1
Liver	9·1	11	174	19	70	7·8
Rest of body	111	0·3	0	0	21	0·19
Total	149	1·2	174	1·2	104	0·70

β-carotene absent from all organs.

TABLE VI. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *PARASEPIA ELEGANS*

(Specimens caught near Plymouth on 10. vi. 1952.)

Organ	No.	Weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
				μg/spec.	μg/g	μg/spec.	μg/g	
Ink sac	14	0·25	0·4	0	0	0·6	2·3	0
Eyes (pairs)	14	1·5	0·9	0	0	2·0	1·3	0
Alimentary canal	14	1·1	1·2	0	0	7·2	6·6	0
Liver	14	1·3	2·5	0·32	0·24	7·5	5·8	Trace
Nidamental glands	11	2·3	1·7	0	0	0	0	0
Ovary	11	2·8	2·6	0	0	2·8	1·0	0
Testis	3	1·1	0·5	0	0	2·0	1·8	0
Rest of body	14	19	0·3	0	0	1·9	0·10	0
Total	14	28	0·8	0·32	0·01	22	0·79	Trace

TABLE VII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *ROSSIA MACROSOMA*

(Specimen caught in Firth of Clyde on 17. vi. 1954.)

Organ	Weight (g)	Oil (%)	Vitamin A		Carotenoids	
			μg/spec.	μg/g	μg/spec.	μg/g
Eyes (pair)	2·4	0·66	0	0	2·7	1·1
Liver	1·1	5·5	12	11	14	13
Rest of body	21	1·1	0	0	8·3	0·4
Eggs	0·8	21	0	0	3·2	4·0
Total	25	1·9	12	0·48	31	1·2

β-carotene absent from all organs.

Sepia officinalis (Linnaeus)

Three groups of the common cuttlefish, all collected near Plymouth, were analysed with results shown in Table VIII. As in most of the previous species, the liver was the richest in vitamin A and carotenoids, with vitamin A alcohol slightly in excess of vitamin A ester. The carotenoids included both carotenes and xanthophylls. The ovaries contained ripe ova, which were rich in vitamin A alcohol. Vitamin A was present in the alimentary canal as the ester and in the eyes and muscle, brain, skin and tentacles as the alcohol. Next to the liver, the ink sac had the highest concentration of carotenoids, these being mostly xanthophylls.

The eyes were dissected from fourteen much smaller cuttlefish, collected at the same time as the previous specimens, and the analytical results for these are shown in Table VIII. The vitamin A was all in the alcohol form.

As Table VIII shows, the results for specimens collected in November were similar to those taken in June, but the ratio of vitamin A ester to alcohol in the liver was 4:1. As before, the eyes contained only vitamin A alcohol

TABLE VIII. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *SEPIA OFFICINALIS*

(All specimens caught near Plymouth.)

Date	Organ	No.	Average weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene (μg/g)
					μg/spec.	μg/g	μg/spec.	μg/g	
7. vi. 51	Eyes (pairs)	2	16	0·67	1·3	0·08	11	0·68	0
	Gills and hearts	2	18	1·6	0	0	18	0·93	Trace
	Ink sac	2	6·6	0·33	0	0	29	4·4	0
	Liver	2	34	4·2	72	2·1	1255	37	2·6
	Female reproductive system	2	49	1·8	0	0	0	0	0
	Alimentary canal	2	19	0·91	3·3	0·18	31	1·6	Trace
	Muscle, brain, skin and tentacles	2	432	0·67	5·6	0·01	77	0·18	0·01
	Ovary	2	57	2·0	100	1·7	0	0	0
	Total	2	632	1·1	182	0·29	1421	2·3	0·15
7. vi. 51	Eyes (pairs)	14	4·4	0·47	0·20	0·05	3·6	0·82	0
16. xi. 51	Eyes (pairs)	2	14	0·60	4·4	0·32	2·2	0·16	0
	Liver	2	34	7·4	67	2·0	210	6·2	0·57
	Caecum	2	6·6	1·8	0	0	0	0	0
	Alimentary canal	2	12	1·3	0·57	0·05	9·1	0·78	0
	Ink sac	2	12	0·46	0	0	8·9	0·74	0
	Ovary	2	1·8	2·4	0	0	0	0	0
	Nidamental glands	2	6·9	1·9	0	0	0	0	0
	Gills and hearts	2	17	0·50	0	0	0	0	0
	Muscle, brain, skin and tentacles	2	406	0·51	0	0	24	0·06	0
	Total	2	510	1·0	72	0·14	254	0·50	0·04
10. vi. 52	Eyes (pairs)	9	2·5	1·1	0	0	3·5	1·4	0
	Ink sac	9	0·76	0·66	0	0	1·5	1·9	0
	Alimentary canal	9	3·5	0·94	0	0	6·4	1·8	0
	Liver	9	4·2	4·2	4·9	1·2	116	28	0·08
	Rest of body	9	39	1·0	0	0	0	0	0
	Total	9	50	1·3	4·9	0·10	127	2·5	0·007

and the alimentary canal only ester. The ovaries were immature and had no vitamin A. Carotenoid concentrations were lower than in the summer specimens.

The smaller specimens in the last group had vitamin A only in the liver, where the ratio of ester to alcohol was about 7:2. The carotenoid concentrations were of the same order as in the June specimens of the previous year.

Sepietta oweniana (d'Orbigny)

Analytical results for the groups and single specimens of this cuttlefish are shown in Table IX. Vitamin A was entirely in the ester form in the first group of specimens. The second group, consisting of three much smaller specimens, was analysed whole, and the results in Table IX show that they were even richer in vitamin A than the first group. Because of the small amount of material, vitamin A ester and alcohol were not estimated separately. In neither of these first two groups were carotenoids present in measurable amounts.

TABLE IX. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *SEPIETTA OWENIANA* AND *SEPIOLA* SP.

Date	Locality	Organ	No.	Average weight (g)	Oil (%)	Vitamin A		Carotenoids	
						μg/spec.	μg/g	μg/spec.	μg/g
		Sepietta oweniana							
18. x. 51	Loch Fyne	Whole animals	2	1·3	9·3	4·5	3·4	0	0
9. vii. 52	Loch Fyne	Whole animals	3	0·12	3·1	0·55	4·6	0	0
30. ix. 54	Clyde Main Channel	Whole animals	1	4·3	3·1	5·0	1·1	26	6·0
10. iii. 55	Clyde Main Channel	Whole animals	1	0·93	1·4	3·2	3·5	5·5	5·9
12. iii. 55	Loch Hourn	Eyes (pair)	1	0·32	0·25	0·57	1·8	1·4	4·3
		Liver	1	0·28	0·65	1·4	5·1	1·4	4·9
		Rest of body	1	2·4	0·52	0	0	2·9	1·2
		Total	1	3·0	0·47	2·0	0·66	5·7	1·9
		Sepiola sp.							
2. ii. 52	Monaco	Whole	1	1·4	0·76	0·77	0·54	6·7	4·7

β-carotene absent from all specimens.

The concentration of vitamin A in the large specimen, taken in September 1954, was lower than in the smaller ones but, as in the first group, the vitamin was entirely in the ester form. Measurable amounts of carotenoids were present, but no definite bands appeared on chromatography, so that they were not identified. Another specimen, weighing 0·93 g, was analysed whole. The results in Table IX show that the concentration of vitamin A, all in the ester form, was similar to that in the first two groups. There was again not enough carotenoid pigment for identification.

In the dissected specimen, the liver contained most of the vitamin A, with a smaller amount in the eyes and none in the rest of the body, but the concentration in the whole animal was less than in previous analyses. In the liver there were approximately equal quantities of vitamin A ester and alcohol, whereas in the eyes there was only vitamin A alcohol.

Sepiola sp.

A single specimen was analysed whole with the results shown in Table IX. The small amount of vitamin A was measured only in the ester fraction, since sterols interfered with measurements on the alcohol fraction. There was not enough pigment present in the chromatography for identification of the carotenoid.

Order Octopoda

Eledone cirrosa (Lamarck)

Analytical results for this specimen given in Table X show that, as in the decapods, the liver contained most of the vitamin A. Only the ester was determined. The alcohol fraction gave a red colour in the Carr-Price reaction, probably due to sterols which were not previously removed. These may have interfered in the reaction with any vitamin A alcohol that might have been present. Vitamin A alcohol was slightly in excess of the ester in the eyes. In the other parts of the body vitamin A was all in the ester form, although the presence of sterols may have interfered with the measurement of the alcohol.

TABLE X. DISTRIBUTION OF OIL, VITAMIN A AND CAROTENOIDS IN *ELEDONE CIRROSA*

(Specimen caught near Plymouth on 11. vi. 1952.)

Organ	Weight (g)	Oil (%)	Vitamin A		Total carotenoids		β-carotene
			μg/spec.	μg/g	μg/spec.	μg/g	(μg/g)
Eyes (pair)	2·9	0·6	0·59	0·20	5·8	2·0	0
Liver	24	16	64	2·7	340	14	0·20
Hearts, gills, alimentary and reproductive systems	20	0·8	0·54	0·03	46	2·3	0
Rest of body	281	0·5	4·6	0·02	43	0·15	0
Total	328	1·6	70	0·21	435	1·3	0·02

BIOLOGICAL TESTS

Methods

General

The biological activity of the vitamin A fractions from the eyes and livers of 547 squid, *Loligo forbesi* (see p. 68) was measured by rat-growth tests. These were done in the way described by Fisher *et al.* (1952). The standard used was the International Standard for vitamin A at 10,000 i.u./g in cotton-seed oil suitably diluted for the tests with arachis oil stabilized with hydroquinone. The material to be assayed was prepared from the non-saponifiable residue after removal of the sterols and extraction of the vitamin A fractions as described earlier in this paper. The residue, containing vitamin A in the alcohol form, was diluted with arachis oil for feeding to rats. During the tests the oils were kept under nitrogen in the refrigerator and their vitamin A content was checked periodically by the Carr-Price test.

Squid-eye oil

Groups of sixteen (all ♀) wholly vitamin A-deficient rats received twice weekly vitamin A standard at the nominal rate of 2 and 4 i.u. daily; a group of fourteen similar rats was dosed at a nominal daily rate of 2 i.u. and another group of thirteen rats at nominal rate of 4 i.u of the test preparation.

Squid-liver oil

As before, the test was done on female rats only. Three groups of eighteen were dosed twice a week with the standard and three with the liver extract at nominal rates of 1, 2 and 4 i.u. daily.

TABLE XI. CHEMICAL (ANTIMONY-TRICHLORIDE) AND BIOLOGICAL MEASUREMENTS OF VITAMIN A ACTIVITY IN SQUID OILS FED TO WHOLLY VITAMIN A-DEFICIENT RATS, IN I.U. PER GRAM OF ARACHIS OIL SOLUTION

Organ	Chemical Value	Biological	
		Value	True fiducial limits ($P=0{\cdot}95$)
Eye	195	212	157–279
Liver	210	210	184–241

Results

Table XI shows the results of the two tests. The values for chemical and biological measurements of vitamin A in both eyes and livers show good agreement, indicating that the vitamin was in the all-*trans* form.

DISCUSSION

Although some of the results are unsatisfactory owing to our failure in the early stages of the work to remove sterols from some of the more concentrated extracts and to separate retinene from eye extracts, we believe that they provide some useful information. The supply of specimens suitable for our analyses is so irregular and fortuitous that it may be a long time before we can rectify some of the earlier mistakes. We think it therefore advisable to publish our findings, pointing out results which may be doubtful owing to errors in technique.

We have found in all instances vitamin A in some part of each of the ten species of cephalopod we have studied. The concentration has varied between species, the average being of the same order as the highest found in *Patella*, the richest in vitamin A of other molluscs except *Limacina retroversa* (Fisher *et al.*, 1956).

Except in *Alloteuthis media*, vitamin A was always found in highest concentration in the liver. Usually most of the liver vitamin A was in the ester form, indicating that this organ was acting as a store for vitamin A, as in many vertebrates, but contrasting with the situation in other molluscs where

vitamin A in the digestive gland was in the alcohol form (Fisher *et al.*, 1956). In some specimens, mostly mature females bearing eggs, there was less vitamin A ester than alcohol. This fact, coupled with the presence of large concentrations of vitamin A alcohol in the eggs of, for example, *A. media*, *Sepia officinalis* and *Loligo forbesi*, indicates that the liver reserves of vitamin A are used up in the development of the eggs, possibly even to the point of absolute depletion, as in *Alloteuthis* (see Table I), where five of the six specimens contained ripe eggs and the other no gametes at all, probably being spent.

Vitamin A was found in the eyes of all the cephalopods studied, except *Parasepia elegans*, *Rossia macrosoma* and *Todaropsis eblanae*, and it was predominantly or entirely in the alcohol form, although the aldehyde (retinene) was found in those species, *Loligo forbesi* and *Ommastrephes pteropus*, for which the special technique for its separation was employed. When this technique was used for the eyes of *Loligo forbesi* the values we obtained for vitamin A alcohol were about one-tenth of those given by Wald (1941) for *L. pealii*, but the concentrations of retinene were of the same order in both species. It is difficult to reconcile our findings of vitamin A and carotenoids in other parts of *L. forbesi*, especially the liver, with their complete absence, except for the eyes, in *L. pealii*, but, in conversation, Prof. Wald has told us that his investigation, done some years ago, on those parts other than the eyes might well be repeated. According to Brachi (1953), vitamin A was absent from *L. forbesi* and *Sepia officinalis* at Plymouth, but our analytical technique was probably more sensitive than his, normally applied to the more massive quantities of vitamin A found in whale-liver oils.

In the eyes of *Ommastrephes pteropus* the values for both vitamin A and retinene were similar to those found by Wald (1941) in *Loligo pealii*. There are, thus, no qualitative differences between the vitamin A components in the visual cycles of squids living, like *Ommastrephes*, at great depths and those, like *Loligo*, in shallower waters. What is striking is that not only is there a remarkable resemblance between the structures of the cephalopod and vertebrate eyes, as Berrill (1951, p. 200) has pointed out at some length, but also that they function biochemically in a similar way. We have already noted the storage of vitamin A in the cephalopod liver as a vertebrate characteristic, found so far nowhere else among invertebrates, unless the storage of rich concentrations of vitamin A ester in the eyes of euphausiid Crustacea (Fisher *et al.*, 1952) be taken as similar in purpose.

Another interesting resemblance between the vitamin A metabolism of cephalopods and that of vertebrates is indicated by the presence of vitamin A alcohol in ripe eggs, just as found by Neff, Parrish, Hughes & Payne (1949) in hen eggs, where 73–93% of the total vitamin A was in the alcohol form. Parrish, Williams & Sanford (1951) subsequently showed that it was gradually converted to the ester and stored in the embryonic liver during incubation of

the chick. No similar observations on developing cephalopod eggs have yet been made. The absence of vitamin A from the ova of *Rossia macrosoma* and *Parasepia elegans* was presumably associated with their immaturity. Lönnberg (1935) reported a positive reaction with antimony trichloride which he described as 'surprisingly strong' for a colourless extract from ripe eggs of *Rossia macrosoma*, which would undoubtedly be due to vitamin A.

From our limited findings the cephalopod groups, squids, cuttlefishes or octopods appear to be fairly uniform in their vitamin A content. The concentrations of carotenoids were comparatively low in all species. In fact, Goodwin (1952) says of the cephalopods: 'The most outstanding fact concerning carotenoids in this group is their comparative absence'. Nevertheless, differences could be detected. The squids were, generally, poorer in carotenoids than cuttlefishes or *Eledone*. This difference may be because they are more active and feed, therefore, on pelagic Crustacea, which usually contain fewer carotenoid pigments than benthic species, very often only astaxanthin being present in the swimming Crustacea, whereas carotenes and xanthophylls occur in other species (Fisher *et al.*, 1952, 1953). We never found astaxanthin in cephalopods in more than faint traces, indicating that it is either little absorbed or is broken down quite rapidly.

Cephalopod livers contained the highest concentrations of carotenoids, but these were extremely variable even within a species. The carotenoids in the livers were usually carotenes and xanthophylls, but carotenes were not found in the livers of *Todaropsis* and *Rossia*. The livers of these two species, and of *Ommastrephes*, did, however, contain traces of astaxanthin.

In a study of the carotenoids of *Paroctopus bimaculatus*, Fox & Crane (1942) found xanthophylls and their esters in the ink. They suggested that this might be a unique method of excreting carotenoids. In some of the species we examined, the ink sacs and their contents were analysed, but not the ink separately. No carotenoids were found in the ink sacs of the squids, *Loligo forbesi* and *Todaropsis eblanae*, but xanthophylls or their esters were present in those of the cuttlefishes, *Parasepia elegans* and *Sepia officinalis*. The ink sacs of the other species studied, including the only octopod, *Eledone cirrosa*, were not analysed separately. We have already pointed out that the cuttlefishes and octopods are richer in carotenoids than the squids, which probably, therefore, do not require any special means of excreting the pigments.

The curves in Fig. 1 show that the absorption spectrum of cephalopod vitamin A is similar to that of all-*trans* vitamin A. The biological activity of squid-liver oil agreed with the potency determined by the Carr-Price test and there is no evidence of a discrepancy such as we found (Fisher *et al.*, 1952) in euphausiid Crustacea between the potencies determined physicochemically and biologically. The presence of all-*trans* vitamin A rather than other isomers shows another resemblance between cephalopods and vertebrates and a difference between them and other invertebrates.

Cephalopods feed mainly on Crustacea, most of which contain vitamin A. As we have already mentioned, the pelagic cephalopods probably feed on swimming Crustacea, among which, because of their swarming habits, the euphausiids must form an important part of the diet. Hjort & Ruud (1929) mention that euphausiids are important as food of the squid, *Gonatus fabricii*, which in its turn forms part of the diet of the bottle-nosed whale, *Hyperoodon rostratus*. Our work on vitamin A in Crustacea so far indicates that the euphausiids are the richest in vitamin A of all the Crustacea (Fisher *et al.*, 1955). They may thus be the main source of preformed vitamin A for the cephalopods which store it and pass it on to their predators, especially the toothed whales. It would be difficult to determine whether these whales eat sufficient cephalopods to obtain all their rich liver reserves of vitamin A preformed, since the feeding rates of whales are unknown. The enormous numbers of cephalopod beaks to be seen attached to the stomach lining of a sperm whale probably represent only a small proportion of the total intake, which may well be adequate.

The cephalopods are the most highly organized of the invertebrates and they exhibit several similarities to the vertebrates and few resemblances to other molluscs in their vitamin A metabolism (Fisher *et al.*, 1956). Perhaps it is more than coincidental that they, the richest in vitamin A of all the molluscs except perhaps certain pteropods, form the main food of the toothed whales just as the euphausiids, the richest in vitamin A of all the Crustacea, form the main food of the baleen whales. Furthermore, the euphausiids are probably one of the principal components of the diet of the cephalopods themselves.

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Summary

Ten species of cephalopods, namely, *Alloteuthis media*, *Loligo forbesi*, *Ommastrephes pteropus*, *Todaropsis eblanae*, *Sepia officinalis*, *Parasepia elegans*, *Rossia macrosoma*, *Sepietta oweniana*, *Sepiola* sp. and *Eledone cirrosa*, have

been analysed for fat, vitamin A and carotenoids. Vitamin A was present in all species. Carotenoids were found in most species but concentrations were low.

Vitamin A was found mainly as the ester, in largest amounts in the liver, but in females these reserves were depleted by developing eggs, which were rich in vitamin A alcohol. The vitamin was usually present in the eyes, mostly as the aldehyde (retinene) in those species, *Loligo forbesi* and *Ommastrephes pteropus*, in which it was separated, indicating a visual cycle biochemically resembling that of vertebrates.

Cuttlefishes and octopods were richer in carotenoids than squids, probably owing to the more varied and richer carotenoid content in the benthic Crustacea of their diet than in the pelagic Crustacea forming the food of squids. There is evidence that cuttlefishes and octopods excrete excess carotenoids in their ink, whereas squids do not.

Cephalopod vitamin A is probably all-*trans* vitamin A, and there was complete agreement between the potencies, determined chemically and biologically, of vitamin A from squid livers and eyes.

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THE ECOLOGY OF THE TAMAR ESTUARY

VII. OBSERVATIONS ON THE INTERSTITIAL SALINITY OF INTERTIDAL MUDS IN THE ESTUARINE HABITAT OF *NEREIS DIVERSICOLOR*

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(Text-figs. 1–5)

INTRODUCTION

The polychaete *Nereis diversicolor* F. O. Müller is one of the most characteristic elements of the brackish-water fauna of north-western Europe, and inhabits an extremely wide range of salinities. As a background for comparative physiological studies of chloride regulation in worms from different parts of the geographical range, the writer has attempted to describe the salinities and the variations in salinity endured by populations of *N. diversicolor* in ecologically distinct and geographically well separated areas. Three principal environments have been studied:

(1) *Relatively stable high salinity*. Since it was initially hoped to study *N. diversicolor* in a 'marine' habitat, a study was carried out at Kames Bay, Millport, on a presumably marine beach, but it was found that *N. diversicolor* was restricted to a brackish zone produced by underground seepage of fresh water (Smith, 1955*a*). Since I have been unable at any time to find this species except in areas subject to some lowering of salinity, my general conclusion has been that in a 'marine-dominated' habitat *N. diversicolor* behaves as a brackish-water animal and is to be found in the least saline portion of the available ground. A similar conclusion was reached after studies in the Isefjord, Denmark, where the general level of salinity is about 20‰ and quite stable (Smith, 1955*b*).

(2) *Relatively stable low salinity*. The most obvious contrast to the marine-dominated intertidal habitat is found in the inner Baltic Sea, wherein *N. diversicolor* reaches the less saline limit of its ecological range. In these tideless waters the salinity is low and, over most of the year, fairly constant. Studies carried out near the Zoological Station, Tvärminne, South Finland, have also been reported (Smith, 1955*b*). In this region the distribution of *N. diversicolor* is surprisingly unlike that seen in Britain. Whereas in British estuaries the species has been observed to penetrate into nearly fresh water, and much farther than any of its usual brackish-water associates, in the Baltic the reverse is found. The range of *N. diversicolor* is limited at summer salinities in excess

of 4‰, and a number of its brackish-water associates (*Cardium*, *Mytilus*, *Macoma*, *Balanus improvisus*) penetrate into less saline waters. The answer to this paradox may lie in the fact that although the inner Baltic is of fairly stable salinity during most of the year, there is an annual lowering of salinity as a result of melting snows, occurring when temperatures are still very low, and possibly at the breeding period of *N. diversicolor*. Whether the limiting factor is the effect of low salinity upon the adult population (whose osmoregulatory capabilities may be temporarily reduced by low temperature) or upon some phase of reproduction is not yet clear. We must consider, however, that whatever the mode of action, the special hydrographic characteristics of the inner Baltic are important in limiting the spread of *N. diversicolor* into waters of low salinity.

(3) *The estuarine habitat*. In comparison with the two sorts of environment mentioned above, the estuarine habitat is characterized by marked salinity *variation* resulting from tidal action. The present paper discusses this third type of salinity regimen, and completes the series of three studies on the distribution of *N. diversicolor* in relation to salinity which the writer was able to carry out in 1953–54. Most of the observations have been made in the estuary of the River Tamar, with additional observations near the head of the Kingsbridge Estuary.

Any estuary exhibits a complex pattern of salinity changes, and individual estuaries may differ very greatly from each other. Day's review (1951) provides a most useful general account, while Rochford's classification of Australian estuaries (1951) illustrates the great diversity which may exist among estuaries in respect to their pattern of salinity variation. In attempting to consider the physiological adaptations of an estuarine animal, we must consider not only mean salinity, which over the range of *N. diversicolor* varies from over 25‰ down to nearly fresh water, but the extent of salinity variation as well. The difficulty of characterizing in any simple way the salinity to which a given population is exposed is very great. At each point within an estuary there may be noted maximum and minimum salinities, but the magnitude of these extremes does not tell the whole story, for their duration and the rates of change of salinity must also be considered (Bassindale, 1943). These factors differ not only horizontally as one proceeds up an estuary from the sea and vertically with intertidal height, but also they vary with time, in all but an idealized estuary where tidal ingressions of sea water were regular from day to day, and freshwater inflow constant and uniform. In actuality, tidal ingressions vary daily throughout the lunar tidal cycle, and are markedly affected by local wind conditions, while freshwater inflow is subjected to seasonal or erratic variation in rainfall. The character of estuaries varies topographically according to volume and configuration of the estuary bed, and with the relative volumes of estuary bed and daily freshwater inflow.

For purposes of the present study, the estuary of the River Tamar has offered the advantages of being relatively accessible from the laboratory at Plymouth, of possessing a long salinity gradient inhabited by *N. diversicolor*, and of being an estuary about which much useful published information is available to the newcomer. Much, however, remains to be learned, and it is hoped that the data presented in this paper will extend the existing knowledge somewhat. Time has not permitted a comprehensive or complete survey, but the effort has been made to base these studies upon reliable previous work, and to relate what has been learned to the total picture.

The most useful introduction to the River Tamar is given by Hartley & Spooner (1938), whose paper should be consulted by any reader not familiar with the locality. By reference to the map (Fig. 1) it may be noted that the lower 10 km of the estuary lies below the confluence of the Tamar and the River Tavy, and that this common estuary is joined by the Lynher River some 5 km from the mouth, which opens into Plymouth Sound. Above the entrance of the Tavy, the Tamar gradually narrows and becomes winding. *N. diversicolor* extends to the vicinity of Calstock (22·5 km from Plymouth Sound), above which point the vegetation and bottom fauna are essentially fresh water, although tidal action extends another 7 km to Weir Head.

The fauna of the Tamar Estuary has been described qualitatively by Percival (1929), whose account is generally useful, although little indication of abundance of individual species is given. It may be noted that Percival worked in a dry summer, and recorded upstream limits for planktonic organisms and certain crustaceans (e.g. *Carcinus* present at Calstock) which may be somewhat atypical. Spooner & Moore (1940) have given a thorough quantitative account of the macrofauna of the intertidal muds of the Tamar as far upstream as North Hooe (18 km). I have been able to add very little general information to their excellent account, except for additional observations on *N. diversicolor* at stations upstream from the limit of their survey. Spooner & Moore reported *N. diversicolor* present scatteringly in St John's Lake, 'apparently here confined to the lower tidal levels', increasing in abundance up-river, and reaching its greatest density at their uppermost station, North Hooe. This increase was believed to be independent of substrate, since equivalent muds are available over more than the entire range of the species in the river. In the lower reaches of the estuary, *N. diversicolor* was found at a lower-intertidal position, but over most of its range was reported to have its maximum density above mid-tide.

The pattern of salinity variation in the Tamar is unquestionably complex, and in comparison with the amount of information available on the fauna, the published data on salinity are still far from complete enough to give an overall picture of the salinity of the *N. diversicolor* habitat. Percival (1929) gave salinity values at or near high and low water for a number of stations from the lower estuary up to the head of tidal action, and the distribution of salinities

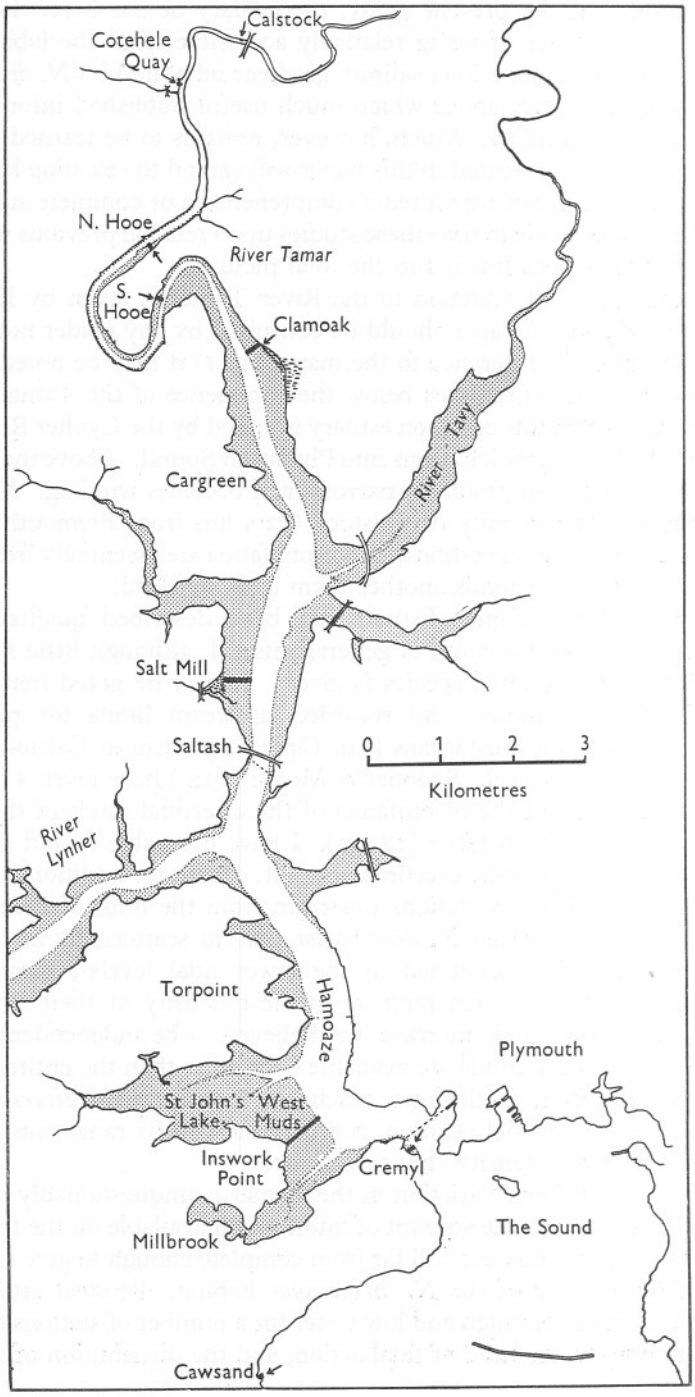

Fig. 1. Sketch-map of the Tamar Estuary showing location of stations and points mentioned in the text. Stippled areas represent intertidal muds between H.W.M. and L.W.M. of ordinary tides. Compiled from various sources. A more detailed map is found in Hartley & Spooner (1938).

correlates well with the reduction of the marine fauna as one passes up-stream. Most of Percival's data were obtained during a summer of 'negligible' rainfall (86 mm at Plymouth in 86 days), hence they probably do not represent the lower and perhaps limiting salinities reached in winter and spring. Percival judged from his salinity data that the main body of inflowing sea water did not pass Cargreen (11 km from the mouth) and that the greatest drop in surface salinity took place between Cargreen and the region about Calstock. In this same stretch of river occurred the greatest differences in salinity between high and low tide. Milne (1938) recorded salinity stratification in May–June on one rising and one falling tide in a cross-section of the estuary 7·7 km from the mouth, between Saltash and Neal Point and thus within the wide portion of the river strongly influenced by salt-water ingression (salinity range *c.* 15–32‰). Milne calculated salinity fluctuation at various intertidal heights, and showed that organisms living near low-water mark must suffer the greatest change of salinity at a tidal cycle; those near high-water mark must experience the least change in salinity; those at mid-tidal positions and those living subtidally are subjected to fluctuations intermediate in magnitude between those at the first two levels. Additional data for the lower estuary are given, and for a few up-stream points values which are in general agreement with Percival's data, emphasizing the marked drop in mean salinity and the considerable salinity variation of the River Tamar up-stream of its union with the River Tavy. However, the available data are insufficient to define the up-stream limits of *N. diversicolor* in terms of salinity. It has, therefore, been necessary to seek additional data in the region above the middle reaches of the estuary.

Methods

Field work was carried out from April to mid-June and from late September into December 1954. Since physiological studies on *N. diversicolor* inhabiting the less saline reaches were the main objective, most observations have been confined to the region from Clamoak to Calstock, with comparative observations in the lower reaches (Salt Mill and St John's Lake) as well as in the 'marine-dominated' Kingsbridge Estuary. In the early weeks of work the extent and complexity of the Tamar Estuary were not fully appreciated; in the last few weeks bad weather contributed to incompleteness of the record.

With the limited available time and the difficulty of securing boats when and where needed, it was felt that the usual method of obtaining samples of water close above the mud at representative intertidal levels and at enough different stages of tide would be impractical. The expedient was therefore adopted of measuring the salinity of water contained in the mud itself by means of a series of samples forming a transect of the exposed shore at each station. It is obvious that the salinity of interstitial water reflects in some

degree the salinity of the water that has lain above the mud. Precisely what the relationship is between interstitial salinity and the varying salinities of the overlying water is not known. At present it certainly cannot be claimed that the salinity of the interstitial water represents the mean salinity of the overflowing water—it can merely be said that the interstitial salinity must be the resultant of the varying overlying salinities and the duration of the exposure. Until the actual time-course of salinity changes of the water just above a given intertidal point is worked out, it does not appear possible to give a significant 'mean', either arithmetic or harmonic, for salinity at this point. If the change of salinity with time followed a symmetrical pattern (e.g. sinusoidal), the median value would approximate a mean value, but if, for example, the time course followed a pattern wherein one extreme (at high water) acted for a much longer time than the opposite extreme (at low water), then the 'mean' of isolated values taken at high and low water would represent only a median value, and not anything like a true mean. We may assume that the values of interstitial salinity in mud would be subject to the time factor of the salinity variation. We may also assume that under conditions of seasonal variation in the 'mean' salinity at a given point in an estuary—as when the freshwater inflow is elevated during prolonged rains—the salinity of the interstitial water would be shifted in corresponding fashion. But it must be emphasized that no precise measurement of the lability of the interstitial salinity has yet been attempted. The present study was timed, although not intentionally, in such a way that the variability of interstitial salinity with seasonal rainfall has been indicated.

Method of Determining Interstitial Salinity in Mud

The mud of the *N. diversicolor* habitat in the Tamar is soft and usually of a sticky consistency, apparently with considerable admixture of clay. Draining, sucking, or centrifuging water from it is hardly practical. In this survey the simplest methods were adopted, it having been felt not worth while to invest time in more elaborate procedures or equipment until a preliminary survey had indicated the usefulness of such an approach. Samples were taken at low tide at a series of points from the highest level of the mud-flat down to the water-line. The top inch or so of mud was scraped aside, several samples were scooped up with the fingertips from the area thus exposed, and these were packed together in a 175 ml. honey jar, which was filled to capacity to prevent any evaporation. In the laboratory two 30–40 g samples were spooned out from each collecting jar into wide shell vials and weighed immediately to the nearest 0·01 g. Samples were then dried to constant weight in an electric oven at $105 \pm 2^{\circ}$ C, a process requiring 3 or 4 days. The loss of weight represented the water content of each sample. Into each vial was then pipetted 25 ml. of distilled water, each dried sample was crushed with a small, flat-ended glass rod, the samples were covered to check evaporation, and were

allowed to stand for 24 h. The glass pestle was left in each vial, and twice more during the soaking period was used to stir up the contents and to crush any solid lumps. In samples with much clay present this task proved troublesome. Finally, after settling, two 1 ml. aliquots were pipetted from the more or less clear supernatant and titrated with silver nitrate as earlier described (Smith, 1955*a*). From the chloride value obtained on the 25 ml. of added water, and the original water content as obtained by drying, the chloride of the originally contained water was calculated. Salinities were approximated by multiplying the chloride values by 1·81.

The method, as described above, is based upon several assumptions. It is assumed that all samples were taken at about the same depth, roughly the second inch beneath the surface in spots typical of the general area at that level, that no loss of water by evaporation took place before weighing or during the soaking process after drying, that the osmotically active chloride of the originally contained water was quantitatively recovered in the extraction step, and that no such chloride was bound permanently or lost during the drying process. It is further assumed that drying at 105° C did not release constituent water of clay. The method should in theory be unaffected by the actual interstitial water content or by the degree of drainage of the sample, provided no evaporation is permitted. The water-retaining qualities of soils, especially when clay is present, are complex (Baver, 1948), but as far as I have been able to determine, the method described is in principle free of serious theoretical sources of error. In its present form it is admittedly tedious, and, in any future survey, would need to be reduced to a routine to ensure more reproducible results. In particular, a standard method of taking groups of core samples should be employed, and a stricter control of the depth of sample is essential, for we must assume that the salinity of interstitial water in the uppermost layer is more labile than that of deeper-lying water. Further, a standard method of mechanical shaking during the extraction process and a simplified routine for chloride analysis would be necessary before a large-scale survey were undertaken. Present results may indicate whether or not the taking of interstitial salinities is likely to be of value in ecological surveys of estuaries.

Methods of Obtaining Levels

At each sampling station a profile of the exposed shore was determined by means of a dumpy level and a metric surveying staff. Distances of less than 50 yd. were measured with a length of cod line marked off in 5 yd. intervals (always used wet); greater distances were measured by use of the vertical angular adjustment of the dumpy level. The instrument was probably not very accurate for levelling or distance-ranging at distances over 150 m, and its use was not attempted on the wide flats of St John's Lake. In order to assign absolute heights relative to Ordnance Datum, three established Bench

Marks were used. Their heights relative to the newer (Newlyn) datum were furnished by the Ordnance Survey Office:

Location of Bench Mark	National Grid (10 m) Reference	Height above Ordnance Datum (Newlyn)		Height above Chart Datum (Devonport)	
		Feet	Metres	Feet	Metres
South face of west pier of Saltash Bridge to north of ferry landing	SX 4337 5877	6·76	2·06	15·68	4·78
West angle of barn at Clamoak	SX 4378 6421	18·61	5·67	27·53	8·40
South-east face of barn at Cotehele Quay	SX 4236 6803	11·64	3·55	20·56	6·27

At Clamoak and Cotehele Quay, the height of the mud flats could be determined directly. To determine the height of the flat at Salt Mill Creek, markers of the type used by Spooner & Moore, consisting of glass tubes lined with gelatine containing silver chromate and having a capillary at the bottom to damp out wave action, were placed near the Bench Mark at the west end of the Royal Albert Bridge at Saltash. A similar marker was set out on a stake in the mud-flat at Salt Mill Creek. The high-water mark at Salt Mill was thus recorded, and was levelled in relation to the top of the mud flat, top of salting, and to the Admiralty Boundary Stone no. 10, which is conveniently located nearby. The height of this stone is not recorded in Ordnance Survey files, but I have calculated its height, by the method described, as 2·31 m above Ordnance Datum (Newlyn). Returning to Saltash, I related the height of high water as recorded on the indicator to the known height of the Bench Mark, and so was able to assign an absolute level to H.W.M. here and at Salt Mill, and to the profile of the shore at the latter station. Similarly, levels at North and South Hooe were determined with reference to the Bench Mark at Clamoak, and at Calstock with reference to Cotehele Quay. At Calstock and North Hooe the point of high water had to be observed directly, since in December of 1954 salinities above South Hooe were too low to discolour the silver chromate indicator (this difficulty might be eliminated in future by placing a little NaCl solution in each tube before allowing the tide to elevate the water within it). My methods have not differed in principle from those of Spooner & Moore, except that I have used several Bench Marks well up in the estuary rather than a single mark on the shore of Plymouth Sound. The up-river readings were taken at high slack water on windless days, and despite the large freshwater inflow are probably close enough together to rule out error caused by any slope of water in the estuary bed. Like the previous authors, I claim no great accuracy.

Method of Estimating Nereid Population Density

The taking of quantitative worm samples while working alone in the extensive Tamar muds proved difficult, and was attempted only occasionally. At the extremes of the range (St John's Lake and Calstock) only impressions can be given. At Salt Mill, North Hooe, and Cotehele Quay, a metal frame

of 0·1 m^2 area was pressed into the mud, the enclosed mud dug out to a depth of about 10 cm, and washed through a pair of sieves of 3–4 and 1·5 mm mesh. Before sieving, the soil was broken apart by hand and as many worms as possible removed intact. The results are inadequate in a quantitative sense but some idea of relative abundance was gained in the effort to be quantitative.

Field Observations

Weather

The present study was done partly during May and June and partly in the autumn, mostly under wetter-than-normal conditions. While it is difficult to evaluate the effect of rainfall in a region where local as well as year-to-year variation in rainfall is the rule, it may be noted that May and June of 1954 were months of above-normal rainfall, but followed an unusually dry April, so that the River Tamar was not abnormally swollen, and studies at this time probably reflected conditions not far from normal. However, the

Table I

	Rainfall (mm)		
	Average normal	6-year average 1948–53	1954
January	159	153	81
February	147	124	184
March	136	116	167
April	102	107	28
May	88	109	167
June	84	80	157
July	112	112	169
August	137	180	157
September	105	161	201
October	174	158	198
November	188	208	313
December	229	156	164
Totals	1648	1650	1984

summer and autumn of 1954 were, subjectively, among the wettest in local memory, and flooding on the Tamar occurred after a series of autumn storms. Conditions in November 1954 were regarded as severe, and the salinity in the estuary probably approached a minimal level. These subjective estimates are to some extent supported by the rainfall records published in the Monthly Weather Reports of the Meteorological Office. Unfortunately there are no rainfall-gauging stations on the watershed of the River Tamar proper, and it was felt that rainfall at Plymouth or along the lower estuary was less critical than that on the high moors to the north. Accordingly, Table I was compiled from the records for Tavistock and Princetown on the watershed of the River Tavy. The values given are the averages of data from these two stations. Although the records show great variation in

a given month from year to year, and from station to station in any given month, the general character of the rainfall in the study area in 1954 is indicated, as well as the 'normal' pattern.

Distribution of Nereis diversicolor *and salinities at selected stations* (*see Fig.* 1)

'Marine' situations

Careful search was made on each of two occasions in spring at Cawsand and on the shore south of the quay at Cremyl. The substrates consisted of sand among boulders, and at both stations there was clear evidence of some fresh-water seepage. No *N. diversicolor* were found at any intertidal level despite the fact that the substrata appeared in no way unsuitable. At the Plymouth Laboratory *N. diversicolor* is regarded as a mud-dweller rather than a sand-dweller, but at Millport and in the Baltic it is commonly in sand, so that its absence does not appear attributable to a lack of suitable substrate. At no time in the course of over a year's work have I found this species in a situation which could be described as fully 'marine'. Undoubtedly, individuals may be found from time to time in marine water adjacent to populous brackish habitats. Salinities at Cawsand may be assumed to be high; Cremyl may occasionally receive brackish water from the Hamoaze, but no lowered salinities were recorded at this place in ordinary rainy weather. As Spooner & Moore (1940) state: 'In the Plymouth District the status of *Nereis diversicolor* as a marine species is doubtful. Though recorded occasionally from the Sound, it probably never establishes itself in permanent full salinity sea water.'

West Muds (*St John's Lake*)

Two visits were made in May, and the area from Inswork Point north-west to the channel edge studied at low tide. The exposed muds are here over 400 m wide, and a profile was not attempted. The greater part of the distance from the shore is occupied by an imperceptibly sloping, shell-covered, soft mud-flat, which probably lies close to Ordnance Datum as shown by Spooner & Moore's figure 7 which is a profile of a nearby area. At an estimated 300 m from shore the shelly flat changes to a noticeable slope of finer and even softer mud, which drops to a level very close to chart datum (tide-table level 0·0 ft, or 2·7 m below O.D., Newlyn). Here, at about the mean low-water mark of spring tides (M.L.W.S.T.), the mud slope terminates in a firm bench or flat strip of hard ground overlain by 2 or 3 in. of soft deposit. The fauna of the upper flat is as described by Spooner & Moore; that of the mud slope is far scantier, including mainly *Arenicola marina* and *Nephthys* sp.; that of the hard flat at M.L.W.S.T. is more abundant, including *Sabella pavonina*, *Nephthys* sp., *Melinna palmata* in large numbers, and *Nereis* (*Eunereis*) *longissima*, fairly abundant. In contrast with the findings of Spooner & Moore, I note the following. Large specimens (> 0·7 g) of *N. diversicolor* occur scatteringly on the outer portions

of the shell-strewn flat, apparently below mid-tide level, as the above authors found on the St John's Lake flats (st. B-3, B-5), but I found none on the softer mud slopes, nor on the hard flat at the channel edge. Spooner & Moore report the largest counts of *N. diversicolor* on this 'bare mud...rather firm' area (their stations D-4 at −2·2 m and C at −1·8 m). Since they do not mention *N. longissima*, which is conspicuously present at this level, there is a possibility of a misidentification, regarding which I can draw no conclusions. They also report *N. diversicolor* present at levels as low as −2·0 m in the secondary channel of St John's Lake. This is fully consistent with observations I have made in the Salcombe Estuary near Kingsbridge—there is no question but that *N. diversicolor* may be quite abundant at low-intertidal levels in steep banks of erosional channels in marine-dominated mud-flats (Fig. 5), but I have not found it present in the depositional slopes of very soft silty mud such as occur along the main channel at West Muds. The question of its occurrence on the firmer flat bench at the foot of such slopes is a problem which should be re-investigated. It should be noted that *N. diversicolor* is also found abundantly at high levels along the shore at Inswork Point, in sand black with organic matter, associated with *Cardium*, *Macoma*, and capitellids. Its occurrence here is possibly correlated with freshwater seepage from the land. Samples of water from this sand had salinities of 23·1–30·2‰, whereas the salinity in the main channel on the same day varied from 32·4‰ at extreme low water to 34·4‰ at mid-tide, and was probably even higher at high tide. The population at this upper level, estimated at about 1·0 m above O.D., contained medium and small individuals; it is probably a breeding population, and may be the source of the scattered large individuals on the outer flats. Spooner & Moore consider that salinities below 30‰ are not encountered at St John's Lake even in winter, except possibly at L.W.M.S.T., and Milne's data are in agreement. Apart from the high-level population, which is in a somewhat special situation, *N. diversicolor* seems to occupy a marginal position on the West Muds and seems to be near its seaward limit.

Salt Mill

The transect, at 7·8 km from the Sound, corresponds closely with that worked by Spooner & Moore, and may be levelled with reference to Admiralty Boundary Stone no. 10, whose mark I have estimated as 2·31 m above O.D. (Newlyn). The essential results of observations made on 17 June and 12 October are set forth in Fig. 2. *N. diversicolor* is found in small numbers (but of large individuals) from −1·3 to +0·8 m relative to O.D. (Newlyn). The population does not extend inshore to the edge of the salting, despite the presence of suitable substrate, and its maximum density lies above mid-tide level. Its zonal position is a little lower than that indicated by Spooner & Moore and, furthermore, seems to have a natural upper limit, not set by the actual edge of the mud-flat. The salinity profile indicates a stable situation with no significant

seasonal shift; interstitial salinities over the area inhabited by *N. diversicolor* vary only from 25 to 29‰. On 12 October, salinities of the rising surface water were taken as the tide covered the flat. These values (Fig. 2) range from 18 to 25‰, and are lower than those of the interstitial water of the underlying mud. They are also lower than the surface salinities obtained by Milne, in 1937, very near this spot. The low values of the present study undoubtedly reflect the greater rainfall in 1954. The fact that interstitial salinities are higher than those of the advancing water suggests that the former are relatively unaffected by the brief contact with surface water of low salinity during tidal rise and fall, and that at high tide the nereid zone receives water of a salinity greater than 25‰.

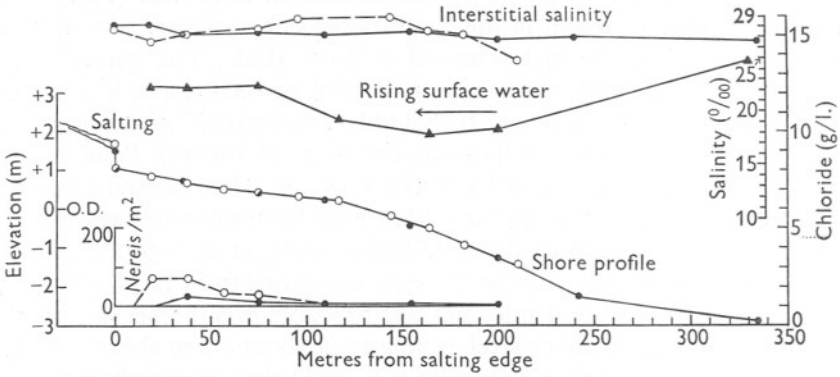

Fig. 2. Salt Mill: upper curves, salinity of interstitial water of mud, below which is a curve (triangles) showing salinity of rising surface water as it covered the flat on 12 October 1954. Middle curve, shore profile. Lower inset, distribution of *N. diversicolor*. Open circles represent values obtained in June; solid black circles, points obtained in October.

Clamoak

This station lies near the upper end of the straight and open part of the estuary, in a region where salinities have started to decline, but where the flats are still quite wide. Saltings are well developed here, 12·5 km from the Sound. *N. diversicolor* is abundant, especially at the upper or inshore levels of the flat and in muddy channels in the salting. It is found from a height of about 0·5 m above O.D. to the foot of the salting at about 0·9 m, and in the channels of the latter to about 1·25 m. No quantitative samples were taken. The salinity profile (Fig. 3A) taken in June showed the salinity across the flat to be as high as that at Salt Mill, except for a pronounced drop near low-water mark. This drop in interstitial salinity coincides with a sharper slope of the outer edge of the mud-flat, suggesting current-scouring of this zone by water of low salinity at low tide. The interstitial salinity of 10 December shows a profound drop to salinities of 5·8‰ inshore and rising to 10·9‰ near L.W.M. This reversal of slope of the salinity profile strongly suggests a salinity stratification such that

the higher levels of the intertidal muds are exposed to much lower salinities than the muds near L.W.M. The possibility of freshwater drainage from the adjacent land may well add to a general lowering of salinity near the banks; on 10 December, following prolonged rains, the flat at Clamoak seemed much wetter than usual at low tide, and one had the impression that it was receiving much freshwater drainage from the near-by saltings. A check of the sheet of surface water showed salinities of less than 3‰, while the river water at low tide was as low as 0·4‰. But from the interstitial salinities it is apparent that ingression of salt water even at this period had provided the *Nereis* zone with salinities at least as high as 10‰ at the lower levels and not less than 6–7‰ at the higher levels. In contrast to the lower estuary, this section of the Tamar is clearly one of great seasonal as well as tidal salinity changes, and the salinity profiles of the interstitial water of the exposed shore seem to provide a reasonable picture of the extent of the changes that occurred between June and December 1954.

South Hooe

A station was selected just around the first major bend of the river, 14·8 km from the mouth. The mud-flat is here distinctly narrower than the preceding; the interstitial salinity profile taken in June is lower and drops more sharply toward L.W.M. (Fig. 3B). The December salinity profile shows both a lowering and a reversal of slope quite comparable to and more exaggerated than that observed at Clamoak. *N. diversicolor* is abundant and occupies a wide band vertically from −1·65 m to +1·18 m, O.D. (Newlyn). No population counts were made.

North Hooe

This spot lies 3 km upstream of South Hooe, around the second major bend of the river. At North Hooe the salinity profiles of 6 June and 10 December are still lower, and the seasonal difference is very great, amounting to about 18‰ difference between summer and winter values for the main nereid zone (Fig. 3C). Salinities near the low-water mark are considerably more stable, although not very high: 9–11‰ in summer and 3·5–4·5‰ in winter flood. A count of *N. diversicolor* had been made in May, 400 m downstream, where the mud-flat is somewhat wider. The nereid population was most dense in the upper levels, exceeding 800 per m^2 even before reaching the highest level where the burrows were more numerous than in the spots sampled. A few small *Nephthys* sp. were taken at this point, and dead shells of *Scrobicularia* were seen at North Hooe. Spooner & Moore reported the highest density of *N. diversicolor* at the latter point, where their survey ended.

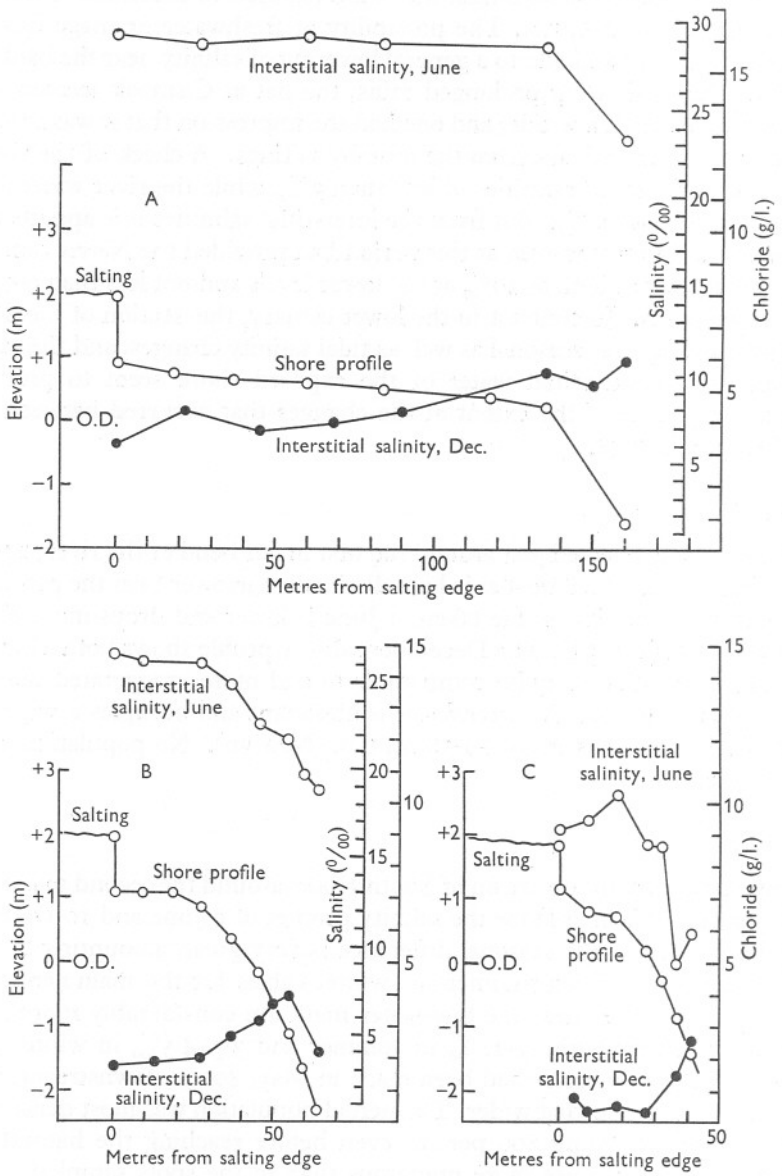

Fig. 3. Shore profiles (June) and interstitial salinity profiles for June (open circles) and December (solid circles). A, Clamoak; B, South Hooe; C, North Hooe.

Cotehele Quay

At this point, 20·5 km from the mouth of the estuary, the river and the bordering mud-banks have become distinctly narrow, and reeds (*Phragmites*) have supplanted typical saltings vegetation. On the stone quay grows a broad band of *Fucus vesiculosus* (det. Dr Elsie Burrows), extending vertically from 1·02 m above O.D. down to 0·52 m below O.D. (Newlyn). In terms of Chart Datum (Devonport) this is from +12·26 ft. to +7·22 ft. tidal height. Percival listed North Hooe as the upstream limit of *Fucus*; this may indicate that the species has spread 2·5 km upstream since 1929, for the growth is extensive enough to have attracted attention had it been growing at Cotehele Quay at that time. Below the *Fucus* is a rich growth of the hydroid *Cordylophora*, typical of oligohaline waters. *N. diversicolor* occurs in considerable density although the available habitat is of small area. Observations were made on a small flat protected from erosion by stone quays (Fig. 4A). The lower part of the steeply sloped mud-bank is poorly consolidated and much gullied. The maximum density of nereids (*c.* 600 per m^2) occurred at the edge of the land vegetation at 1·3 m above O.D., and the population was reduced to scattered individuals at about O.D. level. The population seemed healthy and, judging by the number of small worms present in June, had been breeding. Salinities in the mud were low, varying from 2·6‰ in June to about 0·3‰ in November after a period of flood.

At this time an observation was made which suggested that salinity stratification in these upper reaches of the estuary may be an intermittent phenomenon. Salinity samples were taken at the surface as the tide fell past the zone of *Fucus* (Fig. 4B). A salinity of 0·2‰ was found at high water, when the river lay smoothly, with a gentle downstream drift apparent on the surface. As the tide fell the current gathered strength, a counter-current developed along the quay face where grew the *Fucus*, and the salinity rose to 1·0‰ by 2 h past high tide, by which time the river presented a strong downstream sweep of current. Surface salinity then dropped again until at 3 h past high tide it had reached 0·11‰. The only explanation I can offer is that at high water there is salinity stratification, and that as the ebb develops frictional turbulence is set up, bringing water of higher salinity to the surface. This provides a maximum surface salinity at about the middle of the *Fucus* zone. Later in the ebb the saline water has moved downstream, and the salinity drops sharply. A study of the stability of salinity stratification near Cotehele Quay might prove of interest in connexion with the algal zonation.

At this point, although salinities are generally very low, the tidal and seasonal salinity variation is also nearly minimal. It should be noted that the nereid population at Cotehele Quay, although far denser than any observed in Finnish waters, is living at a salinity of less than half that which characterizes the limit of the species in the Baltic Sea. In the Tamar, as on the south

Finnish coast, both *N. diversicolor* and attached *Fucus vesiculosus* stop together at about the same summer salinity, although this value is much lower in the Tamar than in the Baltic.

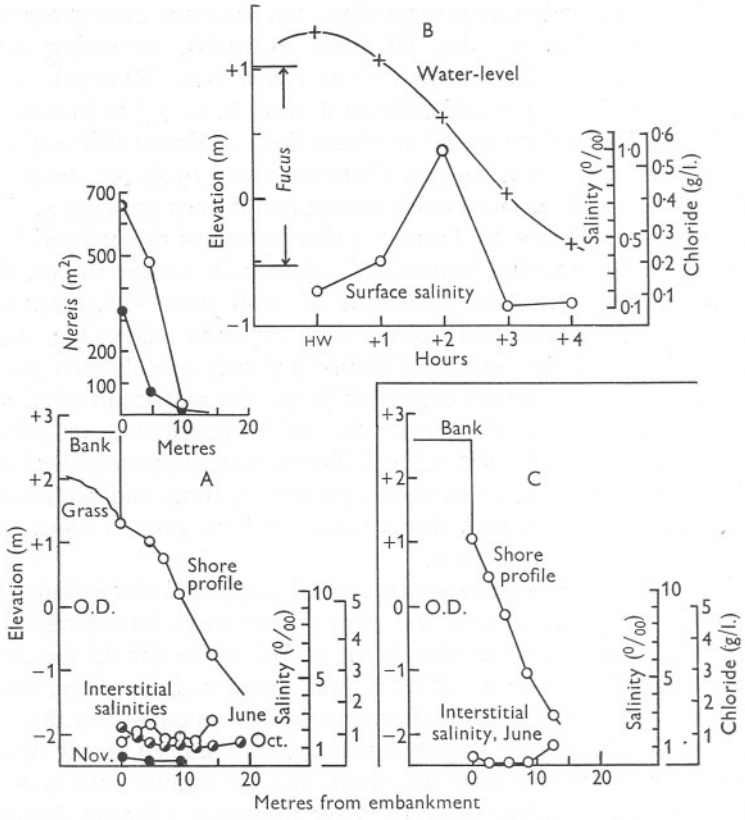

Fig. 4. A, Cotehele Quay: (lower) shore profile and interstitial salinities; (upper) distribution of *N. diversicolor*. B, Cotehele Quay: zonation of *Fucus vesiculosus*. Surface salinities (open circles) during falling tide (height shown by crosses) on 4 November 1954. C, Calstock: shore profile and interstitial salinities, June. Note doubling of horizontal scale in A and C compared with previous figs.

Calstock

Here, 22 km from Plymouth Sound, a few scattered but healthy individuals of *N. diversicolor* were collected on the upper mud slopes just below the railway bridge, always in spots where the mud was of somewhat firmer consistency. The worms were restricted to a narrow vertical band, from O.D. to about 0·5 m higher (Fig. 4C). Associated with them were very abundant tubificid worms, dolichopodid dipteran larvae, and leeches (unidentified) found buried in the mud. Salinities in the mud of the nereid zone were almost nil (less than

0·5‰) but increased to 1·1‰ near low-water mark where the mud was so disturbed as to be apparently devoid of fauna. Percival reported the up-stream limit of *N. diversicolor* as half a mile above Calstock. Careful search at Okeltor, 1 mile upstream, failed to reveal it in a suitable substrate. No other animals or plants of marine affinity were seen at Calstock.

Discussion

The interstitial salinity profiles in Figs. 2–4c show a clear pattern of salinity variation in the River Tamar. If one compares the spring salinities, which were obtained under fairly 'normal' conditions, with those obtained in autumn, three major sections of the estuary can be recognized. (1) A lower estuary, which may be called 'marine dominated', is characterized by wide flats, relatively high salinities, and interstitial salinities little decreased with elevation intertidally and little affected by periods of freshwater flooding. (2) A middle region is characterized by intermediate salinities, with marked variation in salinity with intertidal level, and with a marked lowering of interstitial salinity and a reversal of slope of the interstitial salinity profile at times of freshwater flooding. It may be that excess freshwater discharge is strongly stratified, so that the upper intertidal is more particularly affected; possibly the 'salt-water piston' of the summer is transformed to a deep-lying wedge of lesser volume by the sheer mass of fresh water flowing down above it; perhaps lateral freshwater intrusion from the banks at low tide plays a part. The down-stream limit of this suggested middle estuarine region is perhaps below Clamoak, a point which in summer would seem to be much like the lower estuary but which in winter flood is clearly comparable to points up-stream. The up-stream limit must lie above North Hooe. (3) The upper reaches, characterized by virtual absence of 'flats', show low salinities at all seasons, and little change of salinity either with intertidal level or with season. The down-stream limit in the Tamar is near or below Cotehele Quay. However, these limits cannot be sharp, and it might be best to regard them as entirely labile. If one could imagine a year of low enough rainfall, salinities characteristic of the lower estuary would extend to Calstock, in what we now regard as the upper reaches. Indeed, if we turn not far away, to the Kingsbridge Estuary, we can see this possibility realized.

The freshwater inflow into the Kingsbridge Estuary is so slight, relative to the volume of the estuary bed, that the fauna near Kingsbridge, almost at the very head, is strikingly comparable to that of St John's Lake. A series of observations were made on the flats 1 mile below Kingsbridge (Fig. 5) at a point where the main mud flat is separated from the stony beach by a secondary channel. Because of failure to locate a Bench Mark, the levels shown in Fig. 5 were estimated from the predicted heights of high and low water on 10 October 1954 as corrected for Kingsbridge. This method is particularly subject to error

at a point so removed from the sea, and the results may be inaccurate to the extent of perhaps ±0·3 m. The general level of the mud-flat is estimated as close to O.D. (Newlyn) and is thus approximately the same as the flat of St John's Lake as described by Spooner & Moore (1940). *N. diversicolor* was found to be extremely abundant low in the relatively firm erosional slopes of the secondary channel, a position comparable to its occurrence in St John's Lake as reported by the latter authors. In agreement with my findings at West Muds, *N. diversicolor* is also present on the higher shores, where smaller

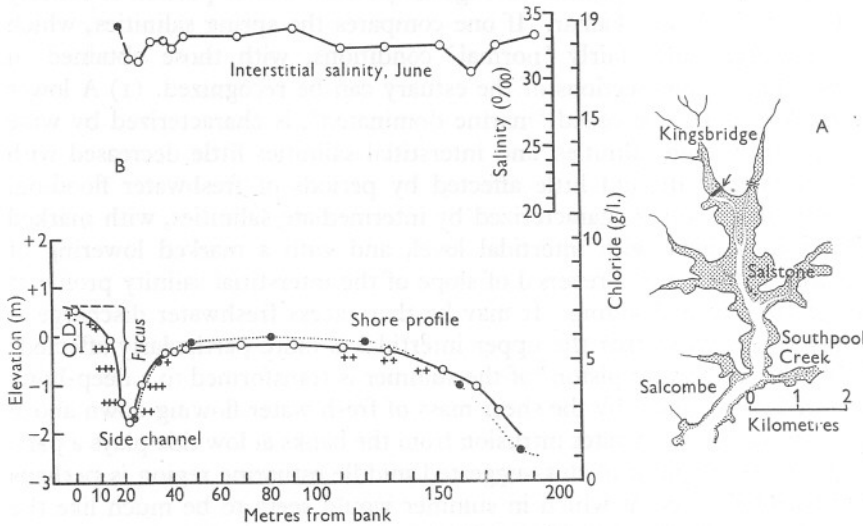

Fig. 5. A, Sketch map of the Kingsbridge (Salcombe) Estuary to show collecting station and points mentioned in text. Stippled areas represent intertidal exposed between H.W.M. and L.W.M. of ordinary tides. B, at top, interstitial salinity profile (June) across flats below Kingsbridge. One point (solid circle) taken in October. At bottom, shore profiles: open circles, June; closed circles, October. Vertical height of *Fucus* zone shown by bracket; estimated relative abundance of *N. diversicolor* shown by groups of crosses. Vertical bar by O.D. represents a possible estimated error in level of ±0·3 m.

individuals are numerous in muddy sand under rocks up to the upper limit of *Fucus* (+0·6 m), and also in pockets of soft mud held in the remains of hulks on the shore. Along the main channel *N. diversicolor* seemed common on the edge of the flat, but was absent from the soft outer slope and from the firmer bench at L.W.M., quite as at West Muds. The salinity profile (June) is higher than that for Salt Mill, but was not studied after the November rains. There is some indication of a drop in interstitial salinity at the edges of the main flat and in the banks of the secondary channel, which is consistent with the distribution of the estuarine *N. diversicolor*. In general, the salinities at this station were high; the lowest observed was no less than 28‰, obtained in the water of the main channel at low tide in June, and a similar value in the

secondary channel in October, both at times of fair weather. However, the nearness of the site to the head of the estuary makes it likely that occasional periods of heavy freshwater run-off might effect a greater lowering of salinity than my brief studies encountered.

Lower in the estuary, near the Salstone, *N. diversicolor* is also present, but scarce. A few large individuals were taken in the muddy banks of channels behind the Salstone reef; their position was roughly mid-tidal, comparable to the position observed at West Muds. The species, where found, was associated with the polychaetes *Arenicola*, *Melinna*, *Nephthys*, and capitellids. It was absent from the lower levels inhabited by *Sabella*, *Lanice* and *Eunereis longissima*, and from the very soft mud occupied by *Myxicola*. It is evident that the Kingsbridge Estuary as a whole is, in Rochford's terminology, 'marine-dominated', and only near its head is *N. diversicolor* abundant. Existing reports suggest that this species is scarce in the Kingsbridge (Salcombe) Estuary; Marine Biological Association (1931), following Allen & Todd (1900) reports it numerous only in Southpool Lake, 'in a small gully traversed by a stream of fresh water'. In view of these earlier reports, the abundance of *N. diversicolor* near Kingsbridge deserves mention. As is well known, the marine-dominated Kingsbridge Estuary stands in marked contrast to the Tamar Estuary, which for the greater part of its length is 'gradient-dominated', and characterized by large salinity variations, both spatial and seasonal.

As for the use of interstitial salinities of intertidal mud as indicators of the salinity characteristics of an estuary, it is felt that the results give a picture of what is happening even though the quantitative relationship between salinity in the substratum and the variable salinity of the overlying water is still not understood. The method may be of value to the individual field worker who must sample several localities on the same day, especially if he must travel by land. Certainly less labile than salinities of tidal water, the interstitial salinities are still labile enough to give some picture of changes within past weeks, although how responsive these interstitial salinities actually are, or how stable, remains to be determined. Possibly one could use the surface layer of mud as an indicator of recent changes in salinity, and deeper layers as indicative of general conditions over a longer period. Mud-dwelling animals, by the irrigation of their numerous burrows, may profoundly influence the speed of adjustment of interstitial salinities to variations in the environmental salinity.

Muds represent extremely complex physico-chemical systems (Baver, 1948), of which the detailed composition doubtless varies a great deal within the bounds of a single estuary, so that generalizations as to their water-holding properties should be made with caution. As pointed out by Bourcart & Francis-Boeuf (1942) in their useful discussion of the properties of mud, the chloride values of re-wetted dried samples of marine muds may differ from those based upon the natural water content. These authors make the statement

(p. 27) that the chloride of interstitial water of marine sediments is always higher than that of the overlying water, and suggest that this may be attributable to desiccation during periods of low tide. While this may be a factor in certain estuaries in regions of low rainfall, the intertidal flats of the Tamar would seem as likely to experience lowering of salinity by dilution with rainwater as concentration by evaporation. In situations where, as at Salt Mill, interstitial salinities higher than that of the overlying water are observed, I am inclined to regard this as the result of failure to measure salinity in the lower strata of water at flood tide. In any estuary where salinity stratification is marked, surface salinities will be expected to be lower than interstitial salinities over all but the highest intertidal shores. The presence of a given interstitial salinity at any spot on an intertidal mud slope probably indicates that the spot is bathed by water of at least that salinity at high tide. In certain situations, as described for Kames Bay (Smith, 1955*a*), subterranean intrusion of fresh water may produce local lowering of interstitial salinity, but no instance of this has been seen in the Plymouth area. Instances of intertidal muds retaining a high salinity beneath freshwater streams at low tide are too well known to be cited specifically; they are in no way inconsistent with the general view that the retained interstitial salinities in intertidal muds reflect in general the higher salinities of the tidal water which reaches them.

The chief objective of the present study, to characterize the salinity and the pattern of salinity variation of the habitat of *N. diversicolor* in a typical estuary, requires recognition of the region of greatest abundance of the species. This aspect of the problem has proved beyond the author's capabilities in the field studies described, but fortunately the careful quantitative work of Spooner & Moore (1940) is available and has undoubtedly been applicable in 1954. In Table II their data on the distribution of *N. diversicolor* are summarized, the values being based only upon those stations where the species was actually taken.

TABLE II. DATA OF SPOONER & MOORE (1940)

Location	Range of densities in worms per m²	Mean of densities recorded per m²
St John's Lake	2–56	20
Thanckes Lake	1–350	130
Salt Mill and Ernesettle	4–770	186
Cargreen-Weirquay	8–590	251
Clifton-South Hooe	> 22– > 1020	> 786
North Hooe	400–3030	1715

The data of the present study, treated in the same way, are given in Table III. The total data agree in indicating a maximum abundance somewhere in the region of North Hooe, that is to say, in that part of the river where salinity variation is the greatest. One point of caution should be noted: no data on weight of worms per unit of area are available, and it is notable that where

N. diversicolor is at a high density in the Tamar, the population is of small individuals, rarely exceeding 0·15–0·20 g. On the other hand, the scattered worms at Salt Mill and St John's Lake are mostly of very large size. Dales (1951) has pointed out that a population of *N. diversicolor* may have a low percentage of males, not over 10%, and that these must be sought out by the females if reproduction is to be accomplished. Females ordinarily spawn and die at an age of 1 year or less. However, those females which fail to find a male may survive for 18 months or perhaps 2 years, and grow to very large size.

TABLE III. DATA OF THE PRESENT STUDY

Location	Range of densities in worms per m²	Mean of densities recorded per m²
Salt Mill	3–70	28
Clamoak	(Abundant)	—
South Hooe	(Very abundant)	—
North Hooe	45–>800	436
Cotehele Quay	5–660	204
Calstock	(Occasional)	—

The occurrence of scattered large worms may thus be indicative of a non-reproductive population, perhaps rendered so by such wide spacing that encounters between the sexes are unlikely. On this assumption, the large worms at St John's Lake and Salt Mill would not, despite the high weight per unit area they represent, be considered as anything but a marginal population. It must be added, however, that large worms may occur in quite dense populations in mud, as at Millbrook Lake and near Kingsbridge, and the problem requires clarification. In respect to the River Tamar population of *N. diversicolor*, the optimal region seems to lie up-stream from the region reached by the main mass of tidal sea water, and to centre in the region of maximum salinity variation.

In summing up the series of studies, of which this article is the third, on the distribution of *N. diversicolor* in relation to salinity, it is well to point out that no simple relationship has been detected. We have seen (Smith, 1955*a*) that the apparent zonation of the species on the sandy beach at Kames Bay, Millport, is correlated with a zone of lowered interstitial salinity. This may in part be the result of abundance of *N. diversicolor* in areas where less interspecific competition is encountered. A clearer example of this sort of limitation is seen in the Isefjord (Smith, 1955*b*), where the apparent restriction of *N. diversicolor* to zones of very low salinity is the result of its exclusion from a large part of its former range as a result of competition and/or predation by another species of nereid. In the Tamar, *N. diversicolor* is most abundant in areas which other polychaetes are unable to utilize, but it is also abundant in the more saline head of the Kingsbridge Estuary where competition by other, more marine, species must be severe. Interspecific competition is perhaps not a factor of over-riding importance in limiting the distribution of *N. diversicolor*

in the Plymouth region, although it may be so in certain situations elsewhere.

Tidal factors *per se* are also difficult to evaluate, but are probably of secondary or indirect importance. *N. diversicolor* is characteristically an upper mid-tidal form in the Tamar, but can occupy lower positions, as it does in the banks of secondary channels in St John's Lake and near Kingsbridge. Here we find a slightly firmer substrate, as well as somewhat lower salinities, than in adjacent higher areas. Over most of its range in the Tamar, *N. diversicolor* is characteristic of the 'flats', which, as noted by previous workers, show a relatively greater area of intertidal mud above mid-tide level as one goes upstream (Hartley & Spooner, 1938). The level of the saltings which border the upper Tamar at many points is said to be quite constant. The pertinent data on heights of mud-flats and saltings obtained in the present survey are given in Table IV.

TABLE IV. HEIGHTS IN METRES ABOVE O.D., NEWLYN

Location	Top of salting	Highest level of mud	General level of 'flat'
Calstock	(Embanked)	1·04	0·1 to 0·5
Cotehele Quay	(Embanked)	1·30	0·7 to 1·3
North Hooe	1·83 to 1·88	1·15	0·7 to 1·0
South Hooe	1·85 to 1·90	1·18	1·13 to 1·15
Clamoak	1·93 to 1·96	0·90	0·40 to 0·75
Salt Mill	1·50 to 1·70	1·05	0·25 to 0·8
St John's Lake (from Spooner & Moore)			−0·2 to +1·0

These slight figures suggest a constant level of about 1·8–2 m above O.D. for the tops of saltings (the lower salting at Salt Mill may be eroding away). Also fairly constant is the highest point of the mud (i.e. the foot of the salting cliff) at about 1 m above O.D. The general level of the main flat is not precisely determined, but shows an increase in level from the lower estuary up to the first great bend in the river at South Hooe. Above this point, with the narrowing and steepening of the intertidal mud, the 'flats' themselves seem to become more inclined, and the outer edge lower, possibly as the result of current scouring.

At Cotehele Quay and up-stream, flats are almost indefinable, and since at both Cotehele and Calstock the measurements are at spots disturbed by man-made embankments, no conclusions should be drawn. It would seem that the somewhat lower intertidal position of *N. diversicolor* in the lower reaches of the Tamar Estuary reflects the general level of the suitable substrates. This situation may provide the incidental advantage that in the upper reaches of the estuary *N. diversicolor* is placed above the very low salinities of the ebb-tide, and in the marine-dominated lower reaches is exposed to favourably brackish water at low tide. It should be noted that if salinity stratification occurs in the middle or upper reaches of an estuary, as seems to happen in the Tamar, the

upper intertidal and the lower intertidal zones are exposed longer to surface waters of low salinity than the mid-tidal levels, over which the rising or falling surface water passes most rapidly. That exposure at low tide is of direct advantage to *N. diversicolor* would seem unlikely except as it may reduce competition from less exposure-resistant forms. Submergence *per se* has not been shown to be deleterious to *N. diversicolor*, and this species must live permanently submerged in its habitat in the Baltic Sea. Only by considering the several factors of substratum, intertidal position, pattern and degree of daily and seasonal salinity variation, temperature, interspecific competition, and so forth, can the relation of a species to any one factor such as salinity be fully appreciated. The further problem of whether populations of a species in different parts of a wide geographical range are physiologically distinct in their responses to the factor in question should be viewed against the background of the ecology of the species. Certain information on the comparative physiology of *N. diversicolor* in response to low salinity will be presented in a later paper (Smith, 1955*c*).

This study was made possible by a Fulbright grant to the writer as an Exchange Lecturer in Zoology at the University of Glasgow. My work at the Laboratory of the Marine Biological Association, Plymouth, was greatly aided and made pleasant by innumerable kind and helpful acts by the staff, scientific, technical, and administrative, of the Laboratory. Since I cannot thank them individually, I wish to express to the Director, Dr F. S. Russell, my appreciation of what he and his staff have done for me. I am also grateful to my wife for the thankless task of assisting in the surveying of mud-flat levels.

Summary

The estuarine habitat of *Nereis diversicolor* in the River Tamar has been surveyed, and the distribution of this worm related to salinities in a 'normal' spring and a rainy autumn.

The method of evaluating the salinity at given points within an estuary by means of 'salinity profiles' of the interstitial water of the intertidal mud is put forward, and the results of the method shown to be informative and consistent with the results of previously used methods.

N. diversicolor is found to reach its maximum population density in that portion of the Tamar Estuary where the greatest salinity variation, both seasonally and with intertidal height, is the rule. At its up-stream limit it regularly endures salinities of less than 0·5‰.

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ON A NEW SCYPHOMEDUSA, *PARAPHYLLINA RANSONI* N.SP.

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(Plates I and II, and Text-figs. 1–3)

Maas (1903) described two specimens of a deep-water coronate scyphomedusa collected on the Siboga Expedition in the Malay Archipelago. He erected for these a new genus *Paraphyllina* and called the species *P. intermedia*. It differed from *Periphylla* in that the rhopalia were perradial and not interradial. In 1903, Lo Bianco figured a specimen taken in the Mediterranean near Capri which was identified for him by Maas as *P. dodecabostrycha* (see also Maas, 1904, p. 48, footnote); Mayer (1910) saw this specimen and re-described and figured it as *Paraphyllina intermedia*. A fourth specimen ascribed to this species by Ranson (1936) was found washed up on the shore at Villefranche.

All these specimens were the same in their essential characters, but differed in some details, especially in the form of the gonad and in the coloration.

In a deep-water haul with the 2 m ring trawl made by R.V. *Sarsia* on 28 April 1955 with 500 fathoms of wire out off the mouth of the English Channel in 48° 26′ N., 9° 42′ W. some four dozen scyphomedusae were caught which belonged to the genus *Paraphyllina*. Most of the specimens, which ranged from 11 to 35 mm in diameter, were in an excellent state of preservation.

These specimens agreed most closely with the medusa described by Ranson, and at first I was inclined to regard them as *P. intermedia*. Fortunately Dr P. L. Kramp suggested to me that the two original specimens described by Maas might still be in existence. On inquiry they were found to be in the Zoölogisch Museum at Amsterdam, and Professor H. Engel most generously sent these two type specimens to me to see.

Examination showed at once the adequacy of Maas' description, and that my specimens were not *P. intermedia*, and must therefore be regarded as a new species. As they are undoubtedly the same as the medusa described by Ranson (see footnote on p. 110), I have great pleasure in naming this species *Paraphyllina ransoni* after Gilbert Ranson, whose researches have added much to our knowledge of medusae.

Paraphyllina ransoni n.sp.

Umbrella with hemispherical dome-shaped summit, with deep coronal groove situated somewhat nearer to the umbrella margin than to the summit,

with fairly thick jelly, and with rectangular pedalial thickenings separated by deep furrows where the sixteen septal attachments of the upper and lower sides of the umbrella run. Rhopalial pedalia slightly narrower than the tentacular pedalia. Well-developed, continuous, coronal muscle band on subumbrella surface. Sixteen lappets with evenly rounded margins; the lappets on either side of the rhopalia are each a little more than two-thirds the width of those of the intertentacular pairs, and somewhat shorter on their rhopalial margins. Twelve solid marginal tentacles, eight adradial and four interradial; each about the length of the radius of the umbrella. Four perradial rhopalia, each with hood, statocyst and pigmented bulb, but no ocellus with lens. Eight adradial flattened elongated gonads, each forming an asymmetrical W whose interradially situated arms are coiled inwards and whose adradial arms bend outwards towards the perradii.

Stomach wall attached interradially to umbrella dome over four flattened triangular septal plates giving rise to four deep triangular pockets externally. Eight rows, or phacellae, each of about twenty or more simple gastric filaments, one along each side of the triangular septal plates. Four perradial horizontal entrances from stomach cavity into gastrovascular sinus, which is divided by the sixteen septa; each septum running from just above coronal muscle band to half the length of the marginal lappet, the rhopalial septa being slightly the longer. Manubrium slightly folded and reaching about to bases of marginal tentacles with four perradial thickenings and very slightly crenulated margin. Whole subumbrella and marginal tentacles uniformly coloured deep chocolate red,[1] darkest in stomach but reaching right to umbrella margin; gonads colourless. Size up to 35 mm in diameter (25 mm at coronal groove).

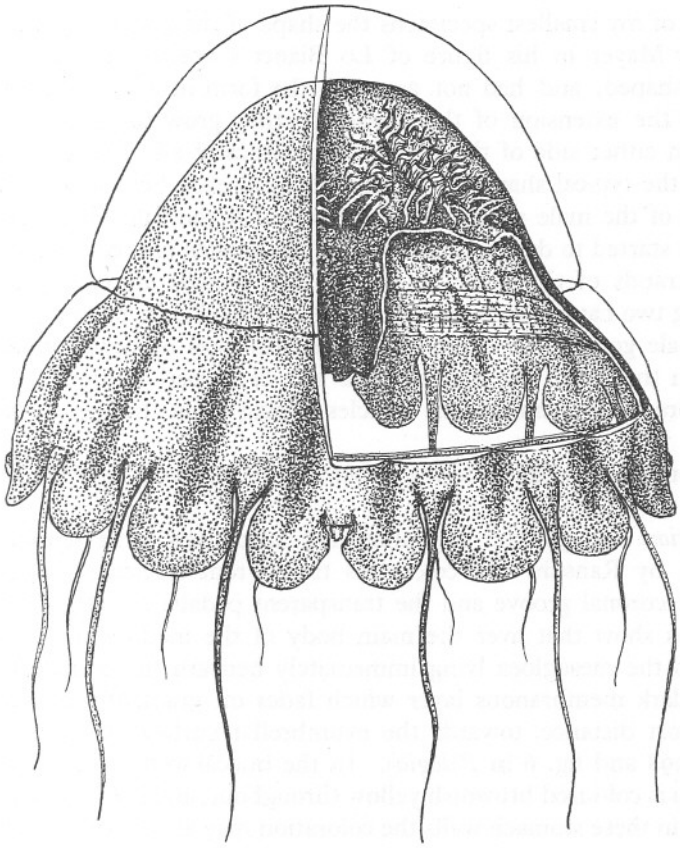
Further Details

Stomach. Each of the gastric filaments, which number about 160 or more in all, arises singly; very occasionally two may arise from one root. There are sometimes two or three terminal filaments at the apex of each triangular septal plate, and occasionally an isolated tuft of filaments on the stomach ceiling, presumably left behind during the downward movement of the areas of attachment as the umbrella grows.

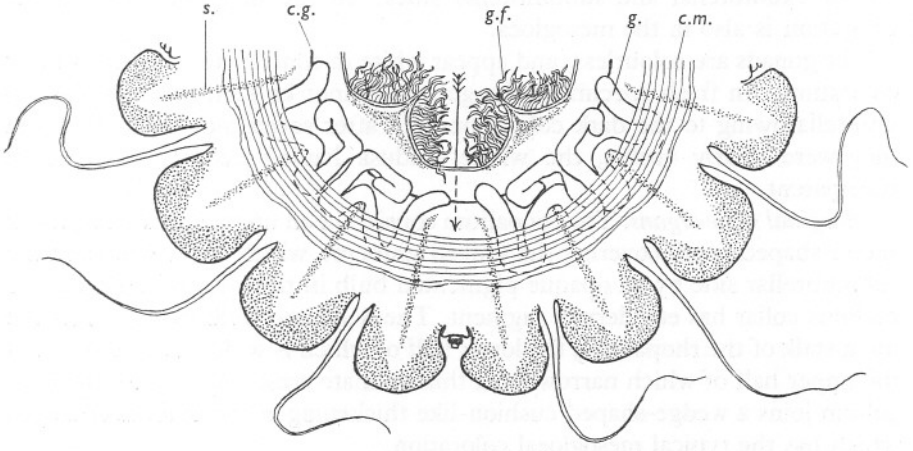
Gonads. The W-shaped form of the gonads is very characteristic. Ranson's description of the gonads in his specimen agrees well with mine except that he said that each divides into three branches interradially. From Ranson's drawing the gonads have the appearance of being very fully developed.

In some of my specimens with most fully developed gonads the interradial areas of the W may recoil upon themselves and overlie or even fuse with their neighbours, so that their true outlines are not very clear, or they may be abnormal in shape.

[1] Specimens preserved in formalin and sea water.



Text-fig. 1. *Paraphyllina ransoni*. Adult medusa with a section of the umbrella and a portion of the manubrium cut away to show the subumbrella and internal anatomy.



Text-fig. 2. *P. ransoni*. Semi-diagrammatic view of subumbrella with buccal walls of stomach cut away. The stippled areas indicate regions of fusion of upper and lower endoderm surfaces. The arrow shows a perradial entrance to the gastro-vascular sinus. *c.g.*, coronal groove; *c.m.*, coronal muscle; *g.*, gonad; *g.f.*, gastric filaments; *s.*, septum.

In one of my smallest specimens the shape of the gonads approached that shown by Mayer in his figure of Lo Bianco's specimen. They were still crescent-shaped, and had not assumed the form of a W which is brought about by the extension of the gonads as they grow towards the umbrella margin on either side of the septa. In practically all specimens, even the smallest, the typical shape of the gonad had already been assumed.

In one of the male specimens it appears that the fully developed gonads may have started to degenerate. Four of the gonads are reduced to crescent-shaped strands of tissue (Pl. II, fig. 2). Two gonads are complete, but the remaining two have been reduced to small pieces (Pl. II, fig. 1).

In female gonads the ova are in different stages of development (Pl. II, fig. 4); in the males the spermatozoa are developed in elongated, oval or slightly branched seminiferous follicles arising from the gonad wall (Text-fig. 3*d*).

The gonads are attached to the upper wall of the gastrovascular sinus along one edge.

Coloration. In general coloration my specimens were similar to that described by Ranson. The colourless transparent thickened dome of jelly above the coronal groove and the transparent pedalia are striking features.

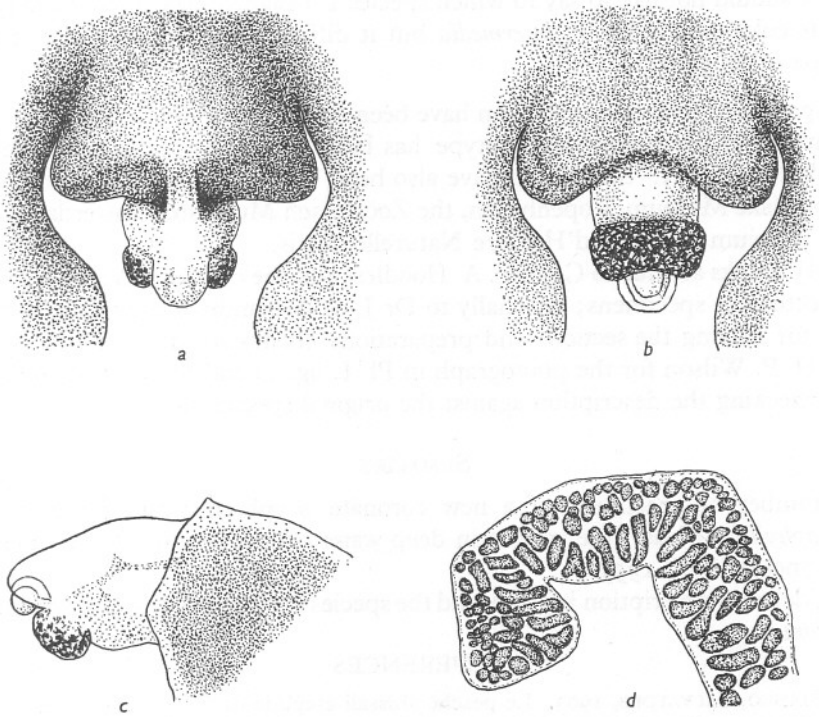
Sections show that over the main body of the medusa the coloration is limited to the mesogloea lying immediately beneath the endoderm; here it forms a dark membranous layer which fades off gradually, but usually for only a short distance, towards the exumbrellar surface (cf. Fox & Millott, 1954, p. 398 and fig. 6 in *Pelagia*). In the buccal walls of the stomach the mesogloea is coloured brownish yellow throughout, and is especially dark near the base; in these stomach walls the coloration may also be dense just beneath the outer ectodermal epithelium. The mesogloea of the marginal lappets is coloured throughout, and the thin dark zone is to be found here both on the exumbrellar and subumbrellar sides. In the marginal tentacles the coloration is also in the mesogloea.

The gonads are colourless, and appear white in contrast to the surrounding coloration. In fresh specimens the gonads cannot be seen through the exumbrella owing to the dark coloration; but after seven months in formalin they were clearly visible, the whole medusa having become much more transparent.

Marginal sense organs. The rhopalium consists of an upper short transparent spoon-shaped hood, covering the stalked statocyst, which is enveloped on the subumbrellar side by an opaque pigmented bulb in the form of a collar; this bulbous collar has ectodermal pigment. The hood and bulb fuse to form the main stalk of the rhopalium, the lower half of which is wide and rounded and the upper half of which narrows to a thin carinate crest. At its base the rhopalium joins a wedge-shaped cushion-like thickening of the umbrella margin, which has the typical mesogloeal coloration.

Text-figs. 3*a–c* show the general structure of the rhopalium, and a photograph of a radial section is given in Pl. II, fig. 3. It is to be noted that the pigment of the bulb is definitely on the outside in the ectoderm, and there is no ocellus with lens such as Maas described in *P. intermedia*.

Umbrella margin. In a few of my specimens the edges of the marginal lappets are undamaged, but in most they are somewhat frayed (see Pl. I, fig. 1).



Text-fig. 3. *P. ransoni*. *a*, *b*, *c*: exumbrellar, subumbrellar, and lateral views respectively of rhopalium (*a*, *b*, reflected light; *c*, transmitted light); *d*, portion of male gonad.

Above the bases of the marginal tentacles and rhopalia there are six to eight, or more, radially directed dark stripes, typical of many coronate medusae. Sections show that these are thickenings of the mesogloea lying under the coronal muscle, which probably play a part by their elasticity in the contraction and expansion of the umbrella margin. They are clearly shown in Pl. I, fig. 2.

Size. The approximate total diameters of the medusae lying free in the formalin and sea water were as follows:

Diameter (mm)	11–14	15–19	20–24	25–29	30	35
No. of specimens	3	13	14	8	2	2

The differences between *P. intermedia* and *P. ransoni* may be summarized as follows:

	P. intermedia	*P. ransoni*
Colour	Limited to part of stomach	Over whole medusa
Gonads	Bean-shaped: above muscle ring	W-shaped: extending under muscle ring
Rhopalium	With ocellus with lens	Without ocellus with lens

It is now known[1] that the medusa described by Ranson is *P. ransoni*, but I should not like to say to which species Lo Bianco's specimen belonged; in its coloration it fits *P. intermedia* but it differs from *P. intermedia* in the shape of its gonad.

Specimens from this collection have been deposited in the British Museum (Natural History) and the holotype has been given the registered number B.M. 1955.11.2.1. Specimens have also been deposited in the Universitetets Zoologiske Museum, Copenhagen, the Zoölogisch Museum, Amsterdam, and the Muséum National d'Histoire Naturelle, Paris.

My thanks are due to Capt. C. A. Hoodless and the crew of R.V. *Sarsia* who collected the specimens; especially to Dr J. S. Alexandrowicz for much help and for making the sections and preparations illustrated in Pls. I and II; to Dr D. P. Wilson for the photograph in Pl. I, fig. 1; and to Dr P. L. Kramp for checking the description against the original specimens.

Summary

A number of specimens of a new coronate scyphomedusa of the genus *Paraphyllina* Maas were caught in deep water off the mouth of the English Channel in April 1955.

A detailed description is given, and the species has been named *Paraphyllina ransoni* n.sp.

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[1] Dr Ranson has confirmed this identity after seeing two of my specimens.

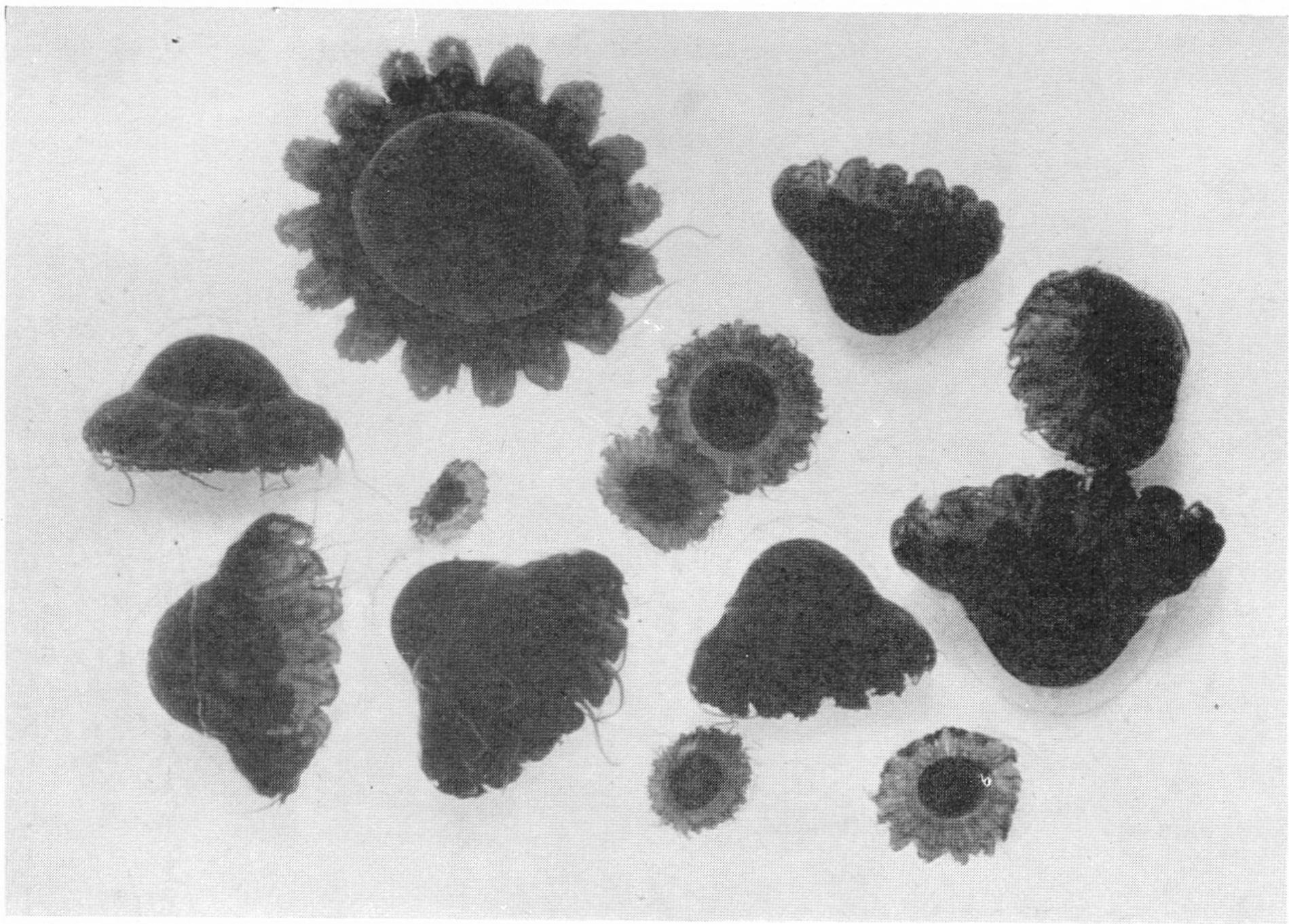

Fig. 1

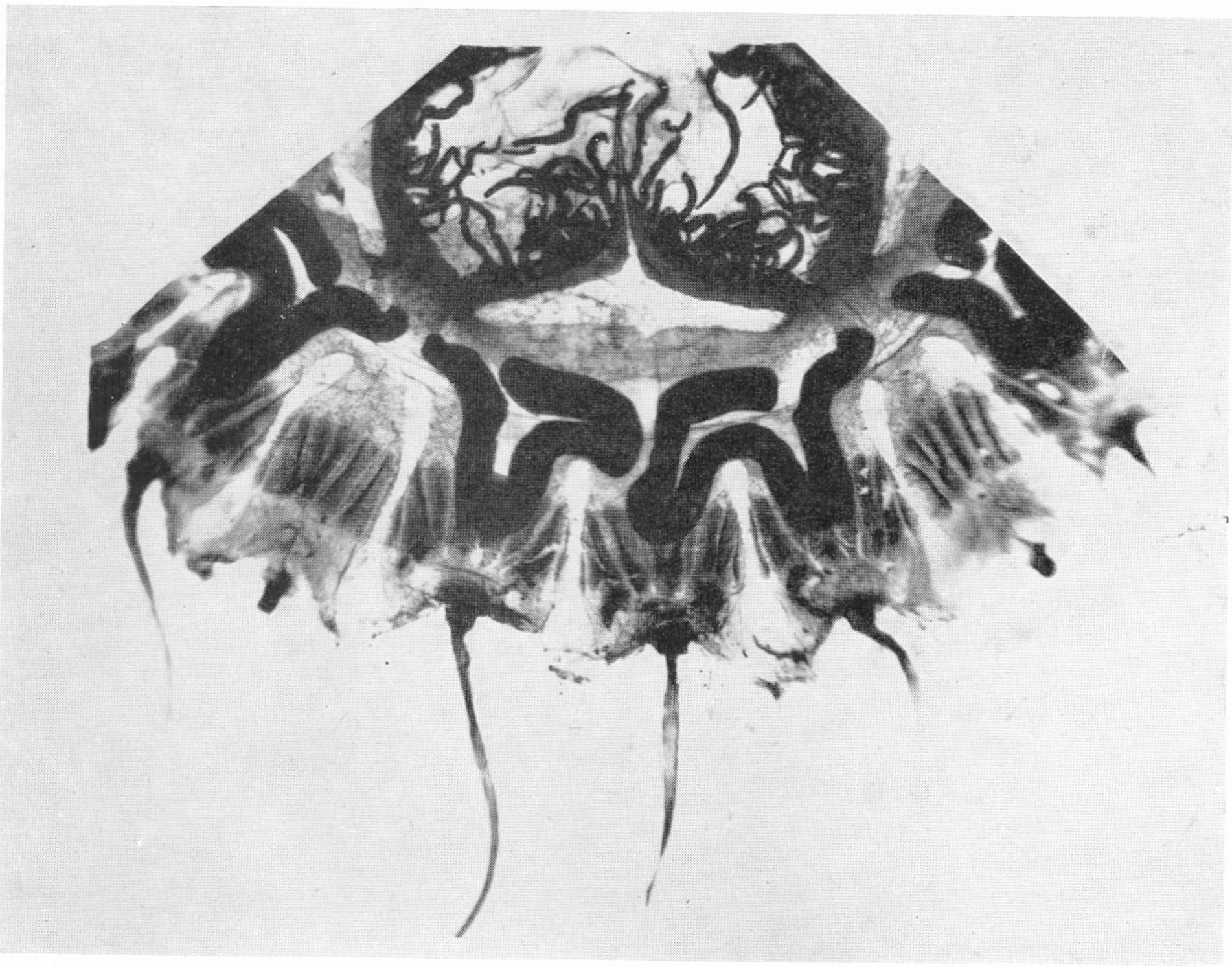

Fig. 2

(*Facing p.* 110)


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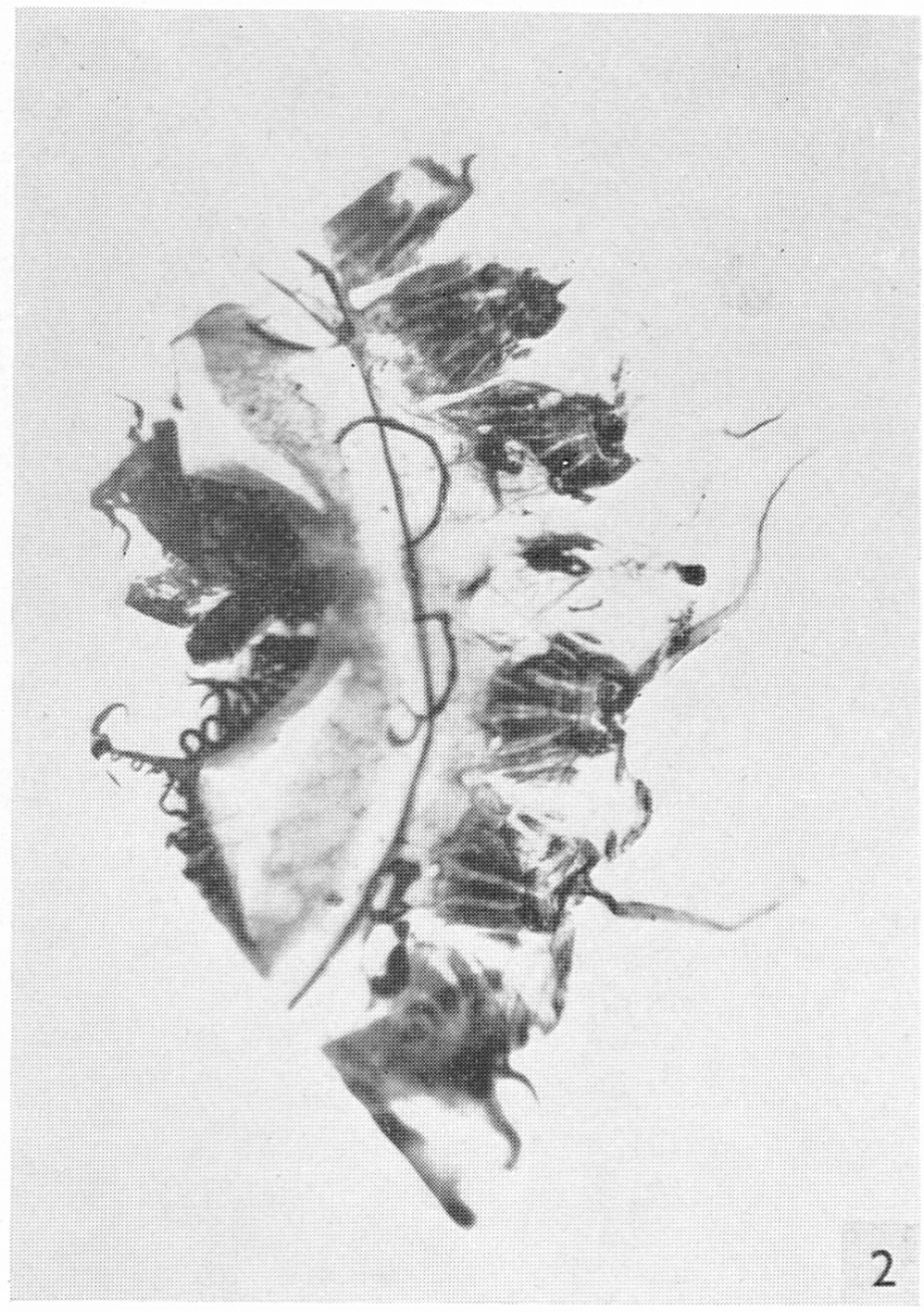
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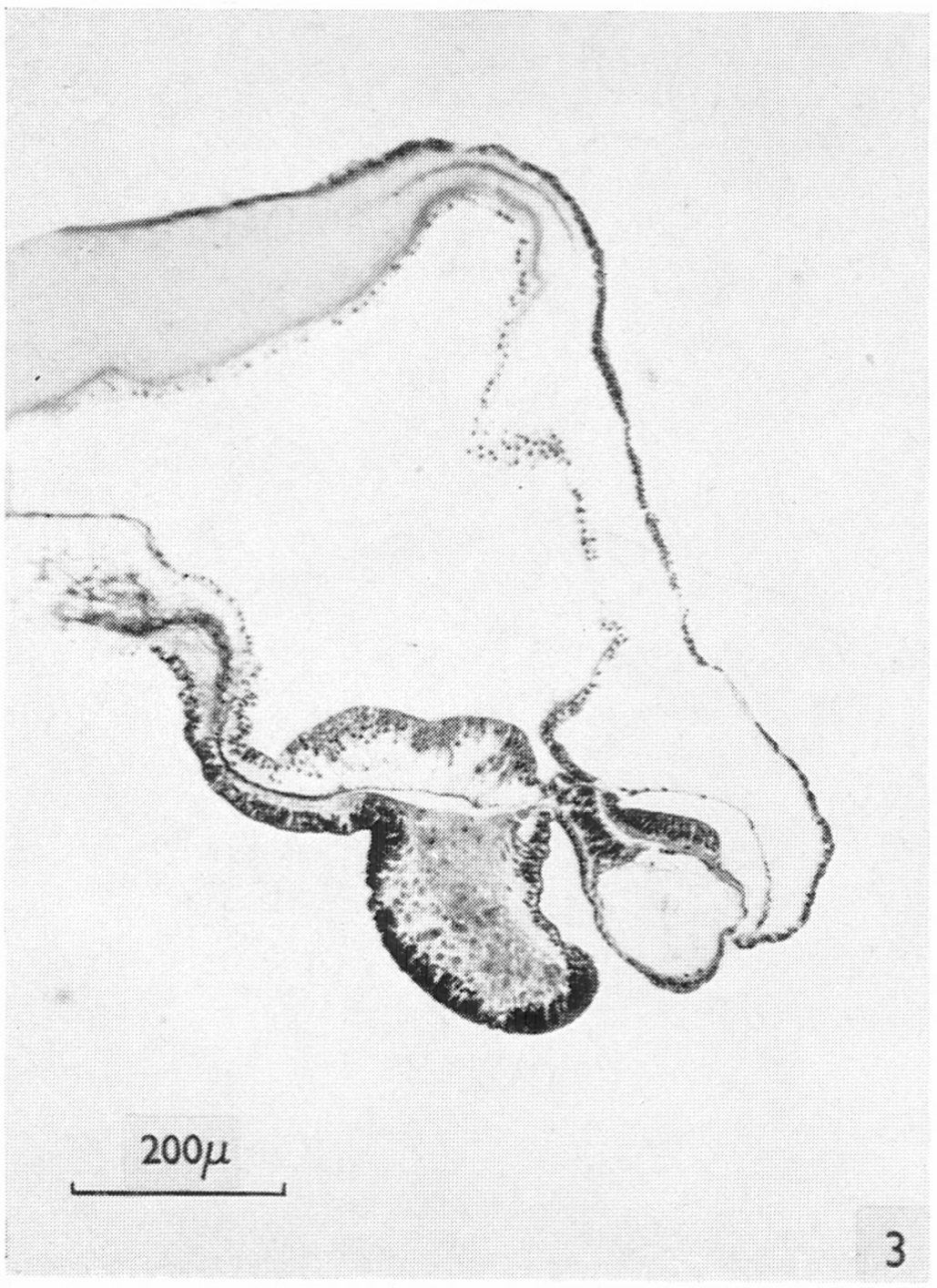
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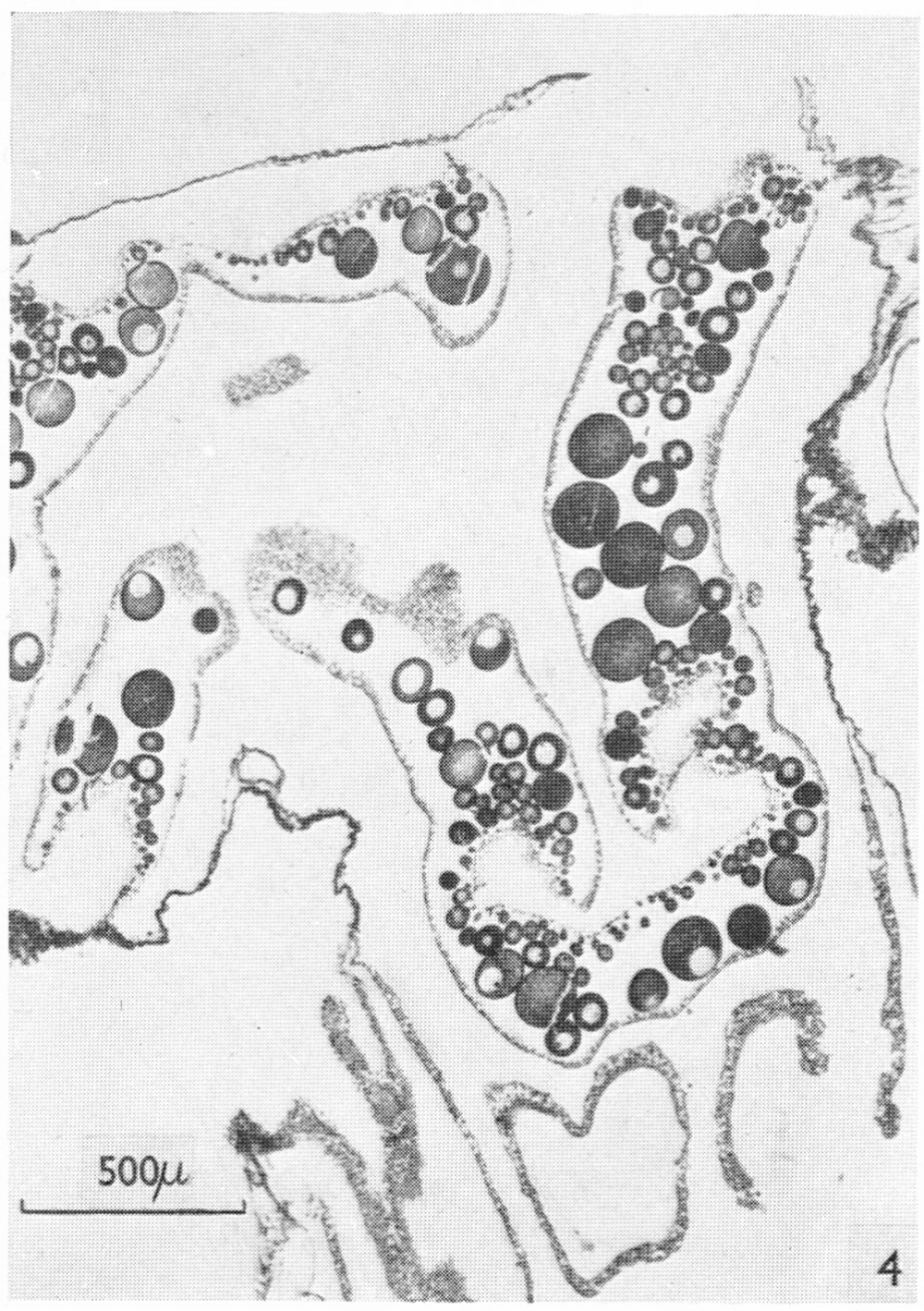
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4

EXPLANATION OF PLATES

PLATE I. *Paraphyllina ransoni*

Fig. 1. Photograph of specimens about four-fifths natural size; one specimen has been pressed on to the bottom of the glass vessel to show the marginal lappets.

Fig. 2. Sector of umbrella bleached by Mayer's chlorine method, stained with borax carmine, and mounted.

PLATE II. *Paraphyllina ransoni*

Figs. 1, 2. Two halves of the umbrella of the same specimen showing reduction of gonads (male); bleached, stained and mounted as in Pl. I, fig. 2.

Fig. 3. Radial section through rhopalium, stained with haematoxylin and eosin.

Fig. 4. Section through female gonad, stained with haematoxylin and eosin.

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THE HOST-SPECIFICITY, MICRO-ECOLOGY, ADHESIVE ATTITUDES, AND COMPARATIVE MORPHOLOGY OF SOME TREMATODE GILL PARASITES

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(Plates I and II, and Text-fig. 1)

In recent years there have been several accounts of the occurrence of diclidophoroidean trematodes parasitic on the gills of fishes, e.g. Price (1943), Sproston (1946), Dawes (1947), Brinkmann (1952), and Chauhan (1953), and these accounts have included descriptions of the morphology of the parasites. The distribution records have revealed a generally high degree of host specificity and, in some species, a preference even for certain gill arches of the particular hosts, while the morphological descriptions have shown that there is a considerable variation in the form of the parasites, extending to various degrees of deviation from bilateral symmetry (Pl. I, figs. 1–11). These morphological variations are present in spite of the fact that the different parasites occupy such broadly similar habitats in the gill chambers of their respective hosts. But, as far as I am aware, this is the first attempt to investigate the distribution and morphology of the parasites in relation to their microhabitats.

Material and Techniques

Most of the material used in the present study was collected at Plymouth during the months of July and August in 1953, 1954 and 1955, but specimens of *Discocotyle sagittata* on *Salmo trutta* were obtained from Breconshire, Carmarthenshire, Monmouthshire and Shropshire. Some material collected previously (Rees & Llewellyn, 1941) from Cardigan Bay, the Irish Atlantic Slope, and the Porcupine Bank has also been used.

The parasites and hosts studied are listed in Table I. The names of the fishes are those used in the *Plymouth Marine Fauna*, 3rd ed. (Marine Biological Association, in Press), and the parasites, with the two exceptions noted below, have been named as in Sproston's synopsis of the Monogenea (1946). The name *Kuhnia scombri* Sproston, 1946, has been shown (Dollfus, 1946; Llewellyn, 1956) to be a synonym of *Octostoma scombri* Kuhn, 1829, and *Cyclocotyla chrysophryi* (van Beneden & Hesse, 1864), Price, 1943, has been used in preference to *Choricotyle chrysophryi* van Beneden & Hesse. The parasite referred to in Table I as 'microcotylid species' awaits precise

identification: over seventy species of *Microcotyle* have so far been reported (Sproston, 1946), and so the possible addition of a new species becomes a considerable taxonomic task. In the present study it is important only that a microcotylid kind of diclidophoroidean has been found to parasitize a host *Trachurus trachurus* already harbouring *Gastrocotyle trachuri* and *Pseudaxine trachuri*.

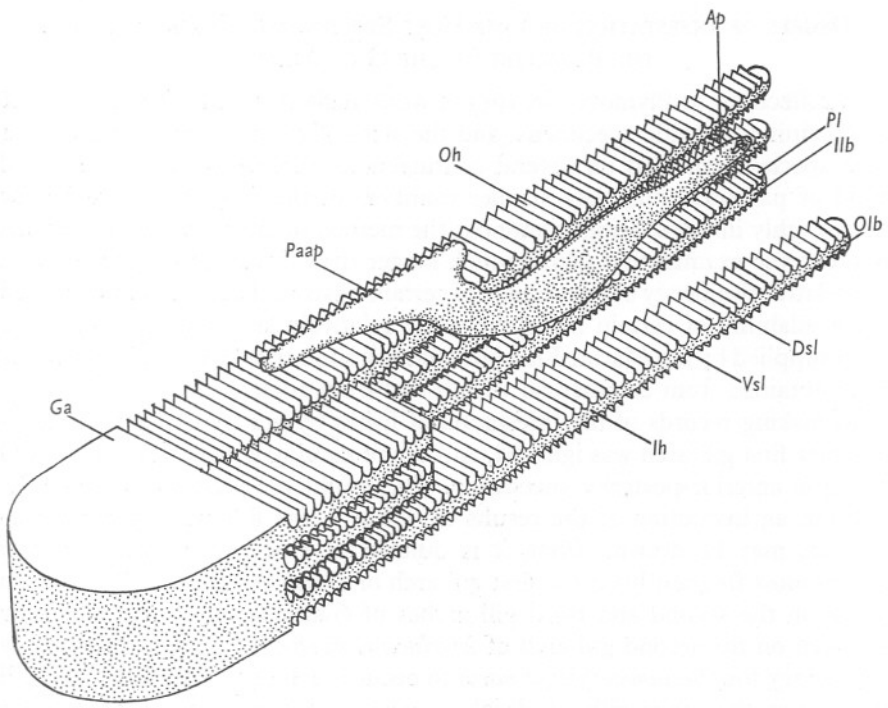
Hosts known from previous records (Baylis & Jones, 1933; Rees & Llewellyn, 1941; Sproston, 1946; Dawes, 1947) to be subject to infection with various diclidophoroideans were examined, as far as was possible, while still alive, the fishes having been brought to the laboratory in baths of sea water. With the parasite of the trout, I have not yet succeeded in examining a living specimen attached to its host: on those occasions when I have taken a microscope to the river bank, my angling friends have not produced a parasitized fish. On other occasions the parasites have died or become detached or have been histologically fixed before examination.

The gills of the fishes were examined by one of two methods, dependent upon previous knowledge of the usual size of the mature parasite. With those fishes, namely *Merluccius merluccius*, *Gadus merlangus* and *G. luscus*, known to be subject to infection with comparatively large parasites, the operculum was lifted and the gills searched while still *in situ*. Sites of infection were then examined carefully with a stereomicroscope. The particular site of infection was noted, i.e. left or right of the fish, serial number of the gill arch, position on the gill arch, inner or outer hemibranch, and dorsal, ventral, or lateral surface of primary lamellae. The importance of a knowledge of all these factors was not realized until the investigation had been in progress for some time, and so early records were incomplete. With hosts likely to harbour smaller parasites, all the gills were removed for microscopic examination. Care was taken so to arrange the excised gill arches that, by inspection, their normal orientation on the fish could be established and so yield records of the precise sites of attachment of the parasites as described above for the larger forms. On only one occasion was a larval trematode observed, but this scarcity could have been due to the loss of larvae after the capture of the hosts, or to insufficiently close observation.

As many as possible of the species of parasites were photographed *in situ* on the gill lamellae, and these have been reproduced in Pl. II. In *Axine belones* the body is bent in two planes, rendering photography very difficult, and a stereogram has been drawn (Text-fig. 1). This figure also illustrates the anatomical terms used for gill structures.

For the preparation of whole mounts for the study of body symmetry, specimens were relaxed either in $7\frac{1}{2}\%$ magnesium chloride solution, or by using menthol, or by simply allowing them to remain in sea water until they were dead. They were then fixed in either Bouin's or Gilson's fluid without the usual pressure of a cover-glass, stained in haemalum, and mounted in

Canada Balsam. Serial paraffin sections were prepared of some specimens of all the parasite species *in situ* on the gills, the sections being stained by Heidenhain's Azan or Masson's trichrome methods.



Text-fig. 1. Stereogram of *Axine belones* on the gills of *Belone belone*. (Very diagrammatic.) *Ap*, anterior end of parasite; *Dsl*, dorsal secondary lamella; *Ga*, gill arch; *Ih*, inner hemibranch; *Ilb*, inner lateral border of primary lamella; *Oh*, outer hemibranch; *Olb*, outer lateral border of primary lamella; *Paap*, posterior adhesive apparatus of parasite; *Pl*, primary lamella; *Vsl*, ventral secondary lamella.

Host Specificity

In the combined earlier investigations of the Irish Sea, the Irish Atlantic Slope, and the Porcupine Bank (Rees & Llewellyn, 1941) and the present Plymouth investigations, 2104 host specimens belonging to seventeen fish species have been examined, and over 900 parasites belonging to eighteen diclidophoroidean species have been collected. Except for *Plectanocotyle gurnardi*, which I have collected from three different species of *Trigla*, namely *T. cuculus*, *T. lineata* and *T. gurnardus*, and *Diclidophora minor* (usually parasitic on *Gadus poutassou*), of which a single specimen was found on a host which I identified as *Gadus merlangus*, all parasite species have been found to be strictly specific to their particular hosts. Only one host has yielded more

than one species of parasite, namely *Trachurus trachurus* often harbouring together both *Gastrocotyle trachuri* and *Pseudaxine trachuri*, and, in addition, on a single occasion one specimen of an unidentified microcotylid species.

Degree of Infestation of some Host Species, and Distribution of the Parasites on the Gill Arches

The collections at Plymouth in 1953–5 were made primarily for the purpose of obtaining parasitic specimens, and the sizes of the samples of the various host species depended on several contingencies. These included the actual yield of parasites, relatively smaller numbers of the hosts found to be the more highly infected being examined; the method of examination of the host, microscopic examination taking much longer than macroscopic (see p. 114); the relative frequency of occurrence of certain hosts in the Plymouth area; and the availability of certain hosts, for while relatively large numbers of fishes were supplied by the Marine Biological Association's trawlers, smaller numbers were obtained from commercial vessels and from anglers.

In making records of the infestation of fishes by gill trematodes the rudimentary first gill arch was ignored, and the remaining arches were numbered 1 to 4 in anterior-posterior succession. The results are included in Table I.

From an inspection of the results in Table I, the following general conclusions may be drawn. First, it is quite clear that *Diclidophora merlangi* occurs most frequently on the first gill arch of *Gadus merlangus*, *Diclidophora luscae* on the second and third gill arches of *Gadus luscus*, and *Anthocotyle merluccii* on the second gill arch of *Merluccius merluccius*. Secondly, there is a tendency for *Plectanocotyle gurnardi* to occur less frequently on the first gill than upon the other gills of *Trigla cuculus*, and similarly a tendency for *Gastrocotyle trachuri* to occur less frequently on the fourth gill than upon the other gills of *Trachurus trachurus*. Thirdly, with *Axine belones* on *Belone belone*, there is a tendency for the first and fourth gills to be less heavily parasitized than the second and third. Insufficient numbers of the remaining species were available for significant comment.

The Morphology and Adhesive Attitudes of the Parasites in Relation to their Hosts

Without exception the parasites were found to be attached with their posterior adhesive organs nearer to the gill arch of the host, and with the anterior end nearer to the distal end of the primary lamellae. In this way the attached ends of the worms lie upstream relative to the gill ventilating current of the host, with the mouth of the parasite downstream.

Beyond this common general adhesive attitude, the parasites exhibited further similarity in that all but two of the species examined were found to attach themselves to the secondary gill lamellae of their hosts (Pl. II, fig. 1).

The exceptional species were *Anthocotyle merluccii* on *Merluccius merluccius* (Pl. II, figs. 5, 6), and *Cyclocotyla chrysophryi* on *Pagellus centrodontus* (Pl. II, fig. 9), where the principal attachment is more directly to primary lamellae. In those parasites which adhere to the secondary lamellae, the opening of each of the attachment organs is directed obliquely postero-ventrally with respect to the parasite, i.e. the plane between the two valves of each attachment organ lies parallel to those which would normally be occupied by the secondary gill lamellae when washed over by the respiratory current of the host (Pl. II, fig. 1).

TABLE I. DEGREE OF INFESTATION OF HOSTS, AND DISTRIBUTION OF PARASITES ON GILL ARCHES, OF FISHES EXAMINED AT PLYMOUTH 1953–55

Category host of sample*	Host	No. of host specimens examined	No. of host specimens infected	Infestation (%)	Parasite	Total numbers of parasites per gill arch 1	2	3	4
A	*Gadus merlangus*	507	44	8·7	*Diclidophora merlangi*	53	8	1	4
	G. luscus	509	108	21·0	*D. luscae*	7	118	91	8
	Merluccius merluccius	500	38	7·6	*Anthocotyle merluccii*	4	28	7	5
B	*Trigla cuculus*	20	19	95·0	*Plectanocotyle gurnardi*	6	22	39	25
	Trachurus trachurus	37	23	62·2	*Gastrocotyle trachuri*	41	56	46	20
			8	21·6	*Pseudaxine trachuri*	8	9	5	2
			1	2·7	Microcotylid species	Not known			
C	*Belone belone*	18	14	77·8	*Axine belones*	19	29	48	22
	Scomber scombrus	8	8	100·0	*Octostoma scombri*	7	15	1	0
D	*Pagellus centrodontus*	47	1	2·1	*Cyclocotyla chrysophryi*	0	0	1	0
	Morone labrax	3	2	66·7	*Microcotyle labracis*	3	0	0	0
	Gadus pollachius	15	1	6·7	*Diclidophora pollachii*	Not known			
	Alosa fallax	2	1	50·0	*Mazocraes alosae*	Not known			

* A, hosts readily available, searched macroscopically; B, hosts readily available, searched microscopically; C, hosts less readily available, searched microscopically; D, hosts less readily available, searched macroscopically.

Beyond the common general features described above, the parasites investigated were found to exhibit considerable variation in their adhesive attitudes. The variable factors include attachment either to one or to more than one primary lamella, attachment to a particular hemibranch, and disposition of the body either between primary lamellae of the same hemibranch or between primary lamellae of different hemibranchs, i.e. between the two hemibranchs of a gill. The pattern of this variation was found to be constant for particular species of parasite and will be described in the following pages, where an attempt is also made to relate the morphology of each of the parasites to its characteristic adhesive attitude.

In *Plectanocotyle gurnardi*, on the gills of *Trigla cuculus* (Pl. II, fig. 2), *Octostoma scombri*, on the gills of *Scomber scombrus*, *Mazocraes alosae*, on the gills of *Alosa fallax*, *Discocotyle sagittata*, on the gills of *Salmo trutta*, and

Microcotyle labracis, on the gills of *Morone labrax*, the posterior adhesive organs are applied either all to the secondary lamellae of the dorsal surface of a primary lamella or all to the secondary lamellae of the ventral surface of a primary lamella, of either an inner or an outer hemibranch, i.e. the trematode is attached to one side or the other of a primary lamella, and never to both sides. In fact the distance between the members of a pair of symmetrical adhesive organs is quite insufficient to span the interval between dorsal and ventral secondary lamellae, i.e. the lateral border, devoid of secondary lamellae, between the dorsal and ventral secondary lamellae.

The longitudinal axis of the parasite, throughout its whole course, always lies parallel to the long axis of the primary lamella, and the whole parasite is bilaterally symmetrical. The attachment organs are all either sessile or borne on very short peduncles. Median ventral posterior hooks (anchors) are present in *Octostoma*, *Mazocraes* and *Plectanocotyle*, but absent in *Discocotyle* and *Microcotyle*.

Further illustrations of the adhesive attitude of the above group of species, as exemplified by *Octostoma scombri*, are given elsewhere (Llewellyn, 1956).

Diclidophora luscae, on the gills of *Gadus luscus* (Pl. II, fig. 3), *D. denticulata*, on the gills of *Gadus virens*, and *D. phycidis*, on the gills of *Urophycis blennoides*, attach themselves to their respective hosts in such a manner that the members of a pair of symmetrical adhesive organs are each applied to opposite sides of the same primary lamella, so that one member of the pair adheres to the secondary lamellae of the dorsal surface and the other to the secondary lamella of the ventral surface. The adhesive organs in these three species of *Diclidophora* are borne on peduncles, the function of which is clearly that of extending the width of the body, and so assisting the parasite to span the distance between the dorsal and ventral surfaces of a primary lamella. As a result of this method of attachment the median longitudinal axis of the worm lies along a lateral border of a primary lamella. The body is bilaterally symmetrical, and no median posterior hooks are present in the adult parasites.

Diclidophora merlangi, on the gills of *Gadus merlangus* (Pl. II, fig. 4), spans both dorsal and ventral surfaces of primary lamellae, as do *Diclidophora denticulata*, *D. luscae*, and *D. phycidis*, but the first-named trematode differs from the other three in that its first and second pairs of adhesive organs are spread over more than one primary lamella, whereas in the other three species all the adhesive organs are applied to the same primary lamella. Characteristically the third and fourth pairs of adhesive organs of *D. merlangi* each grasps the same primary lamella; the second pair grasps the primary lamellae in the same hemibranch immediately on each side of that grasped by the third and fourth pairs; and the first pair of adhesive organs grasps the lamellae that are situated next but one to those grasped by the third and fourth pairs. Occasionally the first pair of adhesive organs may grasp the same lamellae as those grasped by the second pair.

Compatible with this adhesive attitude which involves the spanning of several primary lamellae, the body of *D. merlangi* in the region of the first and second pairs of adhesive organs is relatively wider than the corresponding regions of *D. denticulata* and *D. luscae*, and moreover, the peduncles of the first two pairs of adhesive organs are relatively long. The body is bilaterally symmetrical, and no median posterior hooks are present.

In *Cyclocotyla chrysophryi*, on the gills of *Pagellus centrodontus* (Pl. II, fig. 9), the attachment organs function as suckers and not as clamps (see Llewellyn, 1941), i.e. the adhesive organs do not have opposable jaws for the grasping of pairs of opposite surfaces of primary or secondary lamellae. Instead, each sucker may be applied to any relatively large smooth surface. In the single living specimen that I have had opportunity to examine microscopically, the suckers were applied to the outer lateral borders of primary lamellae, i.e. to regions devoid of secondary lamellae. The four pairs of adhesive organs are borne on relatively long peduncles, and may be spread over two, three, or four adjacent primary lamellae. When forcibly detached from the gill surface, the suckers were able readily to re-establish themselves. When first observed on the living host the parasite was attached to the lateral borders of primary lamellae, but on detachment soon effected new attachment, not only to primary lamellae, but also to the gill arch itself, as shown in Pl. II, fig. 9.

The body is bilaterally symmetrical, and no median posterior hooks are present.

In *Anthocotyle merluccii*, on the gills of *Merluccius merluccius* (Pl. II, figs. 5, 6), the anterior-most of its four pairs of attachment organs is relatively enormously developed so that each of the members of the first pair of organs is itself able to span the distance between the dorsal and ventral surfaces of a primary lamella. The parasite uses this large pair of clamps to grasp the inner border of a primary lamella of an inner hemibranch, the three remaining pairs of small adhesive organs becoming attached to secondary lamellae of the dorsal surface of the same primary lamellae. The body of the parasite passes between primary lamellae of the outer hemibranch and then bends at right angles so that its dorsal surface is in contact with the outer surface of the hemibranch. Were the longitudinal axis of the body to remain in one straight line, the body of the parasite would now lie with its length across the gill ventilating current. However, asymmetrical development has resulted in a bending of the body in the region immediately anterior to the large adhesive organs so that the long axis of the parasite comes to lie more nearly parallel to the direction of the gill ventilating current, and thus the parasite offers less resistance to this current. In a sample of forty-two specimens of *Anthocotyle merluccii* the inclination of the body was found to be towards the animal's right in sixteen (Pl. I, fig. 8, where the animal is seen in ventral view) and to the animal's left in the remaining twenty-six. All twenty-six parasites in which

the inclination of the body was to the left came from gills of the left side of the host fish, and all sixteen with right-inclined bodies came from gills of the right side.

A further feature of asymmetry was found in the relatively greater development of the member of the first pair of adhesive organs which grasps the primary lamella at a position relatively distal to the gill arch (Pl. II, fig. 6). This larger clamp may be on the left or right of the parasite, but is always on the downstream side with respect to the gill-ventilating current of the host, and thus always on the same side towards which the asymmetrical body is inclined. In conformity with the apparently exacting and obligatory requirements of the adhesive attitude of *A. merluccii*, it follows that parasites from the left side of the host fish will be inclined to their left and will have the larger clamp on the left side, and similarly with respect to the right.

Median posterior hooks are present.

In *Axine belones*, on the gills of *Belone belone* (Pl. II, figs. 7, 8A, 8B, and Text-fig. 1), the number of adhesive organs is greatly increased from the more usual six or eight to a number varying between about fifty and seventy. Moreover, in *Axine belones* these posterior adhesive organs, instead of being borne in two symmetrical rows, one on each side of the body, are borne in a single oblique row on what is apparently one margin of the body. This margin is not constantly on the same side of the body in all specimens, at least with respect to the marginal vaginal aperture. This latter organ was found, in a sample of twenty-five specimens, to be without exception on the animal's left. In a sample of 100 specimens of *Axine belones*, the posterior adhesive organs were found on the parasite's right in eighty-seven cases, and on the left in the remaining thirteen cases. Hooks that are presumably homologous with the posterior median hooks of *Octostoma*, *Mazocraes* and *Plectanocotyle* are present approximately mid-way along the row of adhesive organs.

The median longitudinal axis of the body of *Axine* is inclined to the line of the row of adhesive organs at an angle of about 30°.

During attachment to the host, the adhesive organs are applied to secondary lamellae of either the dorsal or the ventral surface of a primary lamella, but always near to the *outer* lateral border of a primary lamella of either the inner or outer hemibranchs. The row of adhesive organs lies parallel to the lateral border of the primary lamella so that the body of the parasite, being inclined to the row of adhesive organs, extends beyond the inner lateral border of the lamella to which it is attached. In the region where the body of the parasite crosses this inner border of the lamella, the body bends at right angles to the plane of the posterior adhesive apparatus so that the greater part of the body of the parasite comes to lie between the two hemibranchs of a gill. As a result of this adhesive attitude the longitudinal axis of the greater part of the body of *Axine*, i.e. the part lying between two hemibranchs of a host gill, lies parallel to the gill-ventilating current of *Belone*. The characteristic disposition of the

body is illustrated in Pl. II, fig. 8, where in fig. 8A the posterior adhesive region is seen in ventral view, and the remainder of the body in lateral view, and where in fig. 8B the body of the same specimen is seen in ventral view.

In *Pseudaxine trachuri*, on the gills of *Trachurus trachurus*, there are about twenty to thirty posterior adhesive organs, all borne in a single row along one margin only of the body. Ventral hooks are present at the extreme posterior end of the row of clamps. The longitudinal axis of the body is inclined to the row of adhesive organs at an angle varying between about 30 and 50°. These adhesive organs are applied near the outer lateral borders of the relatively narrow primary lamellae, and so the body of the parasite soon crosses the inner border of the primary lamella. Here it bends usually through a right angle so that the greater part of the body of the parasite lies between two hemibranchs as in *Axine* (Pl. II, fig. 7; and Text-fig. 1), but more occasionally through 180°, so that the body of the parasite is in contact with the opposite side of the same primary lamella to that to which its adhesive organs are attached.

All twenty-four specimens of *Pseudaxine* that I have collected were found attached near to the distal ends of primary lamellae.

In a sample of sixteen specimens of *P. trachuri*, the adhesive organs were found on the right of eleven animals, and on the left of the remaining 5.

Gastrocotyle trachuri, on the gills of *Trachurus trachurus* (Pl. II, fig. 10), is asymmetrical, the twenty-five to thirty posterior adhesive organs being confined to one side only of the body. The row of adhesive organs lies approximately parallel to the longitudinal axis of the body of the parasite, and ventral hooks are present at the extreme posterior end of the body. In a sample of seventy-eight specimens of *Gastrocotyle trachuri* the adhesive organs were found on the left of thirty-nine animals, and on the right of the other thirty-nine.

The parasite may be attached to any of the secondary gill lamellae, irrespective of whether they be those of the dorsal or ventral surfaces of a primary lamella, or whether the primary lamella belongs to an inner or an outer hemibranch. But invariably the parasite lies with its attachment organs nearer to the outer border of a primary lamella (i.e. the outer border of a hemibranch) so that a (small) part of the body overlaps the inner border of the same primary lamella and comes to lie between two hemibranchs. In the region where it overlaps into the space between the hemibranchs, the body of the parasite is folded ventrally through 90°.

Discussion

The present investigation of the distribution of diclidophoroidean parasites on Plymouth fishes has shown these trematodes to be entirely specific to their particular hosts. My single previous record of *Diclidophora minor* from *Gadus merlangus* very probably involved mis-identification of the fish, for in

an unpublished thesis (1940), in the library of the University of Wales, I referred to this host as '*Gadus merlangus* var. Deep-Sea', probably on account of the trawlermen's name of 'Deep-Sea Whiting' for the fish. Gallien (1937) had previously found *Diclidophora minor* on *Gadus poutassou* in the same locality, and it is almost certain that my specimen of *Diclidophora minor* came from the same host species. It seems distinctly possible also that the record of Baylis & Jones (1933) of *D. denticulata* from *Merluccius merluccius* at Plymouth was also a case of mistaken identity, for the 500 specimens of *M. merluccius* examined in the present study were parasitized only by *Anthocotyle merluccii*. Among the British marine Diclidophoroidea, then, only *Plectanocotyle gurnardi* appears to enjoy the hospitality of more than one host species, and in this case the hosts are closely related. A detailed examination, therefore, should be made of specimens of *Plectanocotyle gurnardi* from *Trigla gurnardus*, *T. lineata*, and *T. cuculus* for signs of incipient speciation.

The descriptions of the adhesive attitudes and of the morphology of the diclidophoroideans studied have shown that there is an exacting topographical relationship between parasite and host, and this is probably an important factor in the mechanism of host specificity.

The present investigation has confirmed Cerfontaine's (1898) observations that the maximum incidence of *Diclidophora merlangi* is on the first gill of *Gadus merlangus*, and that that of *Diclidophora luscae* is upon the second and third gills of *Gadus luscus*. On the other hand, Frankland (1955) found that her observations on a third *Diclidophora* species on a *Gadus* host, namely *Diclidophora denticulata* on *Gadus virens*, gave different results from those of Cerfontaine. The latter author (1898) had found the parasite to be most prevalent on the second and third arches, but Frankland, in a sample of 247 host specimens, found the first and second arches to be the most heavily parasitized. Cerfontaine made no reference to his technique of collecting, and did not record any larval or small forms. Frankland, however, made a microscopic search of the gills, and recorded the presence of larvae. The inference is that there is a change in the maximum incidence of parasite per gill arch with the age of the parasites, the shift, in *Diclidophora denticulata*, being from the first and second arches at initial infestation, to the second and third arches when older. If this is so, it would require either that the parasites are capable of transferring themselves from one gill arch to another, to which there is some contrary evidence (Frankland, 1955), or else that the degree of survival of parasites initially attaching themselves to the second and third gills is greater than for those attaching themselves to the first. In future studies it might be possible to test the latter hypothesis by investigating if there is any correlation between the degree of development of the parasite (size?) and the particular site of infestation.

Cerfontaine thought the explanation of the differential distribution among the gill arches to be in a choice of gill exercised by the parasite at initial

infestation, but in the absence of any evidence that the infective parasite is able to swim well enough to manoeuvre itself in the gill ventilating current, it is difficult to see how any such selective power could operate.

An alternative explanation would be to assume that the infective larvae are swept involuntarily over the gills by the gill-ventilating current. Any variations in the volume of water passing over the different gill arches might then be reflected in the numbers of opportunities for parasites to become attached. However, the greater numbers of larvae brought to those gills receiving the greater ventilation would themselves be committed to a lifelong struggle against the greater current, and the survival rate would be correspondingly lower than on less well-ventilated gills. Those parasites that change their adhesive attitudes during their lifetime, e.g. *D. denticulata* changing from the larval manner of attachment on one side of the primary lamella (Frankland, 1955) to the adult manner of attachment on both sides, appear to be particularly vulnerable at the time of effecting this transfer.

As to the difference in prevalence per gill arch between, on the one hand *D. luscae* and *D. denticulata*, each on the second and third gills of their respective hosts, and on the other hand *D. merlangi*, on the first gill of its host, the most profitable approach for understanding the problem appears to lie in a detailed comparison of the gill ventilating mechanisms of *Gadus luscus*, *G. virens*, and *G. merlangus*.

A common feature of the adhesive attitude found in all the parasites here studied is that the posterior adhesive organs are attached to the gills at a position upstream relative to the gill ventilating current, with the anterior mouth-bearing end downstream. Such an adhesive attitude is obviously well adapted to meet what appear to be the major ecological problems facing these parasites, namely those of clinging to the host in the face of a current, and of feeding, from the highly vascular secondary lamellae, on the blood that forms the main part of their diet (Llewellyn, 1954).

The particular forms of adhesive attitude have been found to vary considerably. The simplest method, e.g. *Octostoma*, *Mazocraes*, *Plectanocotyle*, *Discocotyle* and *Microcotyle*, is for the parasite to apply the whole of its body to the secondary lamellae of one surface of a primary lamella. It thus lies completely between primary lamellae of one hemibranch, and no part of the body is exposed to what presumably are the stronger currents between and outside the hemibranchs.

A variation occurs in *Diclidophora* species. In *D. luscae*, *D. denticulata* and *D. phycidis*, the adhesive organs are applied to both the dorsal and the ventral secondary lamellae of the same primary lamella, and in *D. merlangi* the parasite spans several primary lamellae. This adhesive attitude of *Diclidophora*, however, is achieved at the cost of losing suitable anchorage, in the form of secondary lamellae, for median posterior hooks, which are not found in this genus. The adhesive attitude in *Diclidophora* necessitates a widening of the

span of the pairs of adhesive organs by means of an increase in body width, and in *D. merlangi*, by the development of relatively long peduncles as well.

Cyclocotyla chrysophryi resembles *Diclidophora merlangi* in that it spans several primary lamellae, but differs from it in that instead of using *clamps* to attach itself to secondary lamellae, it uses *suckers* to attach itself to the smooth lateral borders of primary lamellae. The possession of these suckers permits the parasite to wander over other smooth surfaces, and I have observed *Cyclocotyla* to attach itself firmly to the gill arch of a (dead) *Pagellus*. However, the parasite was observed to contain abundant haematin, which has been shown (Llewellyn, 1954) to be evidence of a blood-feeding habit, and it seems probable that the normal habitat of the parasite on the living host is on the gills. Similarly, the numerous previous records of cyclocotylid parasites from the mouth-cavities of their hosts, or even as super-parasites of crustaceans which are themselves parasitic in the mouth-cavity of the host fish (see Sproston, 1946), are probably the result of the wanderings of which these parasites are capable in virtue of their possession of sucker-like adhesive organs and not clamps.

Anthocotyle merluccii is asymmetrical, and the advantage of the asymmetry is obvious since it results in the long axis of the body coming to lie parallel to the gill-ventilating current instead of across it. The need for this asymmetrical adjustment appears to have arisen from the change in the manner of attachment: the adhesive attitude of the ancestral diclidophoroidean probably resembled that of the present-day *Octostoma*, but the adoption of a habit of attachment by means of enlarged anterior clamps applied to the lateral border of a primary lamella necessitated a shift in the orientation of the parasite to a position at right-angles to the gill-ventilating current. A natural consequence would be the development of asymmetry to regain a body disposition offering less resistance to the water current. The reason for the unequal development of the members of the anterior-most pair of clamps is less clear. One would expect the adhesive organ farther upstream to bear the greater burden and so to be the more powerful and presumably the larger. In fact, however, the reverse obtained without exception in a sample of forty-two specimens of *Anthocotyle* observed *in situ* on the gills of *Merluccius*: the clamp nearer to the gill arch was invariably the smaller one of the pair. It has been shown (p. 120) that the side of the parasite on which the larger clamp occurs is directly related to the side of the fish upon which the parasite occurs. Since this appears to be purely a matter of chance, the side of the occurrence of the larger clamp has no taxonomic significance, and Brinkmann's (1952) relegation to synonymy with *Anthocotyle merluccii* of an American species erected on grounds of the side of development of the larger clamp was fully justified.

The asymmetry of *Axine belones*, *Pseudaxine trachuri* and *Gastrocotyle trachuri* fulfils the same function as that in *Anthocotyle*, namely that of bringing the longitudinal axis of the body to lie parallel to the gill-ventilating current,

but the reason why its development should have been necessary is not clear. Presumably it is directly related to the relative disposition of the long axis of the body and the hinge axes of the clamps during early development. This, however, itself presents certain problems, for in a preliminary examination of the orientation of the clamps of *Axine belones*, it was found that the clamps anterior to the hooks, and also those posterior to the hooks, all face in the same direction. The two sets of clamps are thus not mirror images of one another, as would be expected if their disposition were the result simply of the suppression in growth of the longitudinal axis of the posterior adhesive region of the body as has been suggested by Sproston (1946). More complex factors are obviously involved, and await further investigation.

The direction of asymmetry in *Axine belones*, *Pseudaxine trachuri* and *Gastrocotyle trachuri* has been shown to be dependent upon the particular site of infection of the host. In *Gastrocotyle* this is probably a matter of chance (thirty-nine with clamps on the parasite's left, and thirty-nine on the right, in a sample of seventy-eight), but in *Axine* the unequal distribution (eighty-seven with clamps on the parasite's right, and thirteen on the left, in a sample of a hundred) suggests the presence of a dominating factor influencing the position of the parasite along the curved gill arch, or some other variation. On the other hand, Sproston (1946, pp. 453–4) stated that in a sample of at least thirty specimens of *Axine belones* on *Belone belone* at Plymouth, all but one had the adhesive organs on the *left*. It must be pointed out, however, that Sproston (1946, figs. 104*a*, *b*) illustrated a specimen of *Axine belones* in which the vagina was on the animal's right, whereas I found it, without exception, to be on the left in a sample of twenty-five specimens. Lorenz (1878), in a paper on the anatomy of *Axine*, had also found the vagina on the left. Thus further work will be necessary before the precise topographical relationships between *Axine belones* and *Belone belone* can be determined.

Superficially, the three asymmetrical 'microcotylid' species *Axine belones*, *Pseudaxine trachuri* and *Gastrocotyle trachuri* appear to present a series of progressive reduction, in that the adhesive organs which in *Axine* are posterior to the ventral hooks are lost in *Pseudaxine* and *Gastrocotyle*, and that the body itself becomes relatively much smaller in *Gastrocotyle*. However, the morphology of *Axine* itself presents certain problems which should be investigated before worth-while speculations may be made upon evolutionary trends.

I am greatly indebted to the Director and Staff of the Plymouth Laboratory for their valuable assistance. I am also grateful to Dr H. M. T. Frankland for allowing me to read her manuscript on the bionomics and life history of *Diclidophora denticulata* before its publication.

SUMMARY

In a sample of over 2000 fishes belonging to seventeen species, the gills were found to be infested with over 900 parasites belonging to eighteen species of diclidophoroidean trematodes. All the parasites were found to be specific to particular hosts, with the exception of *Plectanocotyle gurnardi*, which may parasitize any one of three species of *Trigla*.

In most cases there was a characteristic differential distribution of the parasite among the gill arches of the host, and it is suggested that this is the result of variations in the flow of water over the different gills rather than of a choice exercised by the parasite.

The parasites were all attached with the posterior adhesive organs upstream relative to the gill-ventilating current and the mouth downstream. Variations in the adhesive attitude, characteristic of species or groups of species, occurred within this common pattern.

Variations in the form of the parasites such as the width of the body, degree of development of the peduncles of the adhesive organs, and asymmetry, could in each case be related to the characteristic adhesive attitude of the species.

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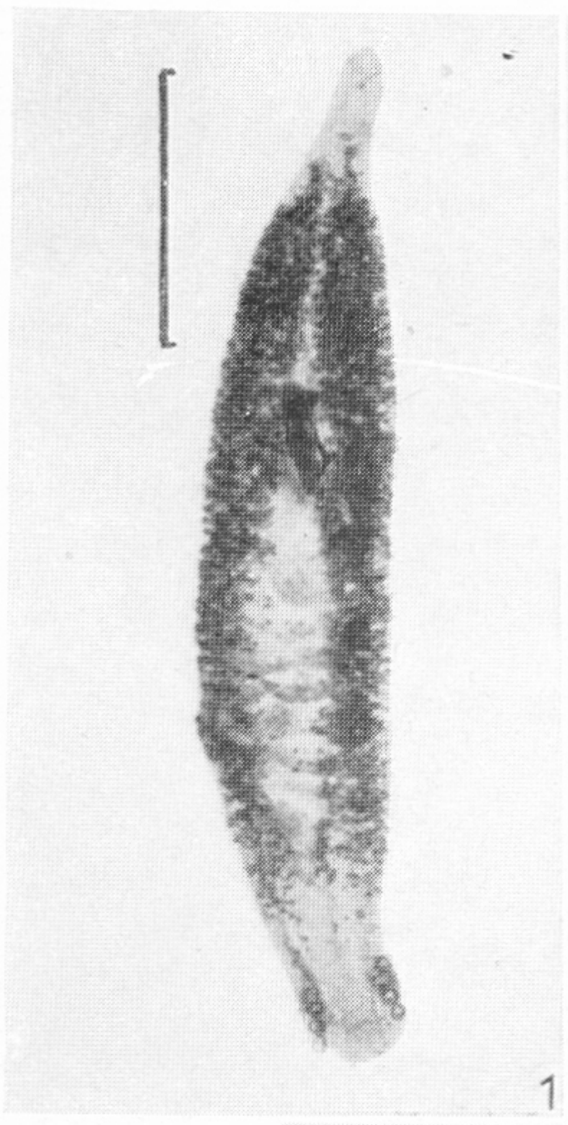
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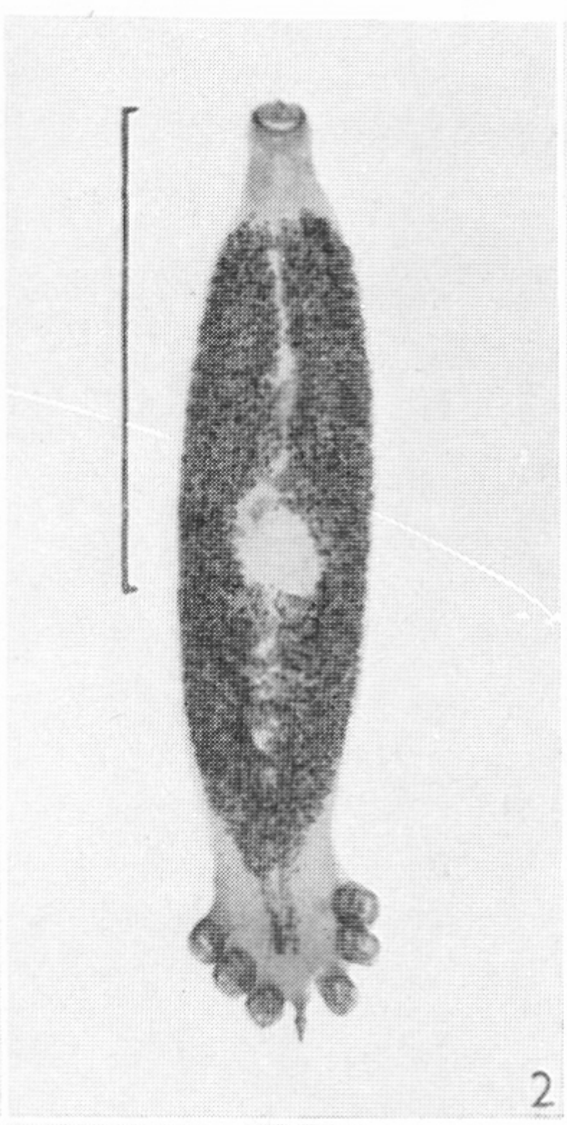
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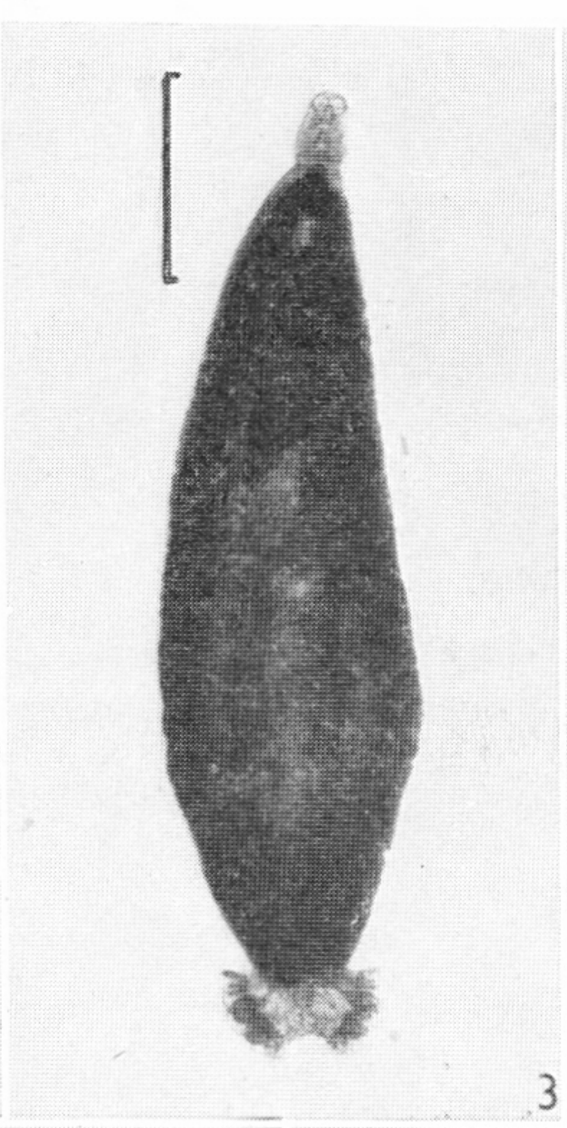
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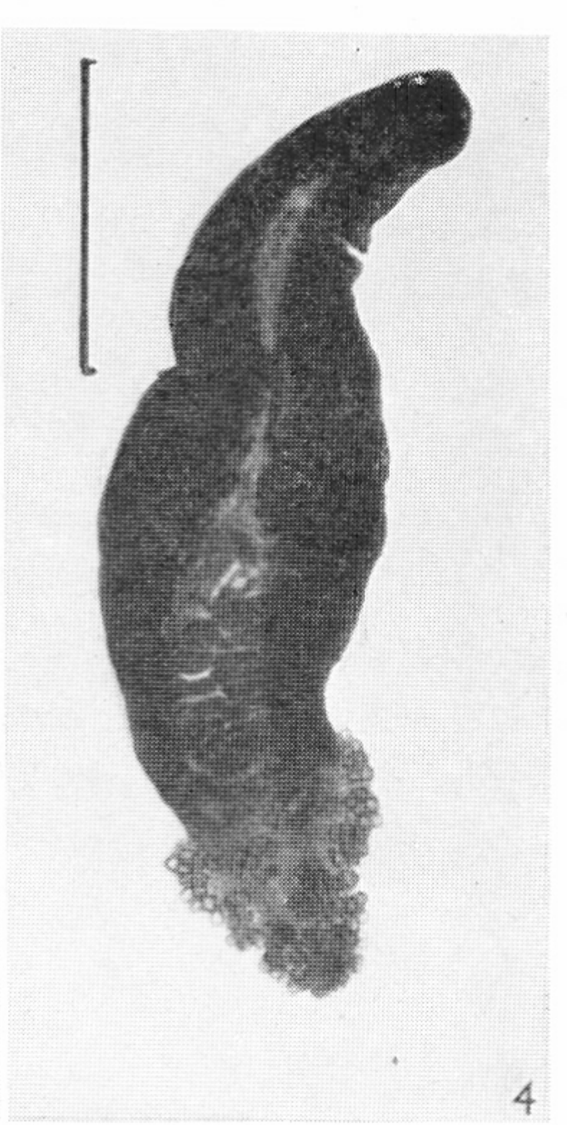
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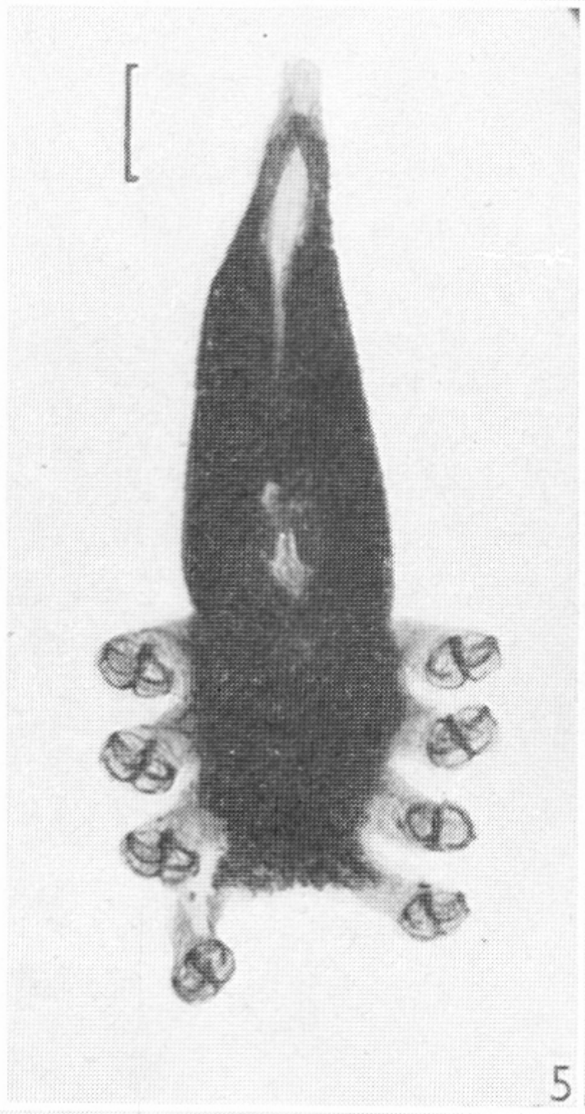
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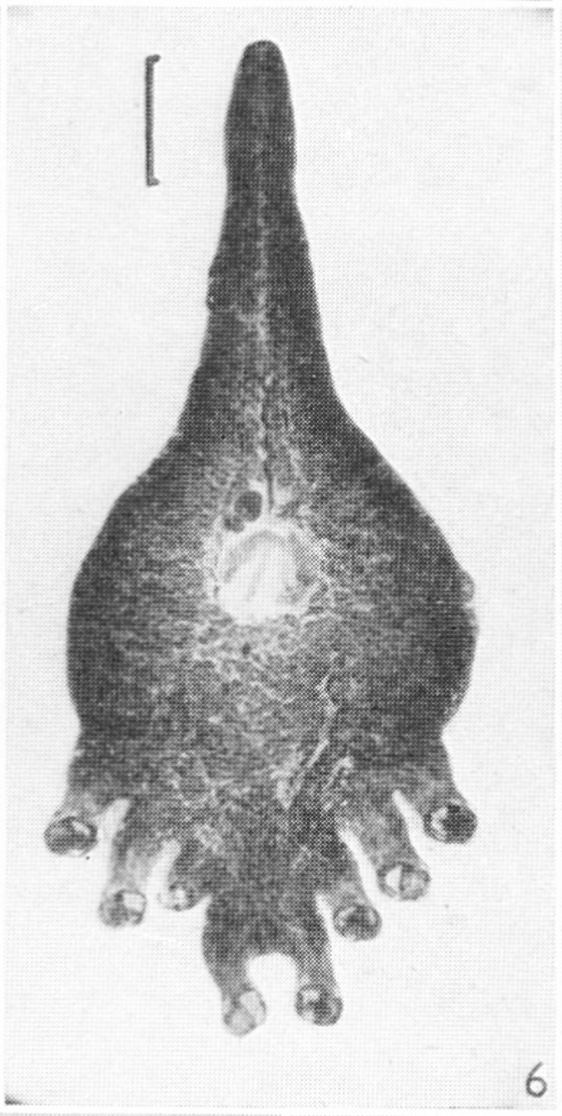
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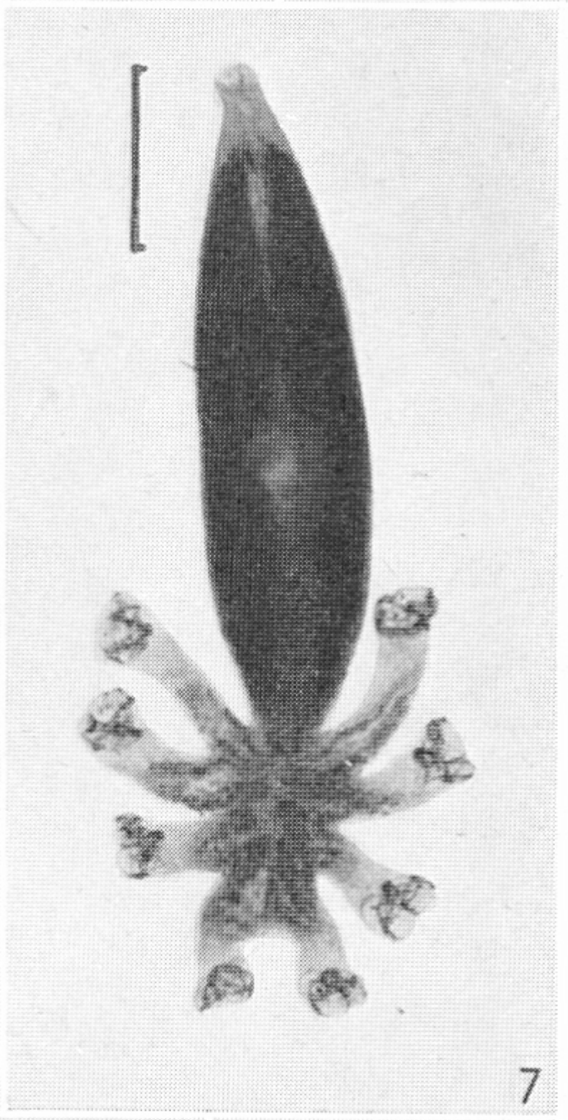
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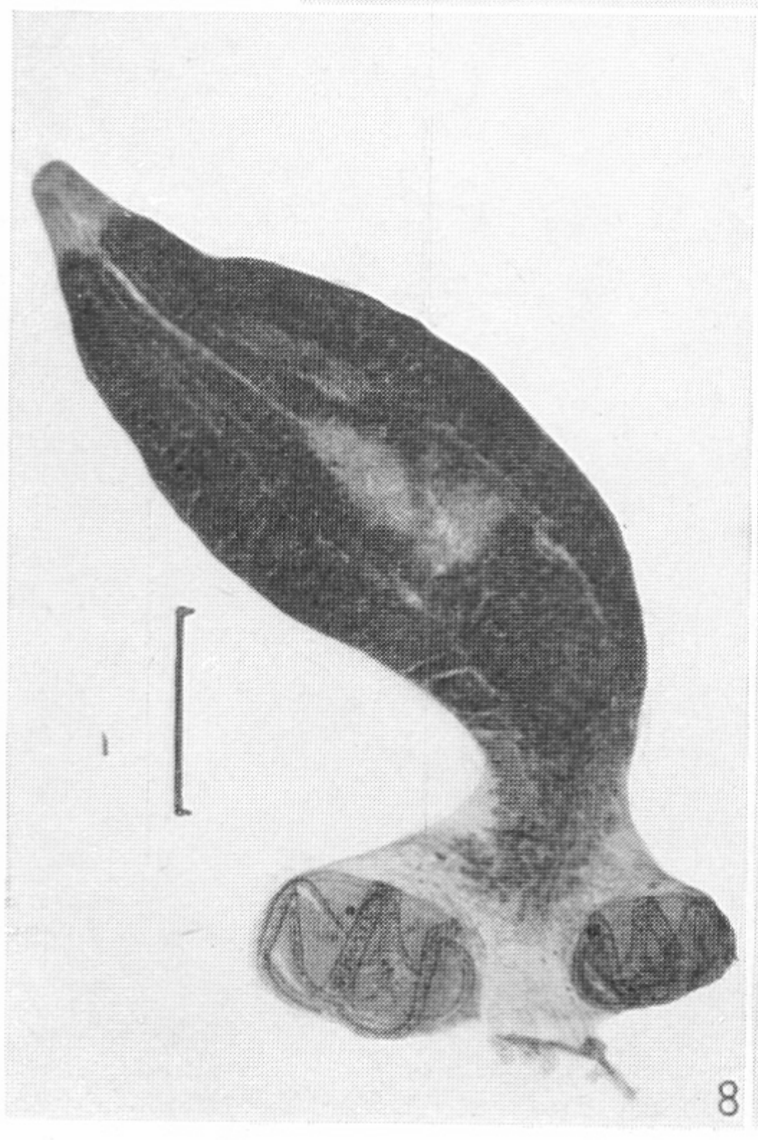
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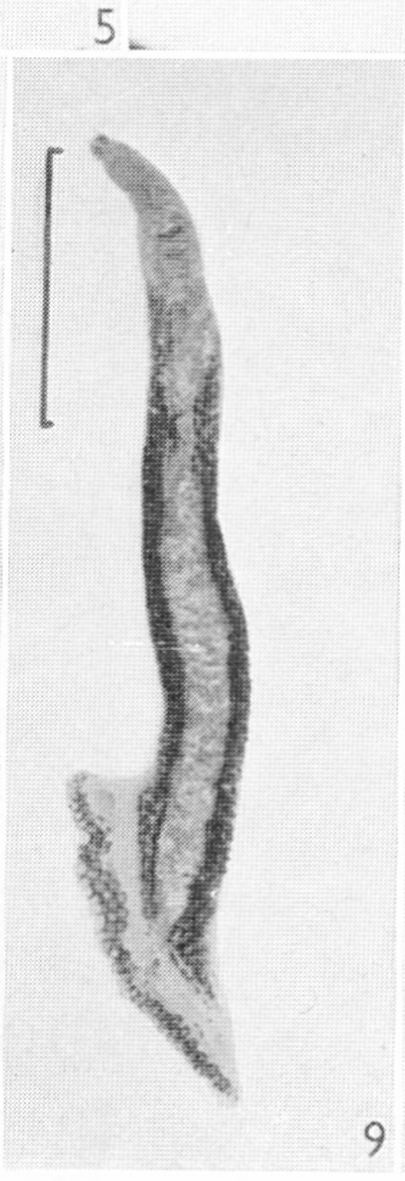
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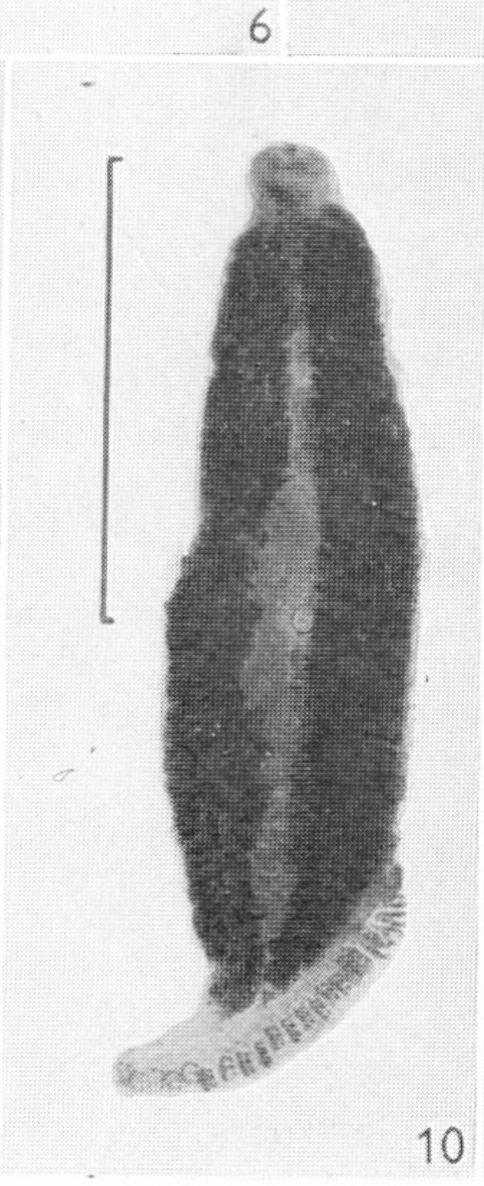
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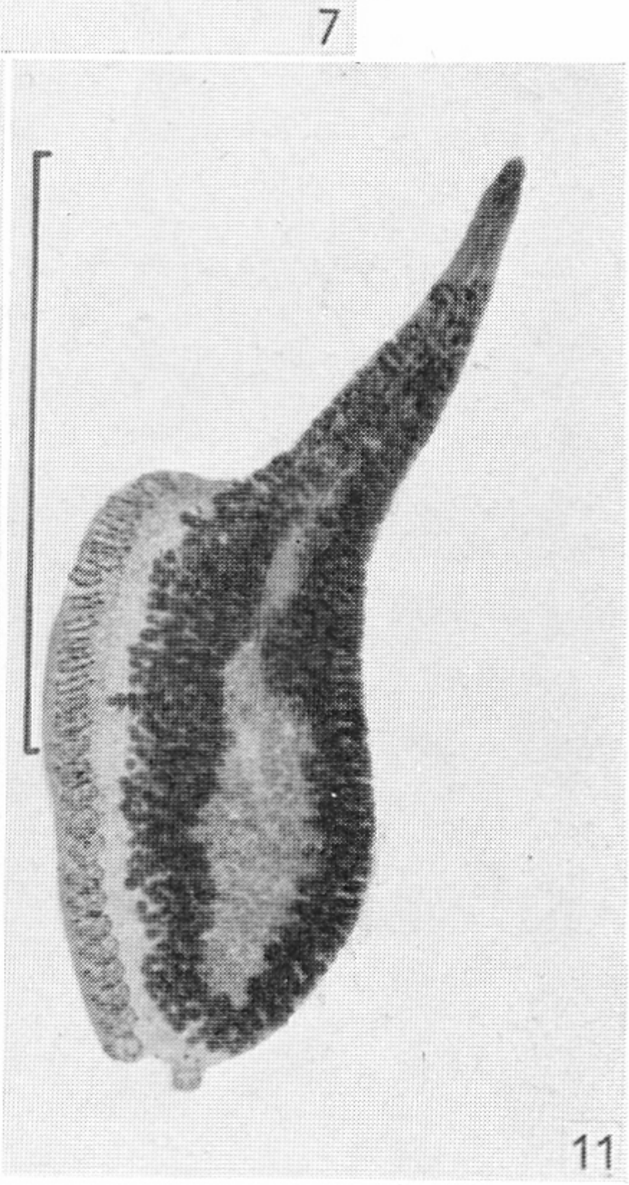
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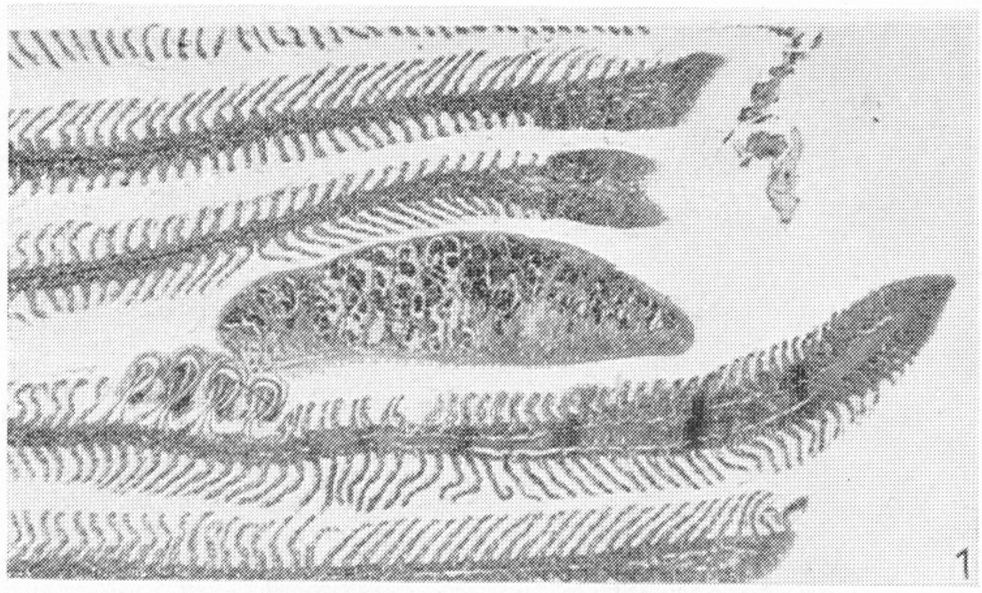
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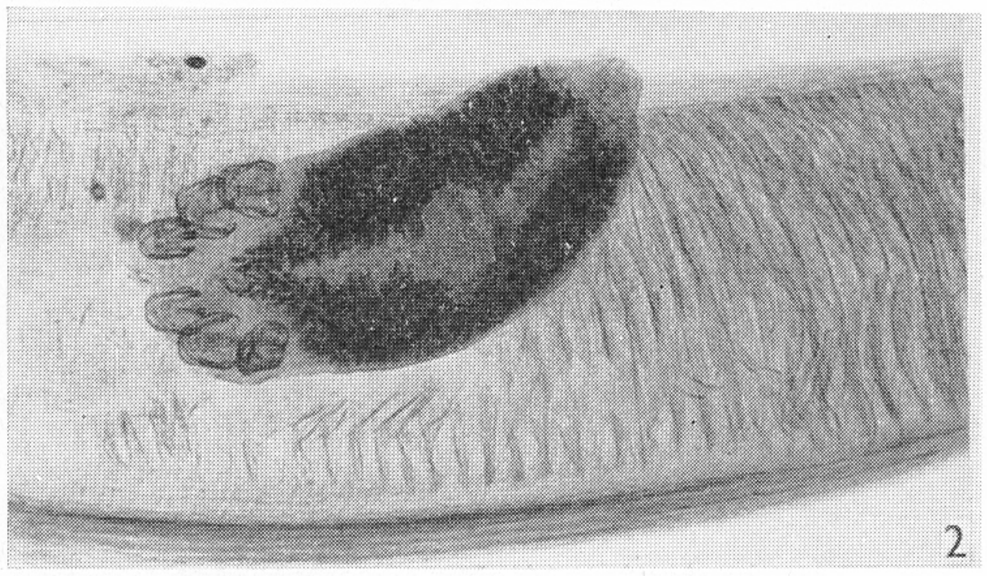
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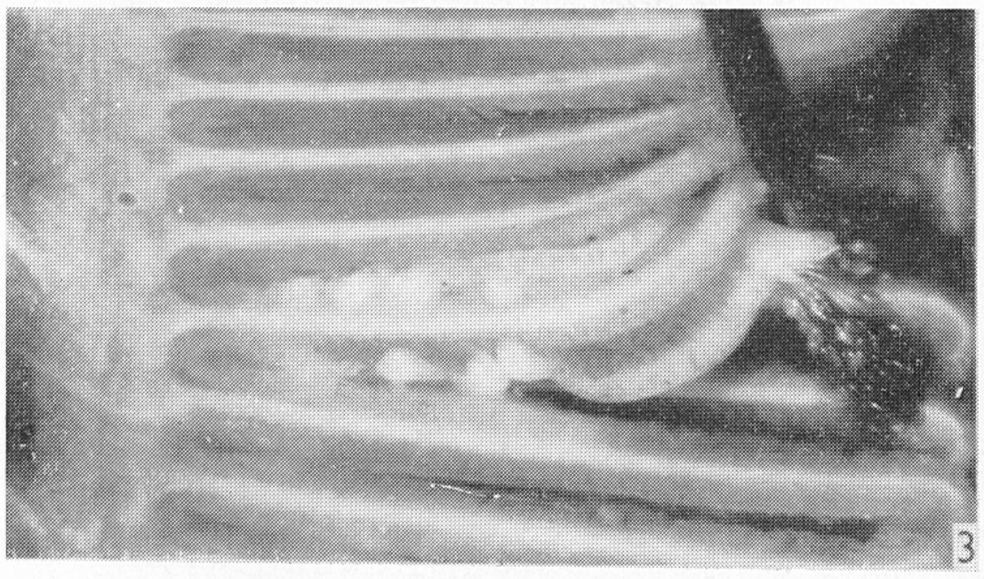
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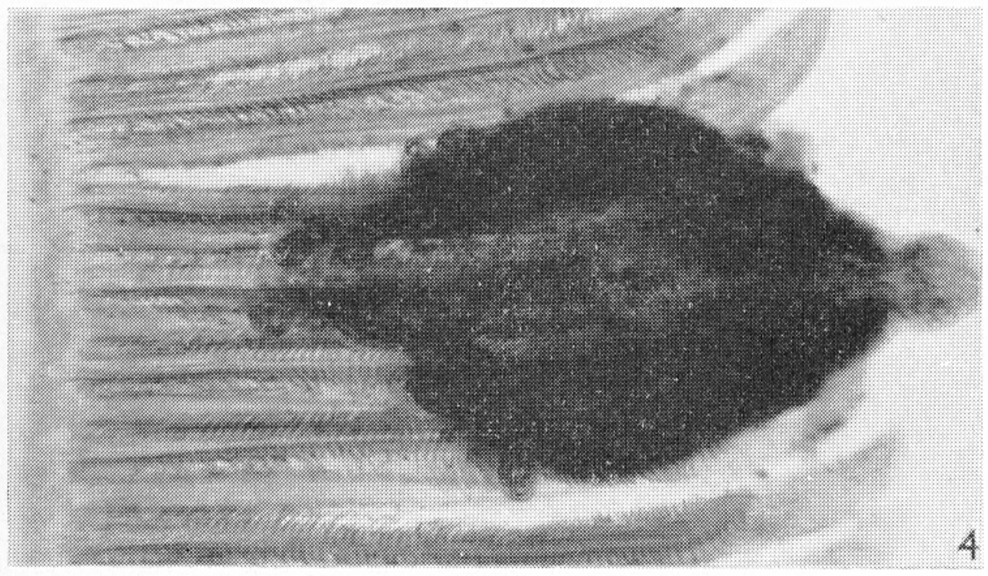
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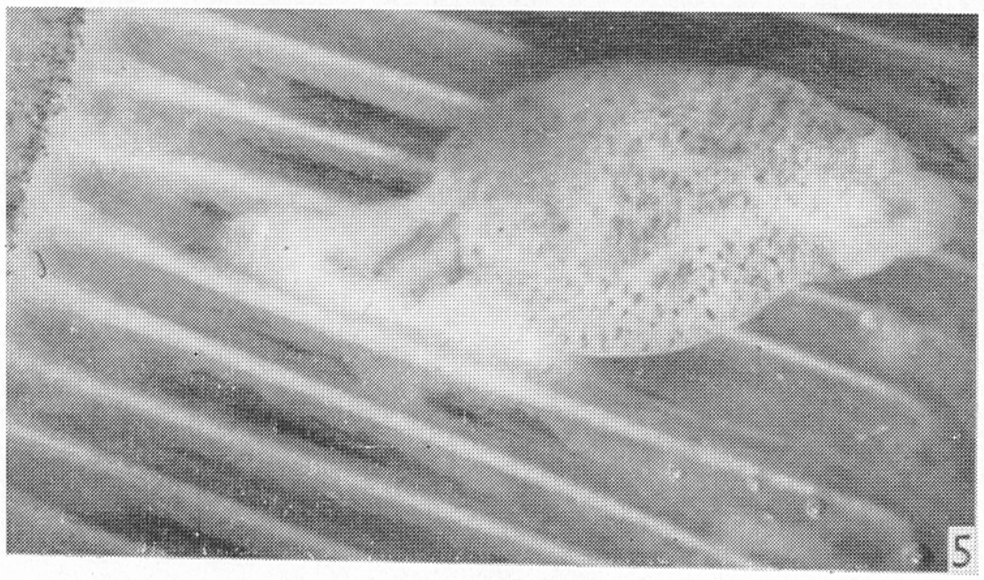
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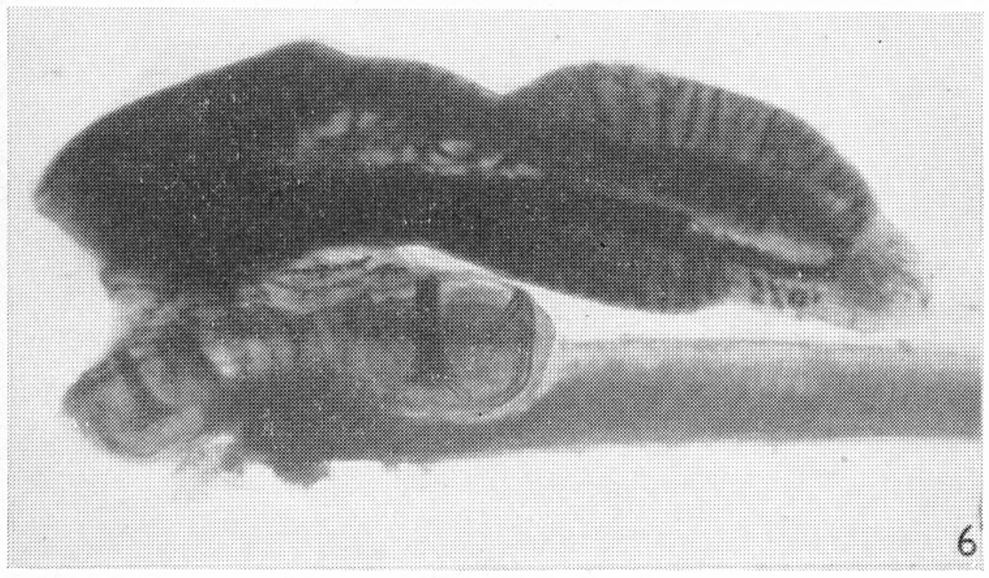
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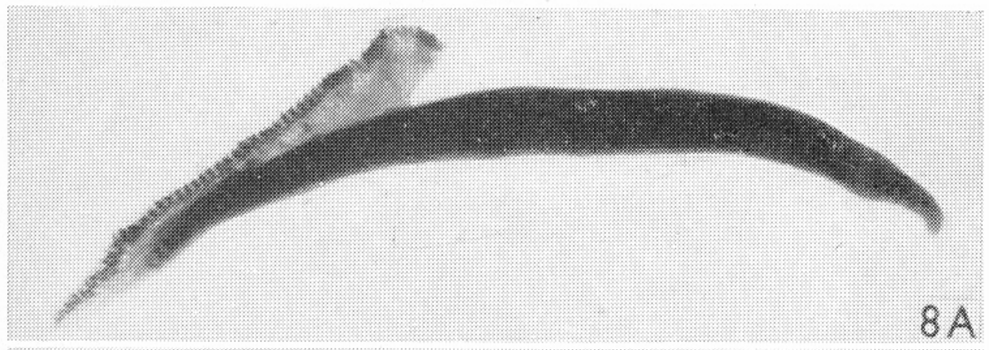
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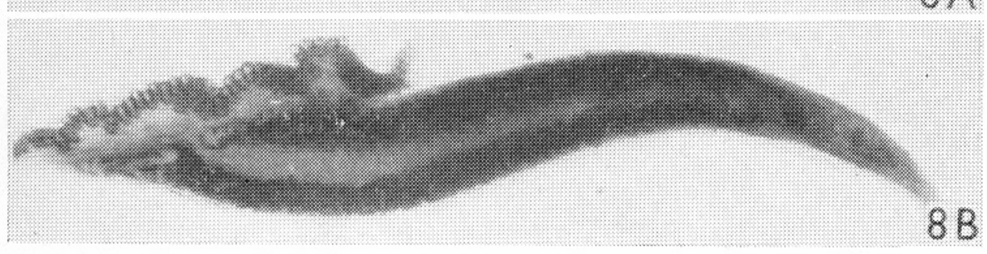
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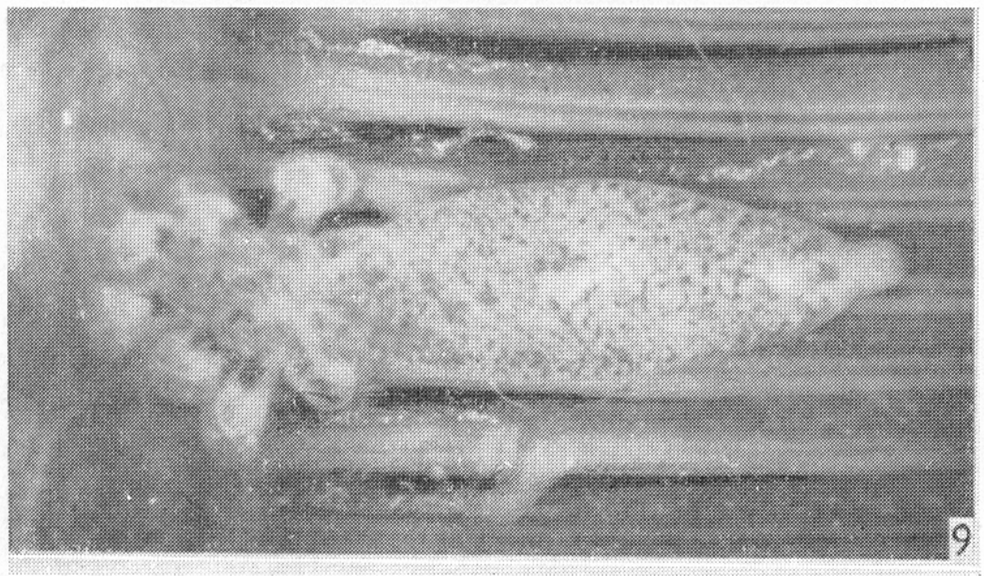
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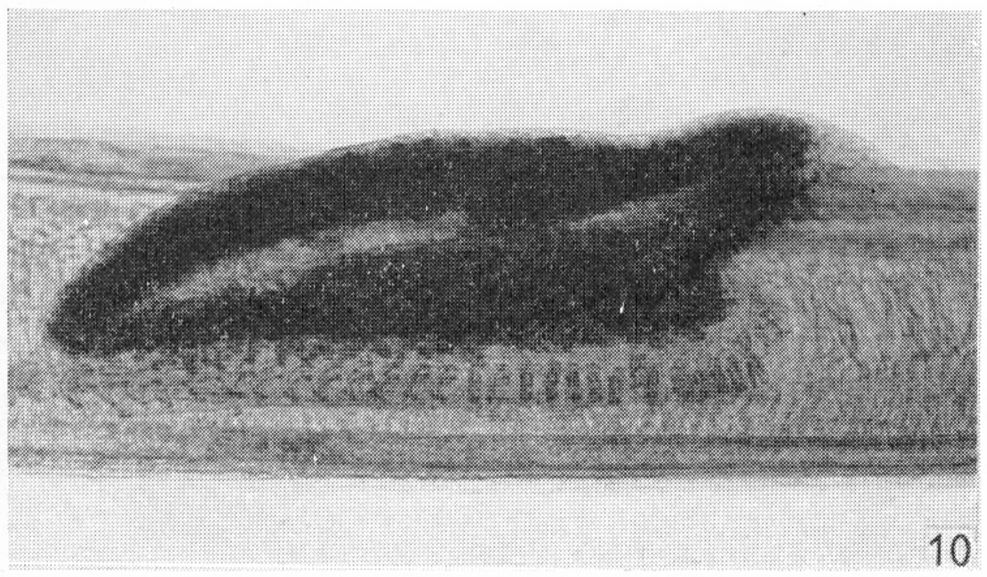
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Direction of gill-ventilating current

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EXPLANATION OF PLATES

PLATE I

The range of form exhibited by representative diclidophoroidean gill trematodes. (Specimens flattened under pressure of cover-glass and mounted in Canada Balsam. Index-line in all figures = 1·0 mm.)

Fig. 1. *Octostoma scombri.*
Fig. 2. *Plectanocotyle gurnardi.*
Fig. 3. *Discocotyle sagittata.*
Fig. 4. *Microcotyle* sp. (from *Serranus cabrilla*).
Fig. 5. *Diclidophora denticulata.*
Fig. 6. *D. merlangi.*
Fig. 7. *Cyclocotyla chrysophryi.*
Fig. 8. *Anthocotyle merluccii.*
Fig. 9. *Axine belones.*
Fig. 10. *Pseudaxine trachuri.*
Fig. 11. *Gastrocotyle trachuri.*

PLATE II

Adhesive attitudes of representative diclidophoroidean gill trematodes. (Figs. 3, 5, 7 and 9: living specimens; Figs. 8A, B: specimens fixed, but not flattened; Figs. 1, 2, 4, 6 and 10: specimens in Canada Balsam.)

Fig. 1. L.S. *Discocotyle sagittata* on *Salmo trutta.*
Fig. 2. *Plectanocotyle gurnardi* on *Trigla cuculus.*
Fig. 3. *Diclidophora luscae* on *Gadus luscus.*
Fig. 4. *D. merlangi* on *G. merlangus.*
Fig. 5. *Anthocotyle merluccii* on *Merluccius merluccius.*
Fig. 6. *A. merluccii* on *Merluccius merluccius.*
Fig. 7. *Axine belones* on *Belone belone.*
Fig. 8A. *A. belones*, body in side view.
Fig. 8B. *A. belones*, body in ventral view (same specimen as 8A).
Fig. 9. *Cyclocotyla chrysophryi* on *Pagellus centrodontus.*
Fig. 10. *Gastrocotyle trachuri* on *Trachurus trachurus.*

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RECEPTOR ELEMENTS IN THE MUSCLES OF *LEANDER SERRATUS*

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(Plates I and II, and Text-figs. 1–4)

In continuation of the studies on receptor elements in the Crustacea the common prawn, *Leander serratus*, has been investigated by the methylene-blue method applied in the same way as described in an earlier publication (Alexandrowicz, 1951). It has been found that there are in *Leander* receptors of the same kind as in those crustaceans previously investigated, some of which exhibit features not yet observed before. As in other crustaceans they are similarly situated on the dorsal side of the body, but since a knowledge of the disposition of the dorsal muscles in the thorax and the abdomen is necessary for locating them, some data about the topography of the muscles are first given.

Topography of the dorsal muscles

The disposition of the muscles as they come to view when exposed from the dorsal side by removal of the carapace and terga is shown in Text-fig. 1. On the left side a part only of the carapace has been removed, and the muscles are left *in situ*. On the right the muscle flanking the heart, which is the first head of the lateral thoracico-abdominal muscle, has been partly removed and the epimeral plate turned with its median surface uppermost. In this way the muscles inserting into this plate become exposed, and on such preparations the receptor elements of the thorax can be examined. It must, however, be borne in mind that in their normal position the muscles follow the curvature of the body and the epimeral plate lies in the dorso-ventral plane.

On pulling aside the posterior part of the first head of the lateral thoracico-abdominal muscle one can see its second head—a flat muscle spreading fanwise with its bundles inserting into the epidermal plate.

In the second large mass of muscles passing from the thorax to the dorsal side of the abdomen three units, called the 1st, 2nd and 3rd dorsal thoracico-abdominal muscles, can be distinguished. All three have their anterior attachments on the epimeral plate. The first dorsal thoracico-abdominal muscle inserts posteriorly into the anterior edge of the first abdominal segment. The second consists of two portions which run side by side anteriorly but separate posteriorly. One portion passes over into the abdomen and fuses end to end with the supplementary extensor muscle of the first abdominal segment; the

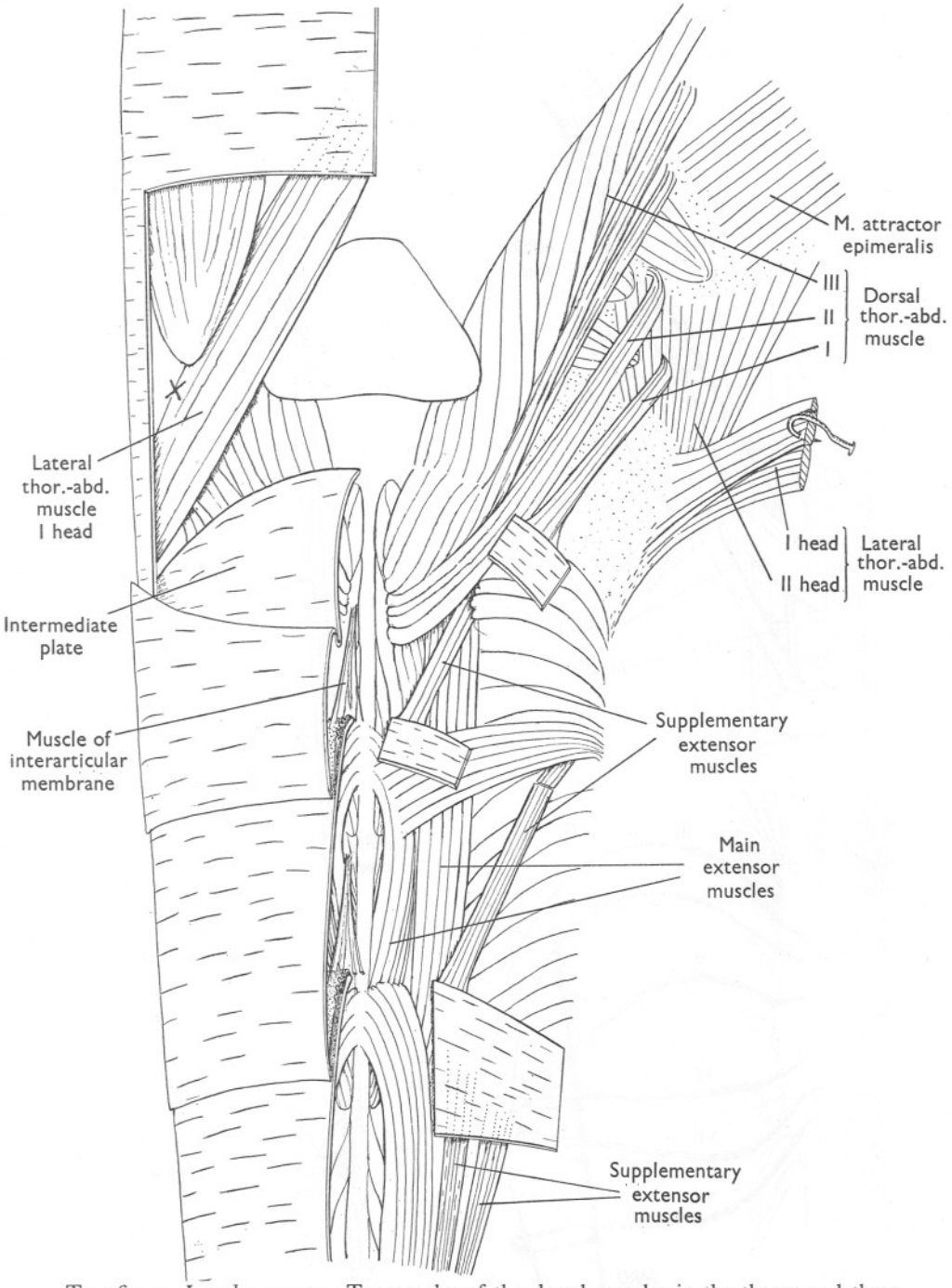
second portion passes under the intermediate plate and helps to form a bulky muscle filling up the concavity of this plate (Text-figs. 1, 2).[1] The 3rd dorsal thoracico-abdominal muscle, the stoutest of all, consists of three portions with separate attachments on the epimeral plate. They pass posteriorly under the intermediate plate, but a part of the stoutest portion is interrupted by a constriction at the anterior edge of this plate. The arrangement of the muscles situated under the intermediate plate is complicated, since it is here that the fibres of various parts of the thoracico-abdominal muscles and also of the extensor muscles of the abdomen meet. The anterior border of the latter is marked by a tendinous intersection which can be seen when the overlying fibres of the thoracic muscles are pulled aside (Text-fig. 2A).

The terms designating the muscles are the same as those used in the work on *Homarus* and *Palinurus* (Alexandrowicz, 1952*a*) and follow, with some minor additions, the nomenclature of Schmidt (1915). It should be emphasized that although there is similarity in the general plan of the musculature of the Macrura there are also differences, and it cannot be claimed that all the muscle units to which the same name has been applied correspond in fact to each other. Besides, the above description is a simplified one and many details in the drawings had to be omitted.

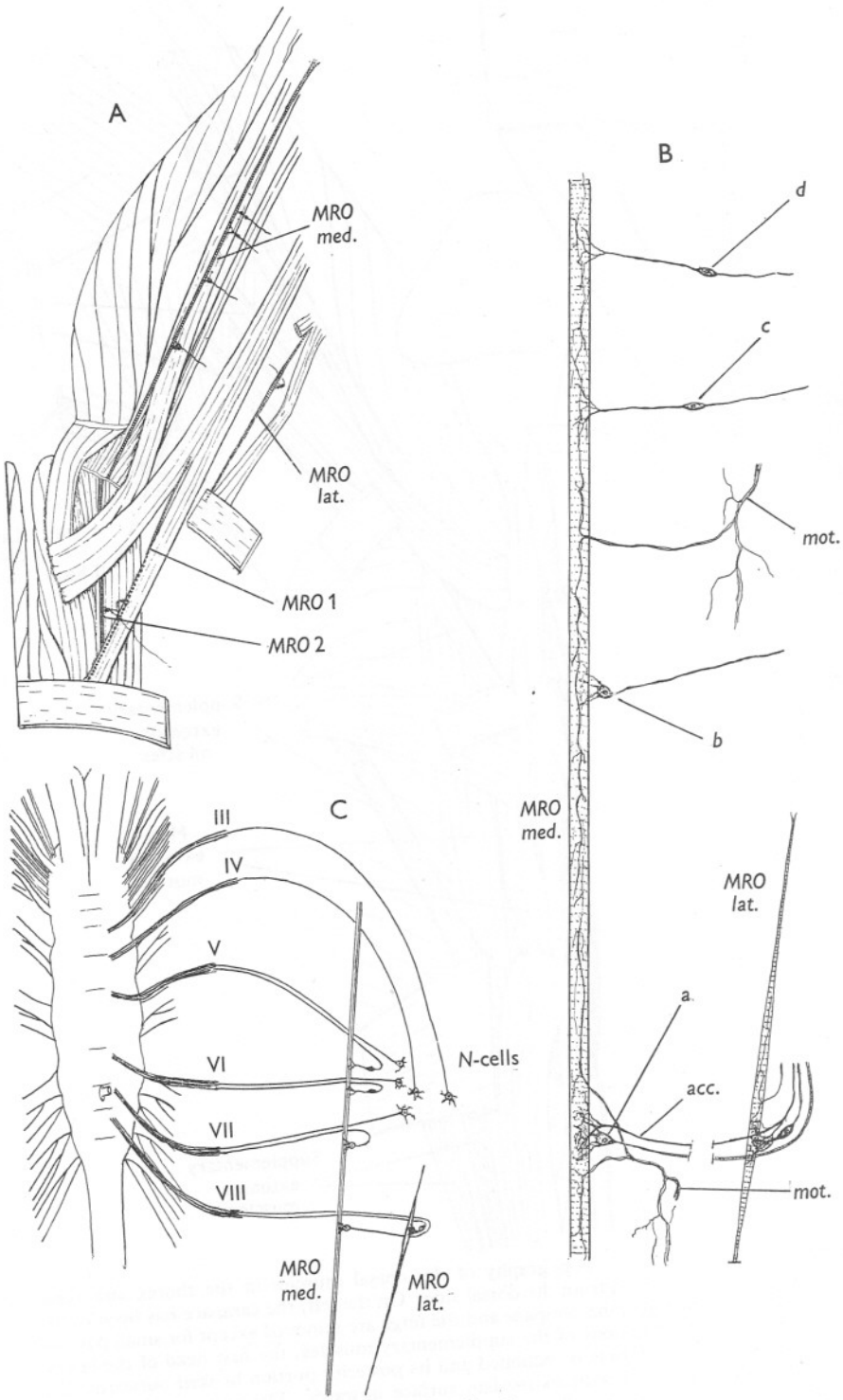
In the abdomen the muscles whose situation should be known when considering the receptor organs are the extensors. Two sets of these muscles which will be called the main and the supplementary extensors can be distinguished. The main extensors, which make up nearly all the mass of this musculature, have their bundles arranged in various ways. Those situated nearer the median line of the body are twisted around each other, while those in the lateral portion take a straighter course. The relations of the bundles of these portions, their courses and modes of attachment are very complicated and cannot be entered upon here.

The term 'supplementary extensors' is proposed for designation of superficially situated flat muscles with parallel or slightly diverging fibres which are never twisted. Posteriorly, in all five segments, they are attached to the anterior edge of the next segment, but they exhibit differences in their length and anterior insertions. In the first segment this muscle is ribbon-shaped and runs forwards and outwards to continue, as aforesaid, with the thoracico-abdominal muscle. In the second segment its shape and direction are the same, but it ends near the anterior border of the segment. In the following

[1] The text-books, even the most comprehensive, skip over this region, and although its chitinous covering, as in *Leander*, forms a conspicuous part of the skeleton it appears not to have a generally adopted name. The term 'intermediate plate' has been used by Calman (1904). Patwardhan (1937), in his monograph of *Palaemon*, calls it in the explanation of his fig. 1 'arthrodial membrane between the thorax and the first segment of the abdomen' and in the text says: 'The posterior half of the inter-tergal membrane connecting the cephalothorax with the tergum is calcified and rigid....' I am grateful to Dr Marie V. Lebour for pointing out to me these two sources of information.



Text-fig. 1. *Leander serratus*. Topography of the dorsal muscles in the thorax and three abdominal segments seen from the dorsal side. On the left, the carapace has been partly sectioned. On the right, the carapace and the terga are removed except for small parts of the latter with the insertions of the supplementary muscles; the first head of the lateral thoracico-abdominal muscle is sectioned and its posterior portion hooked outwards; the epimeral plate is turned with its median surface upwards. The cross on the left side marks the position of an *N*-cell.


A
MRO
med.
MRO
lat.
MRO 1
MRO 2
B
d
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b
MRO
med.
MRO
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a
acc.
mot.
C
III
IV
V
VI
VII
VIII
N-cells
MRO
med.
MRO
lat.

Text-fig. 2.

segments the muscles become gradually shorter, wider, and divide into two or three portions with slightly diverging fibres. They insert into the tergum at a distance from its anterior border which is gradually greater in the subsequent segments (Text-fig. 3). It is to be noted that the histological structure of various units of the dorsal musculature is not the same, viz. the supplementary abdominal extensors and the 1st and 2nd thoracico-abdominal muscles have a more coarse cross-striation.

The term supplementary muscles, used here for facilitating the description, can be misleading as it may imply a subordinate role. It is true that these muscles are weak, but considering their different structure there is a ground for assuming that they must have a special function. Moreover, as similar differences have been observed in other crustaceans (Macrura, Stomatopoda, Amphipoda), it can be inferred that these animals have two systems of the extensor muscles presumably one for slow and the other for fast contractions. It is also for this reason that a special term seems to be appropriate.

Receptor Elements

Two categories of receptors in the muscles of *Leander* can be distinguished.

(1) Muscle receptor organs (*MRO*) consisting of nerve cells connected with special receptor muscles (*RM*). As in other crustaceans previously investigated—(*Homarus vulgaris*, *Palinurus vulgaris*, *Pagurus striatus*, *P. calidus*, *Squilla mantis* (Alexandrowicz, 1951, 1952*a*, *b*, 1954), *Cambarus clarkii* (Wiersma, Furshpan & Florey 1953), they are of two sorts (*MRO* 1, *MRO* 2).

(2) Receptor cells, called *N*-cells, which end in ordinary muscles and have been found, as in *Homarus* and *Palinurus*, in certain thoracic muscles.

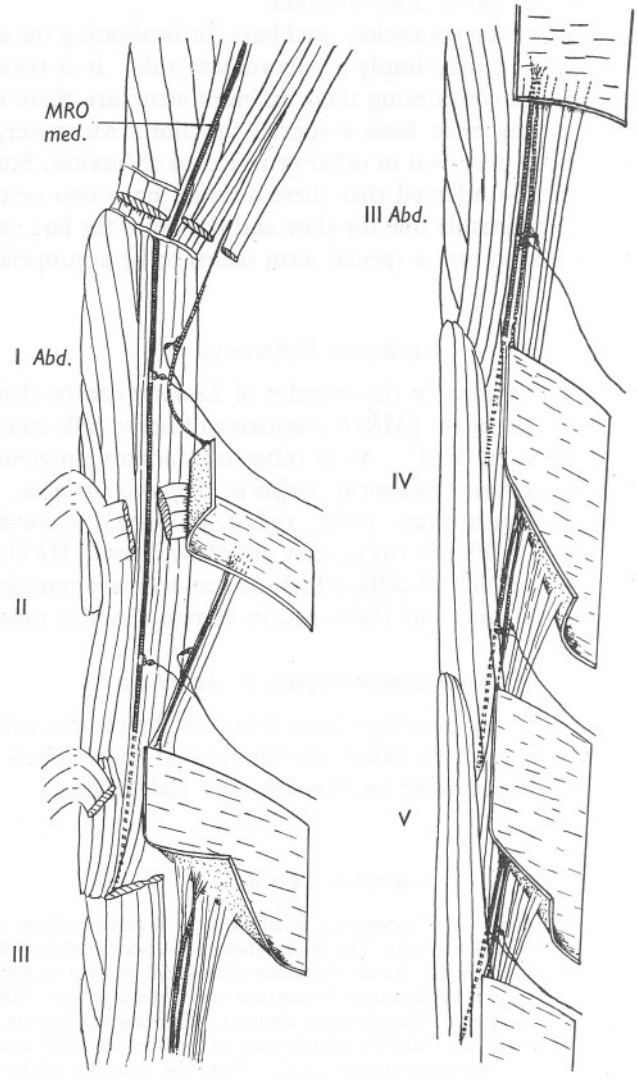
Muscle Receptor Organs in the Thorax

Two muscle receptor organs have been found in the thorax, and according to their situation they can be called the lateral and the median *MRO*, the same terms as used in the work on *Homarus* and *Palinurus*.

Legend to Text-fig. 2

Text-fig. 2. *Leander serratus*. A, Topography of the muscle receptor organs in the thorax and in the first abdominal segment. The dorsal thoracico-abdominal muscles are spread apart (cf. Text-fig. 1); the 1st dorsal thoracico-abdominal muscle is partly removed. *MRO lat.*, *med.*, lateral and median muscle receptor organ of the thorax; *MRO* 1, *MRO* 2, muscle receptor organs of the 1st abdominal segment. B, Muscle receptor organs of the thorax (semi-diagrammatic). Only the middle part of the median *MRO* and the anterior part of the lateral *MRO* are represented (cf. C). Note the situation of the nerve cells *a* and *b* close to the muscle and of the cells *c* and *d* at a distance from it. *mot.*, motor fibres; *acc.*, accessory nerve. C, Diagram showing the connexions of the thoracic receptor cells with the suboesophageal ganglion. III–VIII, nerve trunks of the 3rd–8th segment carrying axons of the receptor cells of the *MRO* and of the *N*-cells.

The lateral *MRO* lies between the bundles of the 1st dorsal thoracico-abdominal muscle (Text-fig. 2A). Its muscle component has in its middle part the shape of a spindle with its thickest part (*c.* 35μ) lying nearer to the anterior end. This spindle thins out in both directions to a fine thread, only *c.* 10μ thick. Both its attachments take place near to those of the 1st dorsal



Text-fig. 3. *Leander serratus.* Topography of the muscle receptor organs in five (I–V) abdominal segments. Note the varying length and shape of the supplementary extensor muscles (removed in the first segment), the gradual shortening of the receptor muscles in the 3rd–5th segment, and the fusion of the muscles of the *MRO*2 in the 1st and 2nd segments. Parts of receptor muscles covered by muscle fibres are drawn in dotted lines.

thoracico-abdominal muscle, but the receptor muscle, although it is so thin and runs between the other muscle fibres, can be traced as an individual unit.

The nerve cell of this *MRO* is bipolar with dendritic processes starting with a common root (Pl. I, fig. 2). They expand in the thicker part of the muscle in dense arborizations (not seen in the photograph). The axon joins the nerve trunk belonging to the 8th thoracic segment. The situation of this receptor is very unfavourable, both for staining and observation. To see its muscle it is necessary to pull aside the muscle fibres covering it; the nerve cell is hidden from view by the nerve fibres curving round the thoracico-abdominal muscle. Moreover, the same nerve trunk conveys some deviating fibres of the pericardial organs which at this point break up in fine branches arranged as small glomeruli. To see the cell as shown in the photograph all these elements have to be removed, but the operation is very delicate.

It is difficult to get a clear picture of the nerves supplying this receptor. There is certainly a motor fibre for the muscle and there are also fibres of the accessory innervation intermingling with the arborizations of the cell-dendrites, but whether they derive from one or two accessory nerves I am unable to say.

The median thoracic *MRO* can be found much more easily. In preparations with the median side of the epimeral plate exposed as in Text-fig. 1 it can be seen on the surface of the middle portion of the 3rd dorsal thoracico-abdominal muscle (Text-fig. 2A). Anteriorly it ends near the insertions of the adjacent muscle; running backwards it passes deeper under the bundles of the 3rd dorsal thoracico-abdominal muscle, and its posterior insertion into the connective tissue septum between the thoracic and abdominal extensors can be seen only after the superficial layer of the muscles in the region of the intermediate plate is pulled aside. The muscle of the median *MRO* is much thicker than that of the lateral one, measuring 50–60 μ in diameter. Nearing the anterior end it becomes thinner. There are a good many connective tissue fibres running longitudinally on the periphery of the muscle and between the bundles of the myofibrils. Towards the anterior end the connective fibres gradually predominate over the muscle elements.

Nerve Cells

Four nerve cells are connected with the median receptor muscle. Their positions as shown in Text-fig. 2B and C are the most frequent, but variations in the situation of the three anterior cells are common. In one preparation all three have been found lying close together (Pl. I, fig. 4).

There are certain differences in the appearance of the cells. The posterior one (Text-fig. 2B*a*; Pl. I, fig. 1) is multipolar; it is situated close to the receptor muscle, sending into it dendrites spreading over an area the length of which is equal to approximately three diameters of the cell. The tuft of arborizations is well delimited on both sides. In this region the myofibrils of the muscle, at least most of them, are replaced by connective tissue fibres.

The next cell *b* is usually multipolar and very similar to cell *a*, but often shows variations in its shape; sometimes with all the dendrites arising with a common root it looks like the anterior cells *c* and *d*. The latter are bipolar with distal processes which are of such a length that the cells as a rule lie much farther from the muscle than the cells *a* and *b* (Pl. I, fig. 3). Here again variations are not uncommon, and one or the other cell can have a multipolar shape and lie close on the muscle.

The dendrites of the cell *b* show the pattern of arborizations similar to those of the cell *a*, but are often more irregularly delimited. The dendrites of the two anterior cells are less densely arranged, but can be much longer than in cell *a*. The expansions of the three cells presumably end on the connective fibres, but the picture is not clear as the myofibril bundles pass through these areas.

The variable appearance of the terminations of the three anterior cells may be partly due to the unevenness of the staining, but evidently also because they are affected by frequent irregularities. As we see later this feature can help us in understanding the nature of these elements.

The axons of the four cells are directed at first outwards and, after joining the nerve trunks running on the median side of the epimeral plate, turn ventrally to proceed towards the suboesophageal ganglion: the axon of the posterior cell on curving round the thoracico-abdominal muscle is joined by the axon of the lateral *MRO*, and both travel in the nerve trunk of the 8th thoracic segment. The three other axons belong most probably to the 5th-7th segments, as shown in Text-fig. 2C. Some uncertainties arise when tracing these axons on account of the anastomoses occurring between the nerve trunks of various segments in which they travel. Moreover, the axons of the cells *b* and *c* or *c* and *d* are sometimes to be seen running side by side; it can be assumed that such associations are accidental and that these fibres in their farther course part company.

The nerves carrying the axons of the receptor organs belong to the set of nerve trunks originating in the suboesophageal ganglion on its dorsal side. They include the motor nerves of the thoracic muscles, the various nerves of the heart, of the pericardial organs and also the axons of the *N*-cells (see below).

Nerves

The motor fibres for the median receptor muscle branch from the nerves of the adjacent muscles. One fibre approaches the receptor muscle near the posterior cell and another in front of it in the region between the two middle cells (Text-fig. 2B). Each of them gives off branches running in the muscle in both directions.

Only the posterior cell has an accessory innervation limited to one fibre, which can be qualified as the thick accessory nerve (Pl. I, fig. 1). The thin accessory nerve seems to be missing.

Muscle Receptor Organs of the Abdomen

In the abdomen, muscle receptor organs have been found in the 1st to 5th segments. In the 6th they are most probably missing, but as the particular arrangement of the muscles in this segment makes the dissection difficult there is no absolute certainty as to this point. In the first two segments the two receptor units of one side (*MRO* 1 and *MRO* 2) are separated from each other, whereas in the 3rd-5th segments they lie close together.

The muscle receptor of the *MRO* 1 of the first segment (*RM* 1) has its forward insertion in the region of the intermediate plate between the bundles of the 2nd dorsal thoracico-abdominal muscle (Text-fig. 2A). From this point it runs obliquely backwards and inwards close to the supplementary extensor passing on its ventral side near its inner edge. Its posterior attachment is on the anterior border of the tergum of the 2nd segment. This *RM* 1, like the lateral thoracic *RM*, has a spindle-shaped middle part continuing in both directions with thin threads. The diameter of this thin part at the mid-point between its anterior attachment and the nerve cell was found to be 8 μ.

The muscle of the second more medially situated receptor *RM* 2 inserts anteriorly quite near the posterior end of the median thoracic *MRO* into the septum separating the thoracic and abdominal extensors (Text-fig. 3). In its backwards course, which is almost parallel to the median line of the body, it lies on the lateral portion of the main extensor muscle. At the border between the 1st segment and the 2nd segment it fuses end to end with the *RM* 2 of the 2nd segment, so that actually there is one receptor muscle running through the first two segments and passing into the anterior part of the third segment where it ends among the bundles of the main extensor muscle. Its total length in middle-sized specimens is about 2 cm, and the diameter which does not change much at various levels is about 50 μ. The position of this *RM* is superficial in these regions, where the muscle bundles on which it is lying are not covered by other muscles. In order to see it in its whole length these overlying muscles must be removed (Text-fig. 3). It is somewhat difficult to isolate the receptor muscle in the region between the two segments, and it is possible that the connective tissue fibres accompanying the *RM* 2 have here some points of attachment, but the continuity of the muscle can be ascertained.

The *RM* 1 of the second segment resembles that of the first segment in its dimensions and direction of its course. It lies on the supplementary muscle near its median margin, and its insertions are near to those of this muscle (Text-fig. 3).

In the 3rd-5th segments the two *MRO* units are situated near to each other, and the anterior attachments of both *RM* are at the same level (Pl. II, fig. 7). Posteriorly each *RM* 1 inserts into the anterior edge of the tergum of the following segment, whereas the *RM* 2 runs farther backwards and passing

deeper into the layer of the main extensor muscle ends between the bundles of the latter. The length of both *RM* decreases from the 3rd to 5th segment and so does the calibre of the *RM* 2. The *RM* 1 retains about the same thickness in its middle part and does not taper so much as in the first segments. Already in the 3rd segment the differences between the two *RM* are not so great as in the first two segments, and in the 5th segment the two muscles are almost of the same calibre. In the diagrammatic drawings (Text-fig. 3) the diameters of the *RM* are out of scale. Their true proportions can best be understood from the photographs (Pl. II, figs. 7, 8) made from the same preparation of the *MRO* of the 3rd segment. The represented parts of the muscles added together include only about half of their total length.

In all segments the receptor muscles are accompanied by connective tissue fibres running longitudinally; even in the thinnest parts of the *RM* 1 the elements of both the muscle and connective tissue are present. Connective tissue also replaces all or almost all the myofibrils of *RM* 2 in the area of expansion of the cell dendrites. In *RM* 1 a good deal of myofibrils can be seen passing over this area. Some difference in cross-striation of the muscles as recorded previously in the large Macrura and Stomatopoda can be observed, viz. the *RM* 1 has a coarser and *RM* 2 a finer cross-striation.

Nerve cells

The nerve cells in the abdominal *MRO* are situated laterally to the muscles, with exception of the cells of the *MRO* 1 in the 1st and 2nd segments, in which they lie either directly above the muscles or medially to them. The particular features of the cells of one pair of *MRO* can be better seen in the posterior segments where both receptors lie close to each other. Here they exhibit great resemblance to the pictures observed in *Homarus* (Pl. I, fig. 5). Cell 1 (of the *MRO* 1), which is always situated in front of cell 2, has wider extending processes than the latter in which these processes and all their ramifications are closer to each other. Consequently, the areas of the respective muscles in which the arborizations of the cells are interlaced with the end-branches of the accessory nerves differ markedly in size and shape (Pl. I, fig. 6).

In Pl. I, fig. 5, representing the receptors of the 5th segment, cell 1 is larger of the two. In the 1st–4th segments the cells are about the same size.

The cells are surrounded by membranaceous connective tissue. There is possibly a cavity separating the cells from this capsule as in large Macrura and Stomatopoda, but it is not distinct enough. Fine nerve fibrils wind round cell 2 forming a so-called basket-work. Similar fine fibres, but not so numerous, can sometimes be noticed on the periphery of cell 1.

Nerves

The pattern of the motor innervation is the usual one, i.e. some of the motor fibres run down alongside the axons of the cells and others branching from the motor nerves of the adjacent muscles reach the *RM* at points lying farther from the cells.

There are many other fibres approaching the cells and entering into relations with their dendrites (Pl. I, fig. 6; Pl. II, fig. 9). Most of them can be traced to their origin from one fibre of stouter calibre—the thick accessory nerve which is conveyed by the same trunk running on the surface of the dorsal muscles into which pass the axons of the receptor cells. In this trunk run also finer fibres which can be regarded as thin accessory nerves.

The thick accessory nerve gives off branches entering into relations with the dendrites of both receptor cells; whether it also supplies both receptor muscles or only the *RM* 1 is not clear. As to the thin accessory nerve it can be assumed that its branches reach the terminations of both cells, but their distribution is not clear. In general, the methylene-blue preparations with *Leander* tissues give less distinct pictures than with the large Macrura. It is therefore not to be expected that such points as appeared dubious in the larger species could be elucidated with this less suitable material.

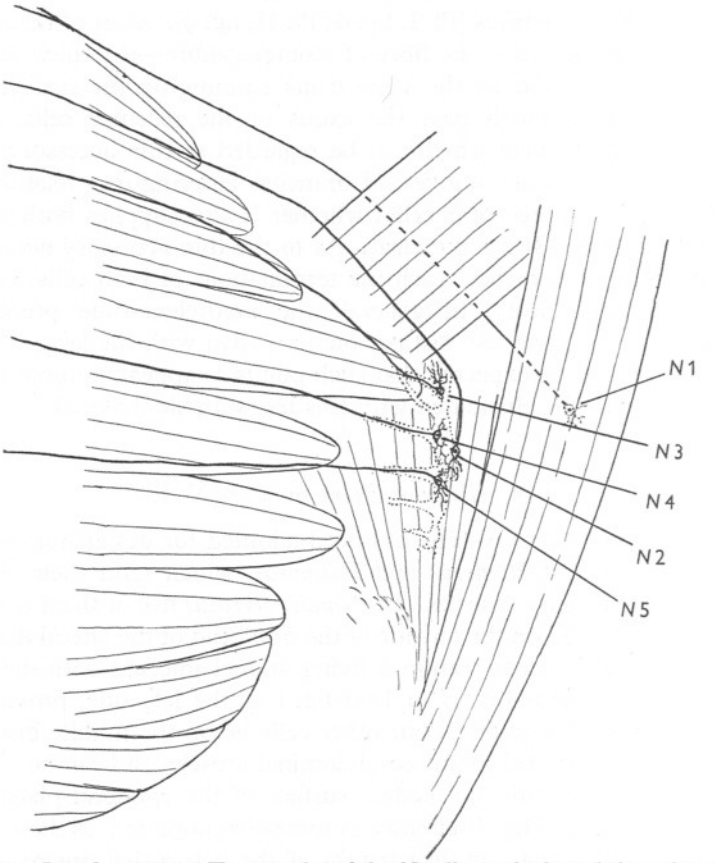
Receptor cells in ordinary muscles (N-cells)

The conventional term *N*-cells has been adopted for designation of nerve cells first described in *Homarus* and *Palinurus*, which send their dendritic expansions into ordinary muscles. In *Leander serratus* five of them have been found. One is situated on the outside of the first head of the lateral thoracico-abdominal muscle; it can be seen in a living animal injected with methylene blue at the point indicated by *X* in Text-fig. 1 on the left side, provided the cuticle is transparent enough. Four other cells lie on the middle bundles of the 2nd head of the lateral thoracico-abdominal muscle, and can be observed only on preparations with the median surface of the epimeral plate turned upwards (Text-fig. 4). The observation is somewhat impaired, as these *N*-cells are situated between the arch-like trunks of the pericardial organs and the muscles and therefore are covered by the former. As there is no connexion between the pericardial organs and the nerve cells it is possible to remove the overlying parts of them without injuring the cells and their processes.

The *N*-cells are about the same size as the nerve cells of the *MRO* or even larger than some of them. They are therefore comparatively larger than in *Homarus* and *Palinurus*, in which their size is always smaller than in *MRO*. The dendrites arising from different points of the cell body penetrate between the bundles of the muscles over a larger area than in the *MRO*. The muscles in these regions do not show any particular changes. The photographs

(Pl. II, figs. 10, 11) give an idea of the general appearance and various forms of the *N*-cells, but cannot show the processes running deeper into the muscles which are out of focus.

Whether the endings of these processes are on the muscle tissue or on connective fibres is difficult to discern, especially as the muscles in crustaceans are not composed of such units as the muscle fibre of vertebrate histology,



Text-fig. 4. *Leander serratus*. Topography of the *N*-cells on the lateral thoracico-abdomina muscle viewed from the inside (cf. Text-fig. 1). Cell *N*1 situated on the outside of th first head of the muscle is seen by transparency. The proximal course of the axons of th *N*-cells is shown in Text-fig. 2C.

but consist of bundles of myofibrils anastomosing with one another. Accor ingly the connective tissue separating the muscle mass into fasciculi a enveloping the myofibril bundles does not form such regular tubular she as the endomysium of the vertebrate muscle.

In the description of *N*-cells in *Homarus* more probability has been g to the assumption that their processes end on connective fibres. A corro

tive evidence is now afforded by the fact that the dendrites of the most anterior cell *N*3 (Text-fig. 4) can be seen branching on a strip of tissue continuing with the muscle but in which no myofibrils can be noticed.

The axons of the *N*-cells in their course towards the suboesophageal ganglion join each a nervous trunk of a different segment (Text-fig. 2c). The axon of the cell *N*1 situated on the outer side of the lateral thoracico-abdominal muscle enters a branch of the nerve of the 3rd segment, passing on the lateral side of the m. attractor epimeralis. The axons of the four remaining cells run in the trunks of the 4th-7th segments. The cell *N*2 belonging to the 4th segment is situated behind the cell *N*3 of the 5th segment. Its axon crossing m. attractor epimeralis on its inner surface near to its ventral attachment can be easily found in every preparation, as it stains readily even while its cell is pale or invisible.

The axons of the cells *N*3–5 run in the trunks of the 5th–7th segments, which are surrounded by the neuropiles of the pericardial organs. As stated in the paper describing these structures (Alexandrowicz, 1953) no relation between the *N*-cells and the pericardial organs has been found.

Discussion

It will be noted from the above description that the structure and arrangement of receptor elements in the muscles of *Leander*, although similar to that of the large Macrura, have certain peculiar features. One of them is the extraordinary thinness to which one receptor muscle, the *RM*1, can be reduced. In the 1st abdominal segment the ratio of its diameter in the anterior part ($8\,\mu$) to the total length of the muscle would be approximately 1:1000. It is, further, interesting that although both receptor muscles vary in their dimensions in different segments these variations are by far greater in *RM*1. Hence it may be concluded that this muscle needs finer adjustments to the mechanism of movements in each of the segments, presumably conditioned by the differences in the arrangement of their extensor muscles.

The receptors of the second set, the *MRO*2, show two unusual features: the fusion of the muscles of the 1st and 2nd abdominal segments and the occurrence of four cells connecting with one muscle in the thorax. The fusion of the receptor muscles has not been observed, either in the Decapoda or in the Stomatopoda. In some other crustaceans, however, on which some investigations are being made, a fusion of the muscles has been found. Thus in the thorax of *Ligia oceanica* (Isopoda) one receptor muscle runs through two segments, but the nerve cells are in one of them only; in *Praunus flexuosus* (Mysidacea) the muscles of both *MRO* pass through three thoracic segments, and in each of them two nerve cells are present. The functional meaning of such fusions of the muscles is difficult to conceive.

The median receptor organ in the thorax, which obviously belongs to the

same set as *MRO* 2 in the abdomen, appears as an anomaly not only because four cells connect with one muscle, but also because one of them has the accessory innervation which the others have not. However, anomalies in the anatomy of the *MRO* in the thorax can be easily explained as resulting from the retrogression of these organs in the segments which have become fused and have lost their motility in the course of the phylogenetic evolution of the Crustacea. As pointed out previously (Alexandrowicz, 1954, p. 105), during this process some *MRO* can disappear and in others certain components can be missing. According to this conception only the last thoracic segment has in *Leander* retained both muscle receptor organs, but in all probability they possess only one of the two accessory nerves. The three anterior cells of the median *MRO* can be regarded as the remnants of receptor organs of the 5th–7th thoracic segments, probably their *MRO* 2. They send their dendrites into the common muscle, that is perhaps made up of fused muscles of several segments, but have lost all their accessory innervation. The irregularities in the shape and position of these cells corroborate the assumption of their being elements of retrograding organs.

The receptor elements of the second category, the *N*-cells, have been found in *Leander* in the same number, five, as in *Homarus* and *Palinurus*. This does not necessarily mean that they are all exactly the same set of homologous elements, because there is no certainty that in each of these animals all *N*-cells have been found. Besides, the arrangement of the cells and the muscles is different, and the homology of the latter has yet to be established.

Examination of the *N*-cells in *Leander* has not helped towards understanding their function. Some features, such as the concentration of four cells on one small strip of muscle, make their role even more enigmatic. In a previous paper (Alexandrowicz, 1952*a*), when surveying the different types of receptor elements, it has been suggested that the *N*-cells might represent a more primitive type from which those with special muscles have developed. However, it is equally conceivable that, when highly specialized muscle receptors are becoming redundant, the reversed process may occur in the course of which their own muscles disappear and the nerve cells enter into relation with the ordinary muscles. In that case the *N*-cells might be the remnants of the retrograding receptor organs, presumably the *MRO* 1. The connexions of their axons with the 3rd–7th thoracic segments would fit into this picture. On the other hand, however, there is nothing in their appearance which would indicate that they are decadent elements. The cells are comparatively large, shapely, and their processes are well developed. In trying to solve the problem of the *N*-cells it would be of much help to know whether they are only in the thorax or whether they occur in other parts of the body also. The present investigation, in which thoracic and abdominal muscles of *c.* 150 specimens were examined, makes the first alternative more probable, but the possibility that some nerve cells have not been detected is still to be reckoned with.

Summary

In *Leander serratus* muscle receptor organs have been found in the thorax and in the 1st–5th segments of the abdomen. In the abdomen they are represented, as in other crustaceans, by two units on each side consisting of a thin muscle connected with nerve cell and supplied by motor and accessory nerves. The two muscles (*RM*1 and *RM*2) of each pair of receptor organs keep separated in the first two abdominal segments, whereas in the 3rd–5th they run close together. The muscles *RM*2 of the 1st and 2nd segments are fused end to end, forming one muscle unit running through the segments and connected in each of them with the receptor cell.

In the thorax two muscle receptor organs, a lateral and a median, are present. The former has the usual components, i.e. one muscle and one nerve cell; the median consists of one muscle and four nerve cells. The axons of the posterior cell of the median receptor and that of the lateral receptor run towards the suboesophageal ganglion in a nerve trunk of the 8th thoracic segment; those of the three anterior cells join the trunks of the 5th–7th segments. It is suggested that these three cells are the remnants of muscle receptor organs which have been reduced following the fusion of the thoracic segments and loss of their motility.

The receptor elements of the second category, the *N*-cells, with the dendrites ending in ordinary muscles, have been found, five in number, in connexion with the lateral thoracico-abdominal muscle. Their axons run into the suboesophageal ganglion with the nerve trunks of the 3rd-7th thoracic segments.

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EXPLANATION OF PLATES I AND II

All photomicrographs were made from preparations of *Leander serratus* stained with methylene blue, fixed in ammonium molybdate and mounted in xylol-dammar.

PLATE I. The scale at the bottom applies to all figures.

Fig. 1. Part of the median thoracic muscle receptor organ with its posterior nerve cell. *mot*, motor fibre; *acc*, accessory nerve.

Fig. 2. Middle part of the lateral thoracic receptor organ. *RC*, receptor cell, *RM*, receptor muscle. The outlines of the dendritic arborizations are blurred by a diffuse staining.

Fig. 3. Three anterior cells (*b*, *c*, *d*, cf. Text-fig. 2B) of the right median thoracic *MRO*.

Fig. 4. Three anterior nerve cells of the median thoracic *MRO* bunched together and showing irregularly arranged processes.

Fig. 5. Muscle receptor organs of the 5th abdominal segment (left side). The nerve cell of the *MRO* 1 is larger and is situated anteriorly.

Fig. 6. Muscle receptor organs of the 5th abdominal segment (right side). Note the differences in size and shape of the networks formed by the cell dendrites and the accessory nerves. The nerve cells are faintly stained and are invisible in the photograph.

PLATE II. The scale at the bottom applies to figs. 9–11.

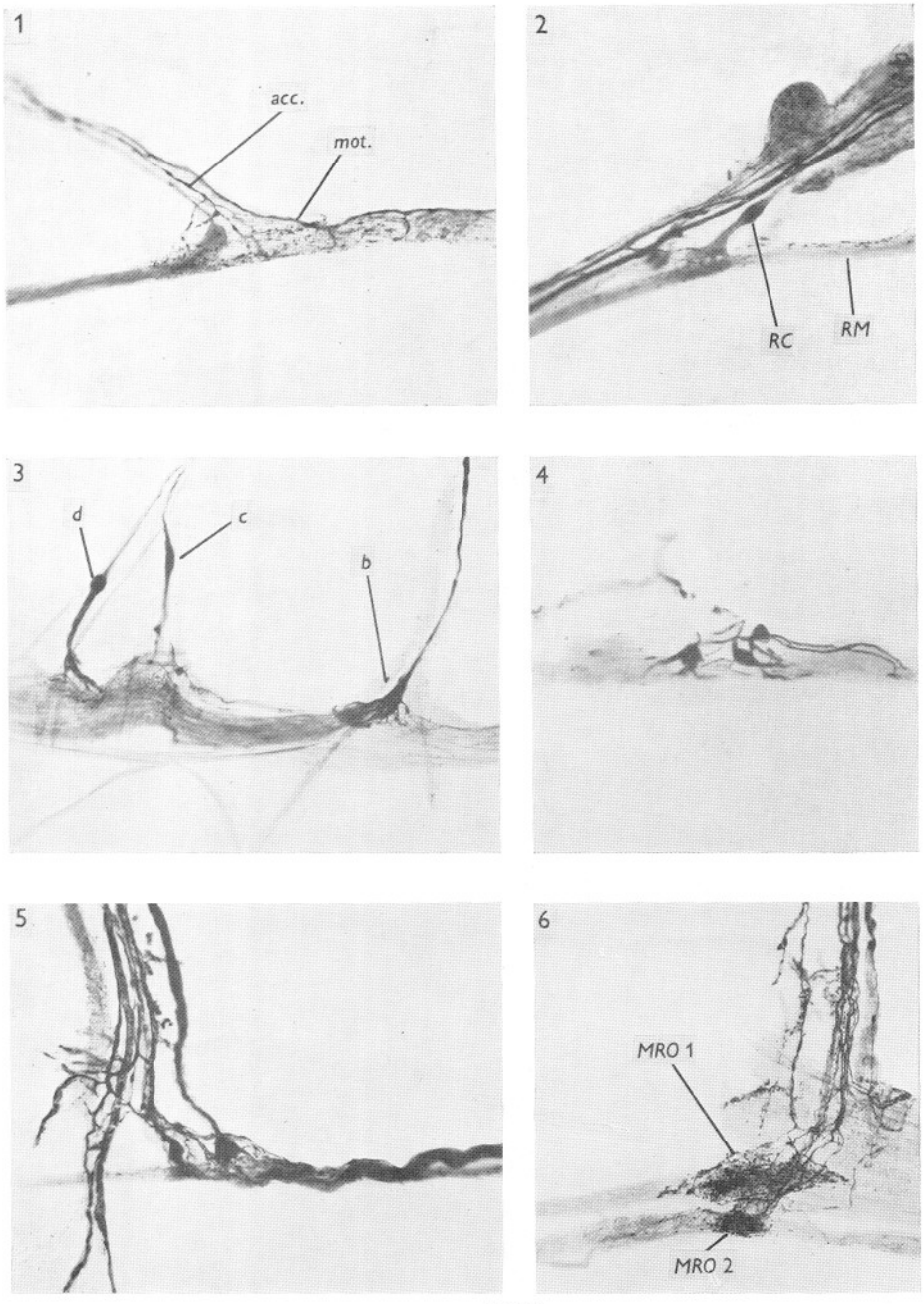
Fig. 7. Middle quarter of the *MRO* of the 3rd abdominal segment of the left side. The receptor muscles became displaced during the mounting of the preparation: in the animal body the *RM* 1 lies dorsal and to the side of the *RM* 2; it passes on its median side in the anterior quarter as seen in fig. 8.

Fig. 8. Same preparation as in fig. 7. Anterior quarter of the *MRO* of the 3rd segment with the attachment of the receptor muscles.

Fig. 9. Motor and accessory nerves of the *MRO* of the 3rd segment. The kink in the muscle and its thickenings are artifacts.

Fig. 10. Two *N*-cells (*N* 2 and *N* 4, Text-fig. 4). The overlying arch-like parts of the pericardial organs are almost completely removed.

Fig. 11. *N*-cell situated on the outside of the first head of the lateral thoracico-abdominal muscle (*N* 1, Text-fig. 4).



Figs. 1–6.

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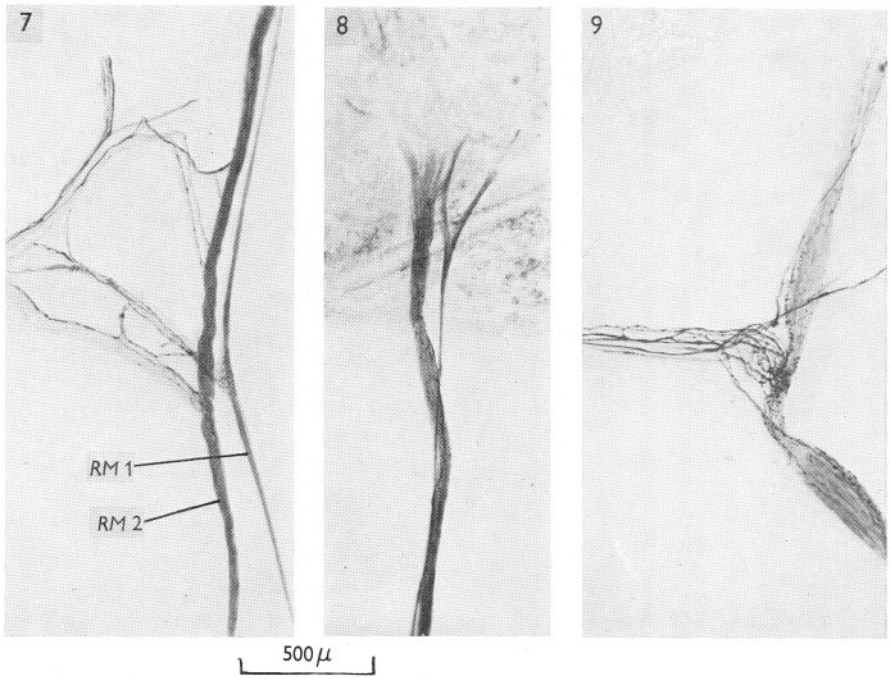

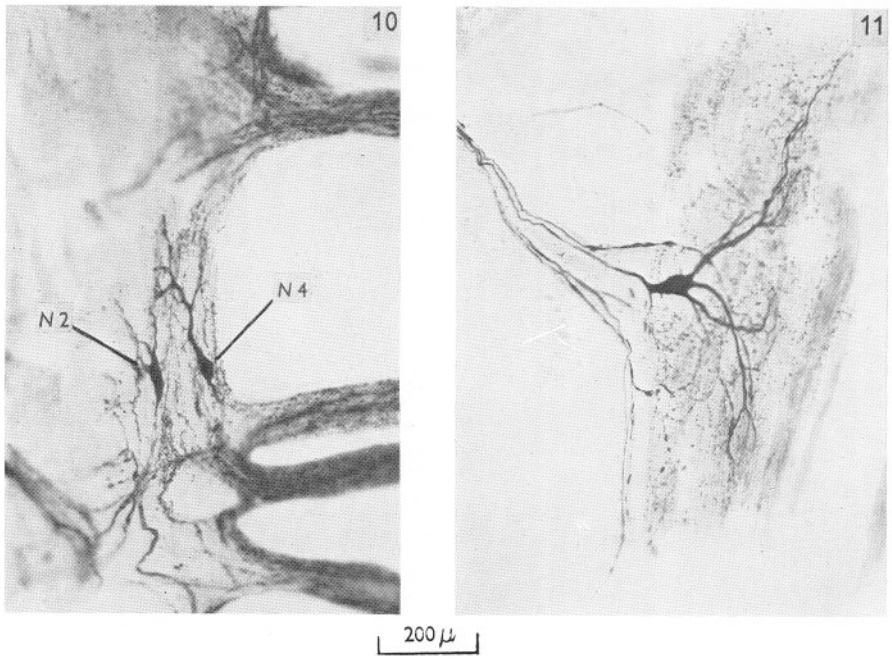

Figs. 7–11.

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STUDIES ON THE BIOLOGY OF LIMPETS

I. THE LATE J. H. ORTON'S WORK ON *PATELLA*

By A. J. Southward and J. M. Dodd

From the Plymouth Laboratory and the Gatty Marine Laboratory, St Andrews

At the time of his death in 1953 Orton had almost completed an extensive study of the biology of the three British species of *Patella*. Although certain aspects of the investigation were dealt with by collaborators and have already been published (Crewe, 1947 on parasites; Goodwin, 1950 on pigments; Jones, 1948 on ecology) most of the breeding and spawning data have been neither analysed nor published. With the knowledge that Orton's records contained the most complete information on breeding yet compiled for a littoral animal, and that these records had considerable bearing on the question of speciation in *Patella*, we have attempted to analyse and present those which are most complete.

Orton first became interested in the biology of limpets during a general study of growth rate in marine animals in which it became necessary to determine the breeding periods of the animals involved (Orton, 1914, 1920*a*). In *Patella* the preponderance of males in the smaller size-groups and of females in the larger size-groups suggested a change of sex during the life-history similar to that found in other gastropods (Orton, 1909). A preliminary account of the sex proportions (Orton, 1920*b*) was followed by a fuller account in 1928, when the results of investigations on the rate of growth and on shell dimensions were also presented (Orton, 1928*a*, *b*). Other papers dealt with ecology and showed the need for further special studies (Orton, 1929, 1932, 1933).

Orton was unable to resume his investigation of these problems until 1945, and a year later he announced some preliminary results. These confirmed the previous evidence on sex change, and demonstrated differences in the breeding periods of the three British species (Orton, 1946). The investigations proceeded rapidly from this date until his death, the aspects under study including size, sex proportions, breeding cycle, spawning stimulus, rate of growth, parasitization, and general ecology (Orton, 1948, 1949).

To prepare for publication some of the great mass of information collected by Orton, a good deal of selection has had to be practised. In the first place, aspects such as rate of growth, on which the data were very incomplete, have been omitted. The breeding records have been reduced considerably by omitting unnecessary duplicate samples, and small samples. The series of records from Cullercoats has been rejected because of discrepancies between successive samples; in any case this particular limpet population has been

studied in detail by other workers (Das & Seshappa, 1947). Records of parasitized neuter individuals have been omitted since the neuter gonad in these cases may be the result of parasitic castration rather than a stage in the normal gonad cycle.

In his later work on the breeding cycle, Orton classified the gonads into ten stages of advancing ripeness and a similar number of post-spawning stages in each sex. These data have been reclassified on the basis of an earlier and simpler scheme which facilitates comparison with other species.

A representative series of gonad stages of *Patella vulgata* was sent to one of us (J. M. D.) by Orton, and these have been used to describe the histology of the gonad. For the macroscopic description of the gonad stages we were fortunate in having available some of Orton's MS. notes and drawings: these also indicated the method which he intended to use for the diagrammatic presentation of the breeding data. The other tables and diagrams are our own, and for these and for the interpretation of the evidence we are wholly responsible.

The nomenclature adopted is based on personal communications from Mr R. Winckworth to Orton, and is the same as that of Brian & Owen (1952). The three British limpets are therefore *P. vulgata* Linnaeus; *P. aspera* Lamarck, 1819 (=*P. athletica* Bean, 1844,=*P. depressa* Jeffreys, 1865); *P. depressa* Pennant, 1777 (=*P. intermedia* Jeffreys, 1865).

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STUDIES ON THE BIOLOGY OF LIMPETS

II. THE BREEDING OF *PATELLA VULGATA* L. IN BRITAIN

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

From the Plymouth Laboratory and the Gatty Marine Laboratory, St Andrews

(Plates I and II and Text-figs. 1–4)

Some preliminary observations in North Cornwall (Orton, 1946) showed that the periods of breeding of the three British species of limpets were different. In order to determine these differences more accurately, and to study the breeding cycle, samples of limpets from six localities in Britain were examined at regular intervals for 5 years. This paper gives the results obtained for the common limpet *Patella vulgata*, which was present at all the localities sampled and was the most intensively studied of the three species. Later papers will deal with the remaining species and with other aspects of the investigations.

The work fell roughly into two stages. For the first season, 1945–46, it was necessary to determine exactly the distribution of sexes in the various size-groups at the different stations, since it was known that at Plymouth the small sizes were preponderantly male (Orton, 1920, 1928) and roughly equal numbers of both sexes are desirable for regular observations on breeding. At the same time the opportunity was taken to re-investigate sex-phenomena which had been noted previously and thought to indicate a change of sex during the life-history (Orton, 1920, 1928). For the remaining period of study emphasis was placed on determining the exact period of spawning in the various localities, and on possible spawning stimuli.

Although J. H. O. collected most of the samples that were taken personally, as distinct from those collected by other professional or amateur collectors, all three authors have taken part in the field work. J. H. O. was mainly responsible for the examination of the gonad stages, their grouping, and the calculation of the various numerical factors, J. M. D. carried out the histological work and described the arbitrary stages, whilst A. J. S. analysed the breeding cycle and explored the relationship between meteorological factors and spawning. This paper has been prepared by A. J. S. and J. M. D., who are responsible for all opinions expressed herein.

Sampling and Examination of Samples

The regular samples were obtained from the Bay of Nigg, Aberdeen; Pier rocks, St Andrews; Millport, Isle of Cumbrae; Kallow Point, Port St Mary, Isle of Man; Trevone, North Cornwall; and the south side of the breakwater at Plymouth. The collections were usually made at about M.T.L.: the Millport samples, however, were generally taken nearer to H.W.N., while the specimens from Plymouth breakwater were from a ledge half-way between M.T.L. and L.W.N. (see Southward & Orton, 1954, fig. 2, sections C–C and D–D). Additional samples were obtained from other localities in the Isle of Man and near Plymouth during the spawning period to investigate the influence of local factors on spawning.

While many samples were collected personally and examined the same day, a large proportion were sent by post in damp sea-weed, and there was sometimes a delay of up to 4 days between collection and examination of the gonads. The limpets do not seem to have suffered from this treatment: as Eslick (1940) found, they survive well out of water.

The length of the shell was used as an approximate index of size and age. There are no known secondary sexual characters in *Patella*, and the gonad was therefore examined directly, and its stage of development determined, by cutting away the foot from most of its attachment to the visceral mass and shell, and pushing it over anteriorly, thus disclosing the gonad lying beneath the visceral mass. The sexes could usually be separated clearly by colour (Orton, 1928), the male gonad being pinkish white or cream, and the female green or brown (Pl. I), but doubtful cases were examined microscopically. There was no way of distinguishing the sexes of those individuals in the resting phase between spawning and development of the gonad except, in some cases, by microscopic section, and these have been classed simply as the neuter resting stage.

The remaining gonad stages could usually be distinguished as to sex by macroscopic examination. They have been grouped into a series of arbitrary stages, of both development and depletion (spawning), each stage being assigned a roman numeral (Table I). This scheme is based on one put forward in 1910 by a subcommittee of the International Council, for an investigation of herring (cf. Orton, 1916). It differs from the original scheme in distinguishing between recently spent individuals and those in a resting phase prior to recovery of the gonad. Moreover, in the present scheme all stages represent definite increments in the size of the gonad, and it is therefore possible, in a single index, to define the mean gonad condition of the population.

The samples were first grouped into the arbitrary stages by the use of the criteria given below; the numbers of the sexes in each group, and those neuter, were then recorded. From the data so obtained four variables have been extracted by means of which it is possible to define the breeding state of

the population sample. One of these is the mean gonad condition, noted above; and the other three are percentages denoting the proportion of the sample in the resting, developing, and spawning condition. The mean gonad condition, or the mean stage of development of the gonad, is determined as follows: the sexes are considered together, since their stage of development is usually identical; the numbers of individuals in each developing gonad stage are multiplied by the number allotted to the stage, the values so obtained being added together. The mean condition of the gonad can then be ascertained by dividing the grand total by the number of individuals in the whole sample.

TABLE I. ARBITRARY SCHEME OF CLASSIFICATION OF GONAD STAGES USED IN THE PRESENT WORK

Stage	Brief description of gonad
Developing	
* o (or N)	Inactive or neuter; either rudimentary virgin stage, or resting after discharge of gametes
I	Beginning to develop, and sex detectable, but only slight increase in size
II	Developing to one-third full size
III	Between one-third and two-thirds full size
IV	Two-thirds full size
V	Fully developed
Spawning	
V+	Full, but actively discharging gametes
IV	Discharging, two-thirds full
III	Discharging, two-thirds to one-third full
II	Discharging, one-third full
I	Almost completely discharged

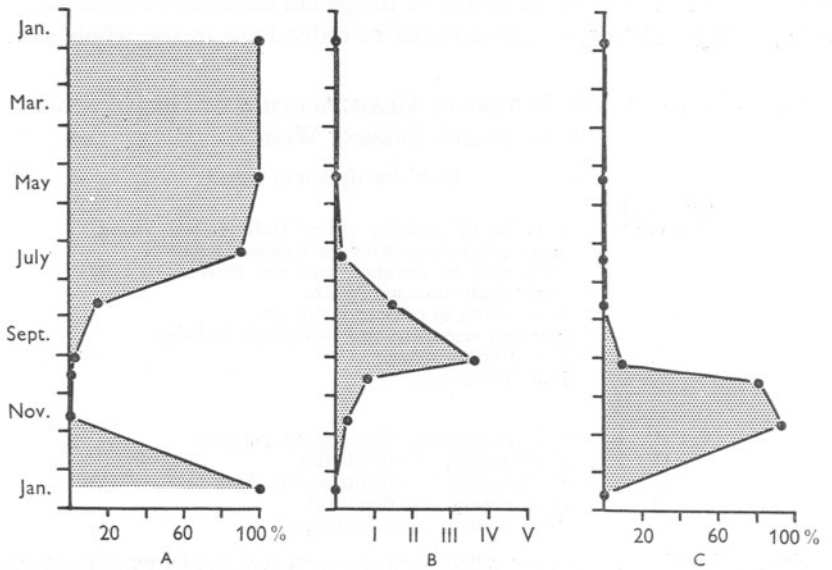
* Virgin and resting stages can be called o and N respectively, but for purposes of calculation of gonad index, both count as o. In the present investigation only the resting stage was concerned in routine work.

An example of a series of samples grouped and treated according to this scheme is given in Text-fig. 1. This method of representation brings out clearly the progression of the reproductive cycle, even when, as in *Patella*, the various stages are prolonged and tend to overlap.

ANATOMICAL RELATIONSHIPS AND HISTOLOGY OF THE GONAD

The limpets used for anatomical and histological description of the gonad stages were fixed in Bouin's fluid in sea water and stored in 70% alcohol. Normal paraffin embedding proved unsatisfactory owing to the size of the mature gonads and the large amounts of reserve material they contained. They were therefore embedded in celloidin which was subsequently impregnated with paraffin wax. Sections were cut at 5, 10, or 15 μ, and stained in either Mallory's triple stain or in Heidenhain's iron-alum haematoxylin followed by eosin or erythrosin Orange-G as a counterstain.

The gonad in both sexes lies on the ventral face of the visceral mass betw this and the foot. It is unpaired and slightly displaced to the left (when viev from above). It varies greatly in size and colour throughout the breed season and its growth causes a good deal of displacement of other organs. I physical reasons it is unable to grow in an anterior direction, consequently anterior margin always lies below the posterior region of the salivary gland

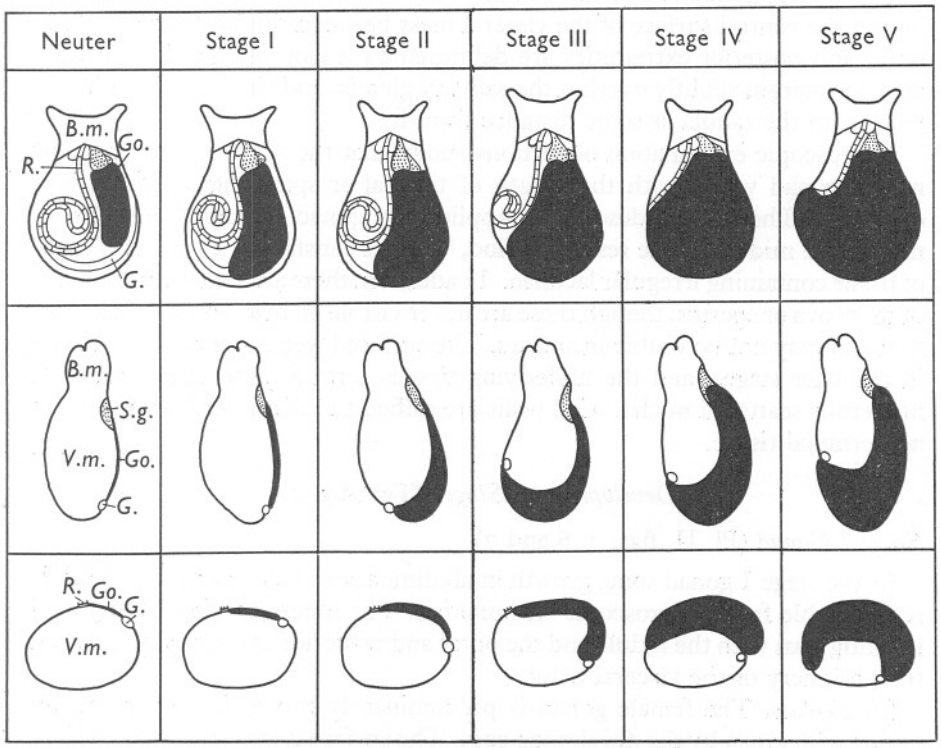


Text-fig. 1. Data defining the breeding state of a population (*Patella vulgata* from Kallow Pt., Port St Mary, Isle of Man, 1946–7; shell length 4·5–5 cm). (A) Percentage with gonad resting (neuter or stage 0). (B) Gonad index denoting the mean state of development of the population. (C) Percentage with gonad in the spawning condition.

The outer lateral margin is delimited by a loop of the hind-gut, and the inner margin by the radula. The lateral and posterior regions of the gonad undergo extensive growth, and a striking increase in thickness is noted as ripeness is approached. Text-fig. 2 shows semi-diagrammatically how the gonad increases in extent and thickness throughout its period of growth; the same diagrams also illustrate the reduction in size which occurs as spawning advances. Concomitant changes which occur in the colour of the gonad are associated largely with changes in the proportions of the reserve material. The latter is orange or brown, the pigment being probably identical with the carotenoids found in almost equal amounts in the gonads of both sexes by Goodwin & Taha (1950) and Goodwin (1950). This pigment becomes dispersed during the development of the gametes. The mature oocytes are coloured green, probably by the 'chromoprotein Y' extracted from female gonads by

Goodwin & Taha, while the increasing whiteness of the developing male gonad is related to the sperm content.

These colour changes are described below and some are illustrated in Pl. I. It must be noted that the coloured illustrations differ from Text-fig. 2 in showing the appearance of the gonad as a result of partly freeing the visceral



Text-fig. 2. *Patella vulgata*: semi-diagrammatic representations of arbitrary stages in the development and depletion of the gonad. Top row: gonad, visceral mass and buccal mass seen from below after removal of the foot. Middle row: longitudinal section of the buccal region and visceral mass passing through the central region of the gonad; plane of section parallel to long axis of body. Bottom row: transverse section of the visceral mass through the widest region of the gonad; plane of section parallel to short axis of body. *B.m.*, buccal mass; *G.*, gut; *Go.*, gonad; *R.*, radula; *S.g.*, salivary glands; *V.m.*, visceral mass.

mass from the foot and turning the latter back as far as it will go while the animal is still in the shell. This was the method used for recognizing the gonad stages in routine analysis; in practised hands it is generally satisfactory. However, as the illustrations in Text-fig. 2 show, a more accurate assessment of gonad state can be made by removing the visceral mass from the foot.

The Neuter Gonad (Text-fig. 2; Pl. II, fig. 1)

In limpets of 1 cm and less in shell length the neuter gonad clearly represents the virgin state: in larger specimens which have already spawned it represents the resting spent stage.

The neuter gonad is a discrete reddish brown kidney-shaped structure. It lies on the ventral surface of the visceral mass between this and the foot. The outer and posterior extremities are delimited by a loop of the hind-gut, the anterior margin slightly overlies the salivary glands, and the inner margin lies parallel to the radula at some distance from it.

Microscopic examination of sections shows that the structure of the spent neuter gonad varies with the length of time after spawning at which it is examined. The present description applies to a gonad examined at approximately the middle of the resting period, when it consists of a very thin layer of tissue containing irregular lacunae. In addition, there are sometimes patches of relict ova or sperms, though these are never visible on macroscopic examination and may not be visible in smears. The surface layer is more obvious than in the later stages, and the underlying tissues form a loose reticulum with numerous scattered nuclei. Cell walls are difficult to distinguish and there is no germinal tissue.

Developmental Stages (Text-fig. 2)

Stage I Gonad (Pl. II, figs. 2, 6 and 7)

In the stage I gonad some growth in all dimensions has occurred and sex is recognizable from macroscopic examination. The inner margin of the gonad is contiguous with the radula and the outer and posterior margins almost reach the periphery of the visceral mass.

(*a*) *Female*. The female gonad is predominantly brown in colour though tinged with green by the developing eggs. The surface is smooth and delimited by an obvious membrane. The largest eggs are few in number and are separated by nests of developing eggs which can be seen only with a lens.

Microscopic examination of the gonad shows very few medium-sized eggs, most of which are in the central region. The gonad is seen to consist mainly of small oocytes in which vitellogenesis is well advanced, and nests of undifferentiated germ cells. The latter are attached to connective tissue trabeculae

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Explanation of Plate I

Patella vulgata: stages in development and depletion of the gonad showing changes in coloration. In these illustrations the *overall* colour of the ventral surfaces of the gonads is shown. The foot has been partly removed from the visceral mass and turned forward.

Fig. 1. Female; spawning stage I.
Fig. 2. Male; spawning stage I.
Fig. 3. Male; spawning stage I–II.
Fig. 4. Male; spawning stage II.
Fig. 5. Female; developmental stage V.
Fig. 6. Male; spawning stage IV.

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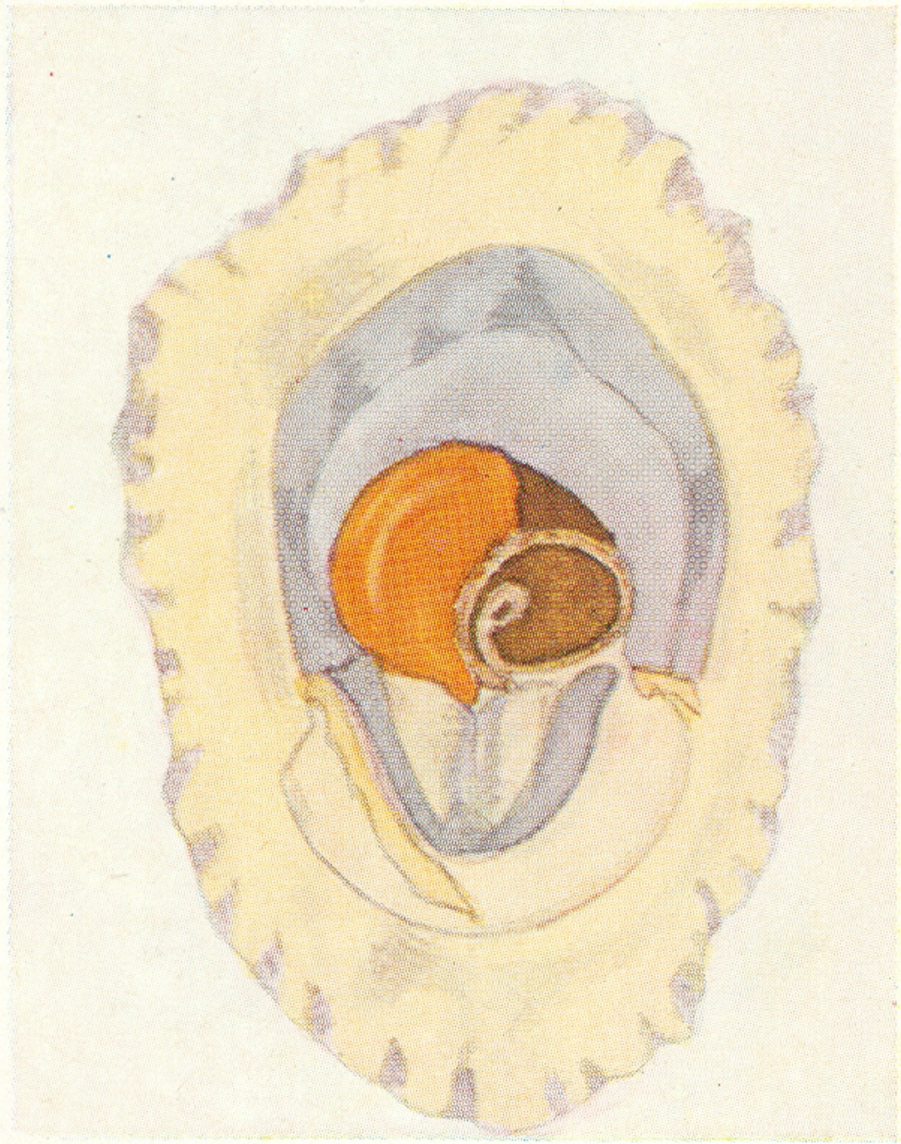

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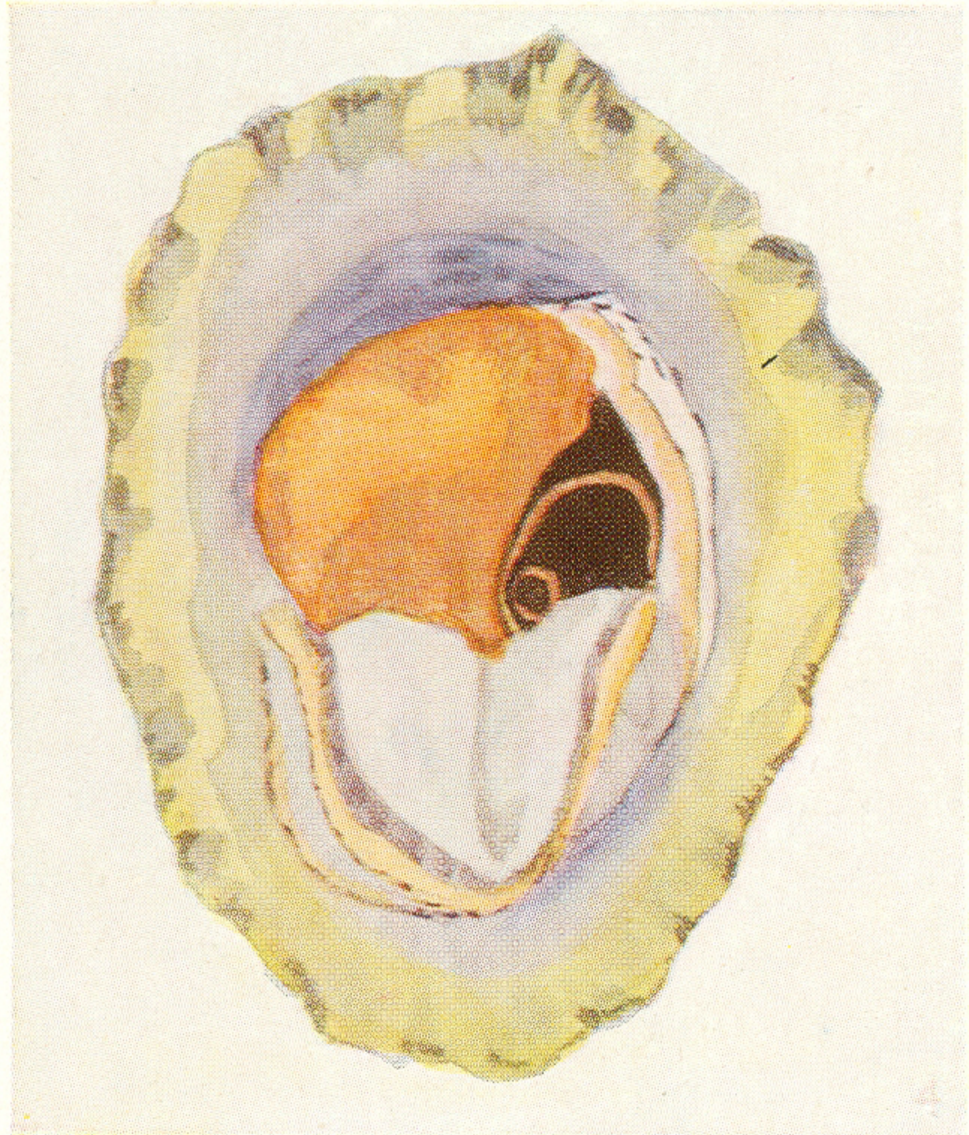

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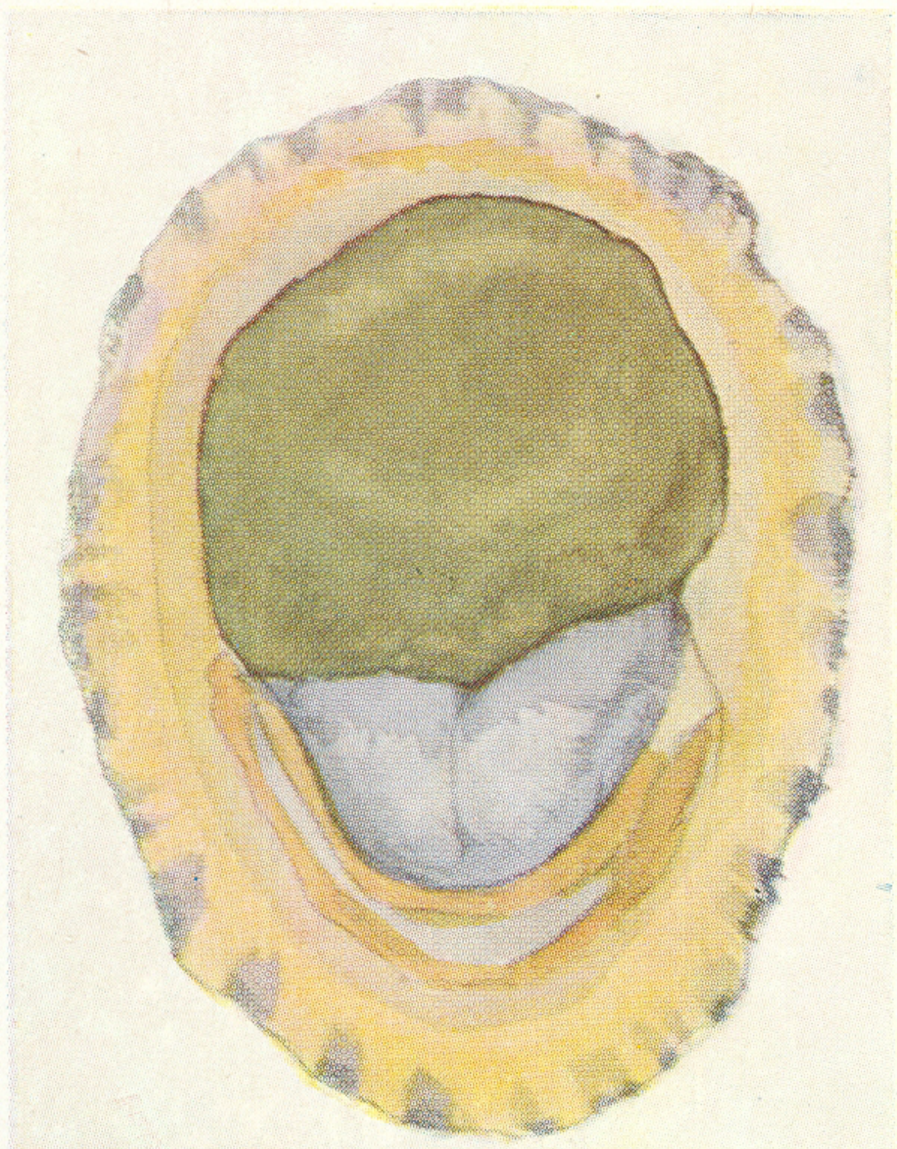

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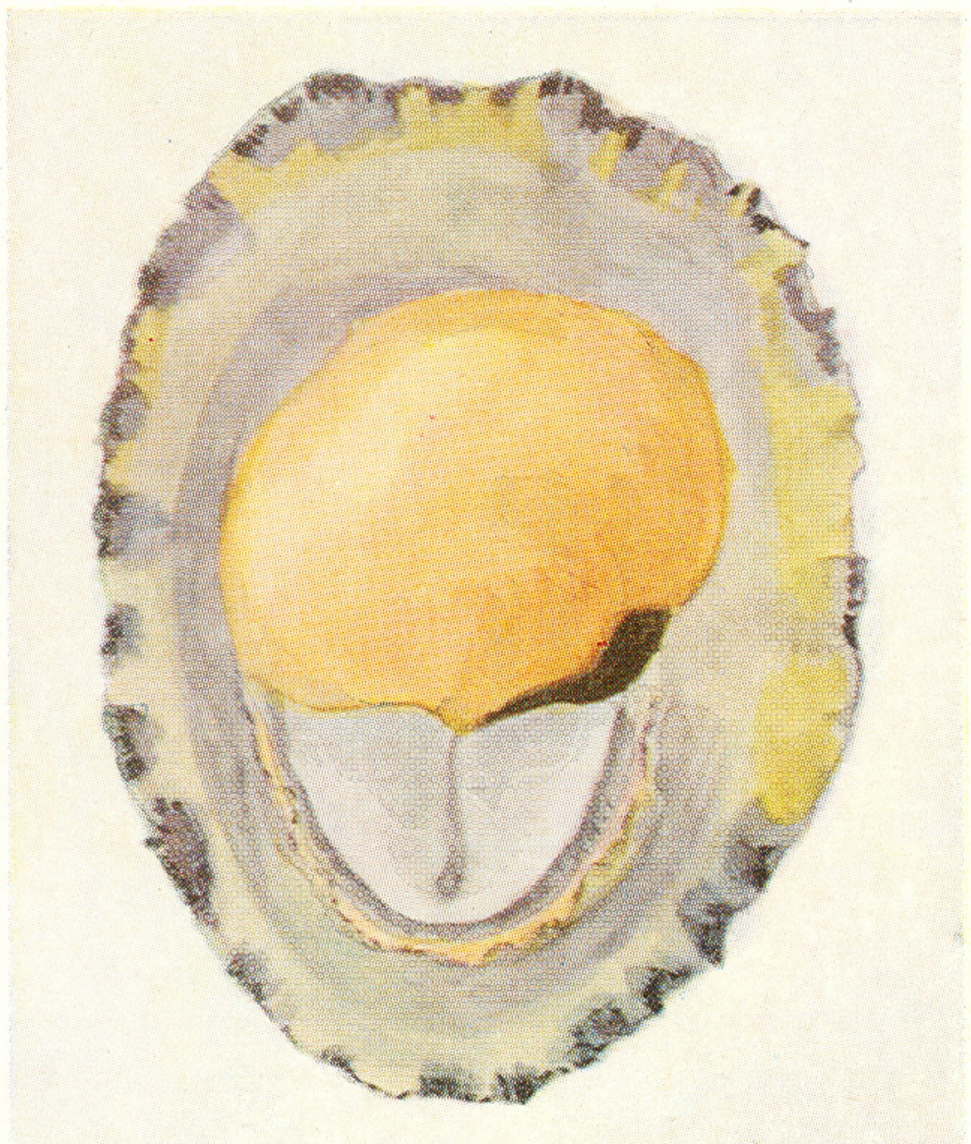

6

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which carry the germinal epithelium into the thickness of the gonad. Interstitial tissue is small in quantity.

(*b*) *Male*. The male stage I gonad is dark yellowish brown in colour with an obvious membrane. The surface is mottled with white, the white areas representing the cavities of tubules which contain some apparently fully developed spermatozoa.

Microscopic examination shows that the seminiferous tubules of the male gonad are already well developed. The tubules extend inwards from the periphery of the gonad and consist of a connective tissue base which supports thick layers of germinal tissue consisting of spermatogonia, spermatocytes, spermatids and spermatozoa, in that order. The spermatozoa present appear fully developed and lie with their tails projecting into the lumen of the tubules. Spermatogenesis is further advanced in the dorsal regions of the gonad (i.e. those in contact with the visceral mass) as compared with the more ventral.

Stage II Gonad

By the time this stage has been reached the sexes are easily recognizable by the colour of the gonad. Growth has occurred mainly in posterior and lateral directions, and the gonad now extends over the outer and posterior margins of the visceral mass. A loop of the hind-gut still delimits the outer extremities and the inner margin lies alongside the radula.

(*a*) *Female*. The ventral surface of the gonad is smooth and glossy in macroscopic appearance, and the green colour of the eggs is already beginning to mask the brown coloration of the reserve material. There are more large oocytes visible at the surface than in stage I.

Microscopic examination of sections of the stage II gonad shows a great advance in development of the oocytes. Many are now medium to large in size and the 'chorion' layer, which is strongly eosinophil, is a very obvious feature of each egg. Connective tissue trabeculae are well developed and the germinal layer which they support shows representative stages in oogenesis.

(*b*) *Male*. The surface of the male stage II gonad shows obvious mottling due to the germinal tubules which reach out to the periphery of the gonad. The peripheral regions of the tubules are brown in colour, whereas the central lumen, full of spermatozoa, is milky white. The arrangement of the tubules gives to the gonad surface an appearance strongly reminiscent of the surface of the mammalian brain, though smooth.

Microscopic examination shows that the proportion of spermatozoa to earlier stages in spermatogenesis is considerably higher than in stage I. Spermatid nuclei are also a prominent feature of the tubule walls. Nuclei which do not stain strongly in Heidenhain's haematoxylin (those of the earliest stages in spermatogenesis), are almost entirely limited to the peripheral regions of the tubules. Active spermatozoa can be obtained from stage II male gonads.

Stage III Gonad (Pl. II, figs. 3, 8)

The stage III gonad shows a considerable increase in thickness when compared with stage II and, also, marked growth of its posterior regions. These have now extended on to the dorsal face of the visceral mass and form a thick extension of its posterior extremity.

(*a*) *Female*. The brown colour of the early gonad is now greatly masked by the green eggs. In some specimens the latter are olive-green, whereas in others they are a bright bluish green: several intermediate shades are also encountered. The eggs at the ventral surface of the gonad show marked differences in size.

The stage III gonad is not greatly different in microscopic appearance from the stage II gonad. There are still many small oocytes present, especially on the trabeculae which ramify throughout the gonad. The proportion of large eggs at the surface is slightly higher than in stage II.

(*b*) *Male*. The stage III male gonad is yellowish brown mottled with white. The white areas become more extensive as the ripe spermatozoa increase.

Microscopic examination shows a higher proportion of fully developed spermatozoa than in the stage II gonad. The walls of the seminiferous tubules consist mainly of spermatozoa and spermatids, though their peripheral regions contain considerable amounts of reserve material and nests of differentiating germ cells. The difference in ripeness of dorsal and ventral regions of the gonad is now less marked, though the proportion of fully developed spermatozoa in the dorsal region is still somewhat higher.

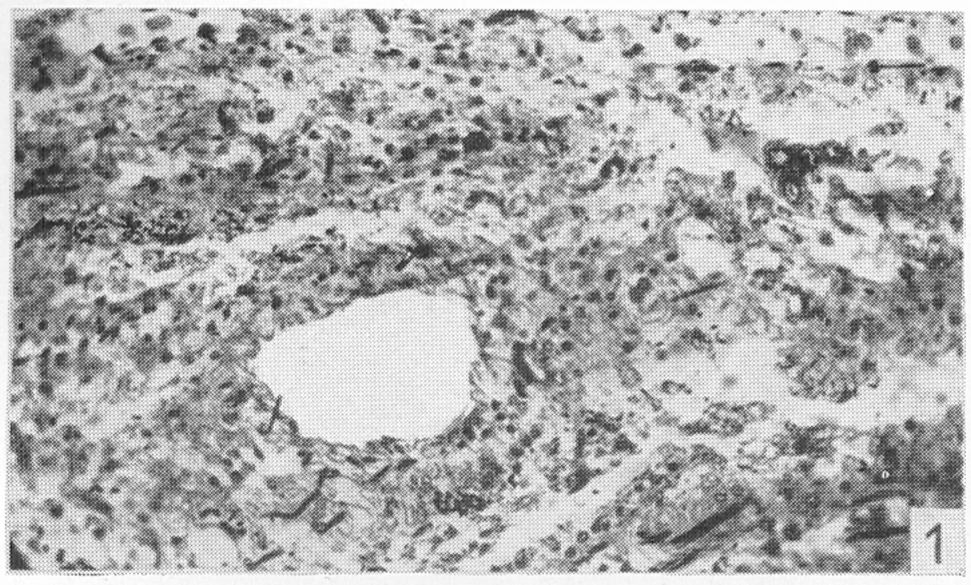
Stage IV Gonad

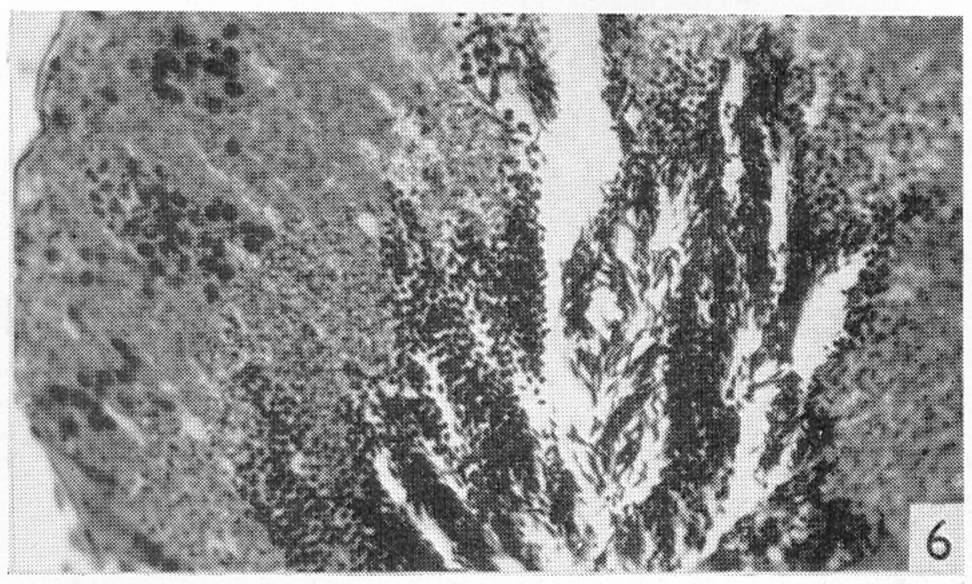
In the stage IV gonad the increase in thickness is yet more marked. This is especially true of the posterior region which now extends well beyond the

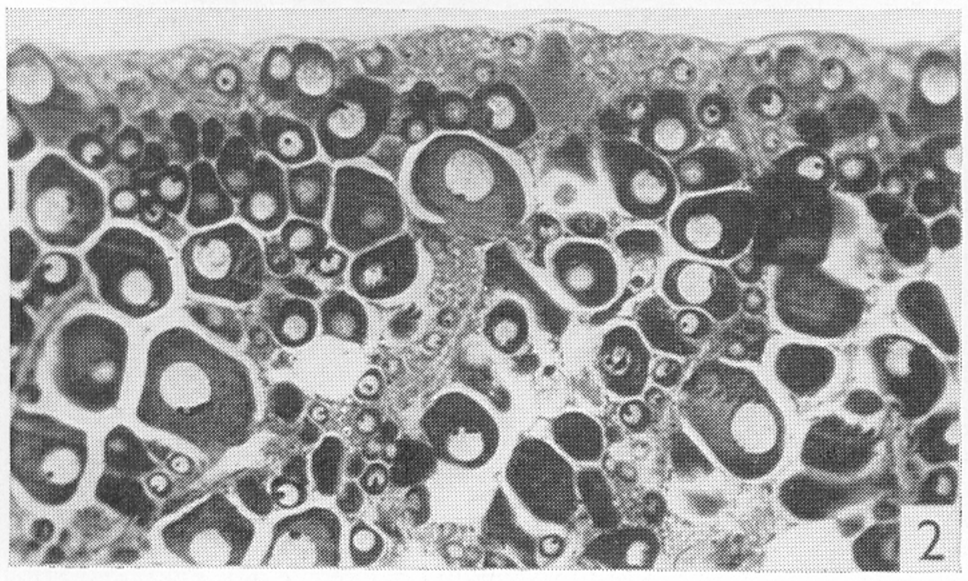
Explanation of Plate II

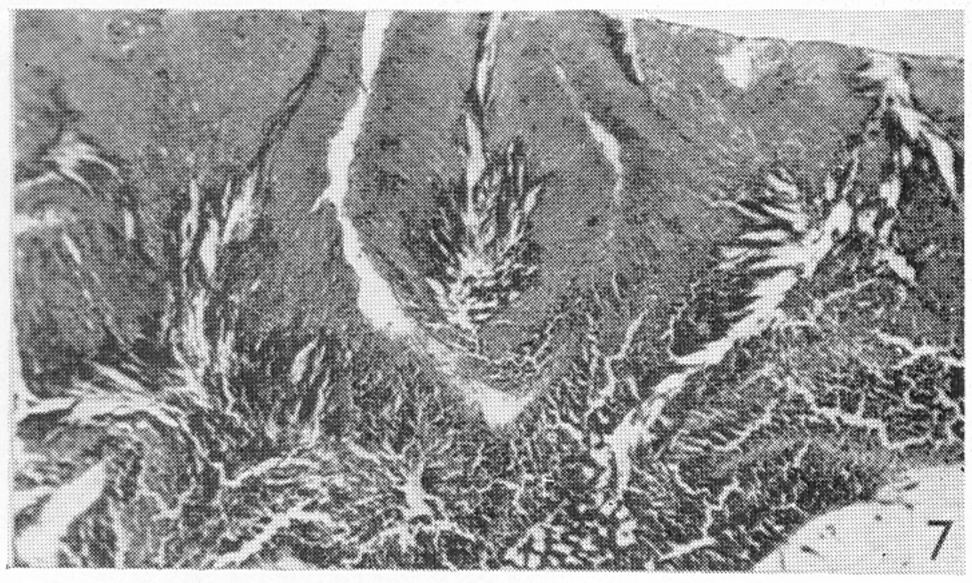
Patella vulgata: Photomicrographs of sections through male and female gonads in various stages of development and depletion. The plane of section is parallel to the dorso-ventral axis of the gonad. Excepting Figs. 1 and 6, the photographs show part of the ventral surface of the gonad and the area immediately adjacent to it. Preparations stained with Heidenhain's haematoxylin and eosin.

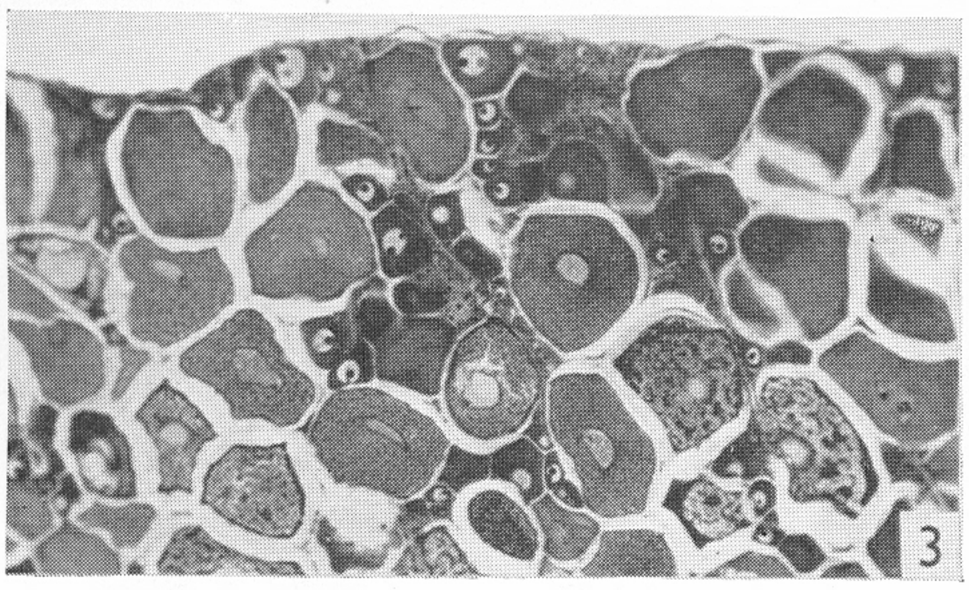
Fig. 1. Neuter gonad. Traces of tubule structure still evident. Note small groups of relict sperms. × 180.
Fig. 2. L.S. ovary; stage I (developing). × 66.
Fig. 3. L.S. ovary; stage III (developing). × 66.
Fig. 4. L.S. ovary; stage V (developing). × 66. Note 'interstitial' tissue.
Fig. 5. L.S. ovary; stage III (spawning). × 66.
Fig. 6. L.S. seminiferous tubule from stage I male (developing). × 300.
Fig. 7. L.S. testis; stage I (developing). × 66. Note salivary gland tissue in lower right-hand corner.
Fig. 8. L.S. testis; stage III (developing). × 66.
Fig. 9. L.S. testis; stage V (developing). × 66.
Fig. 10. L.S. testis; stage III (spawning). × 66.

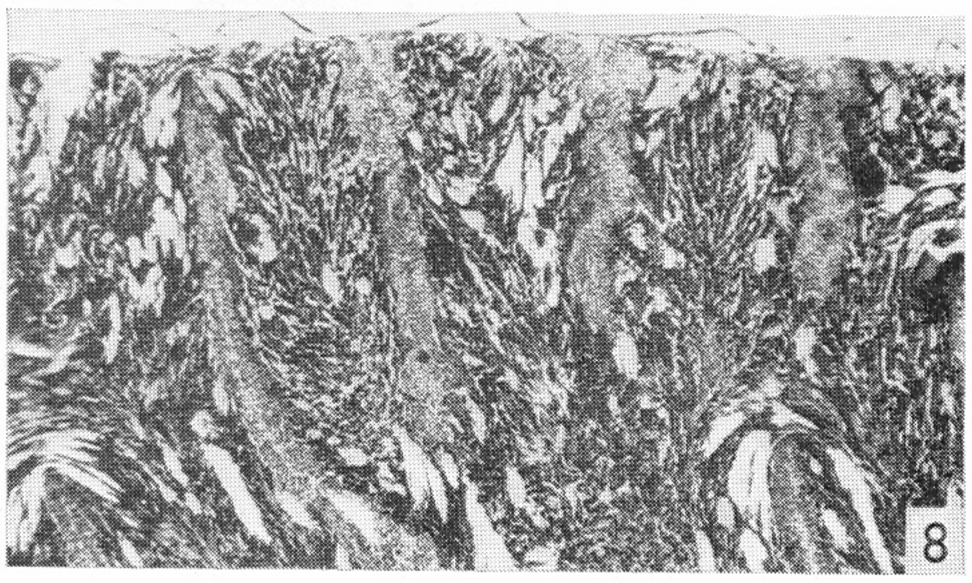


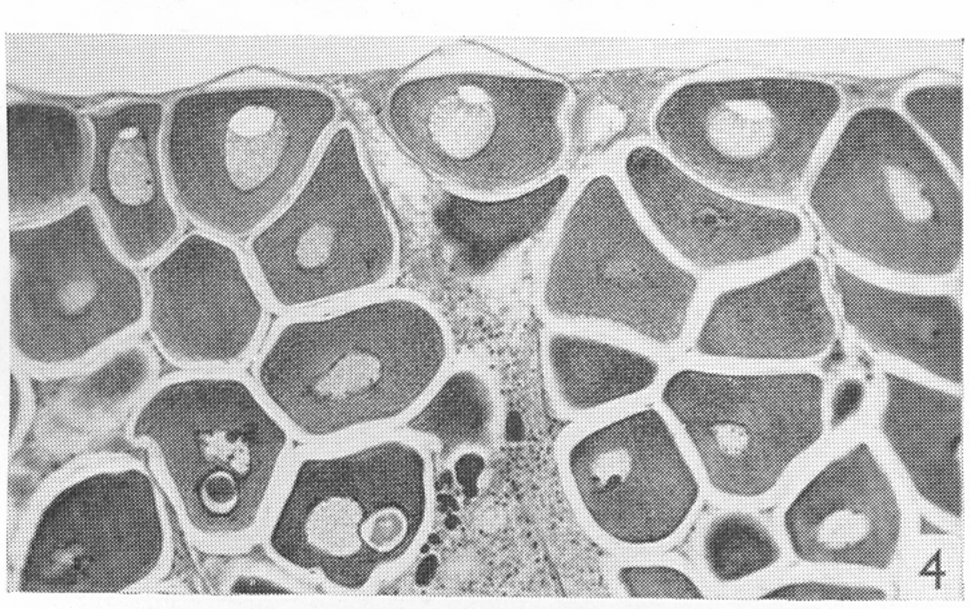


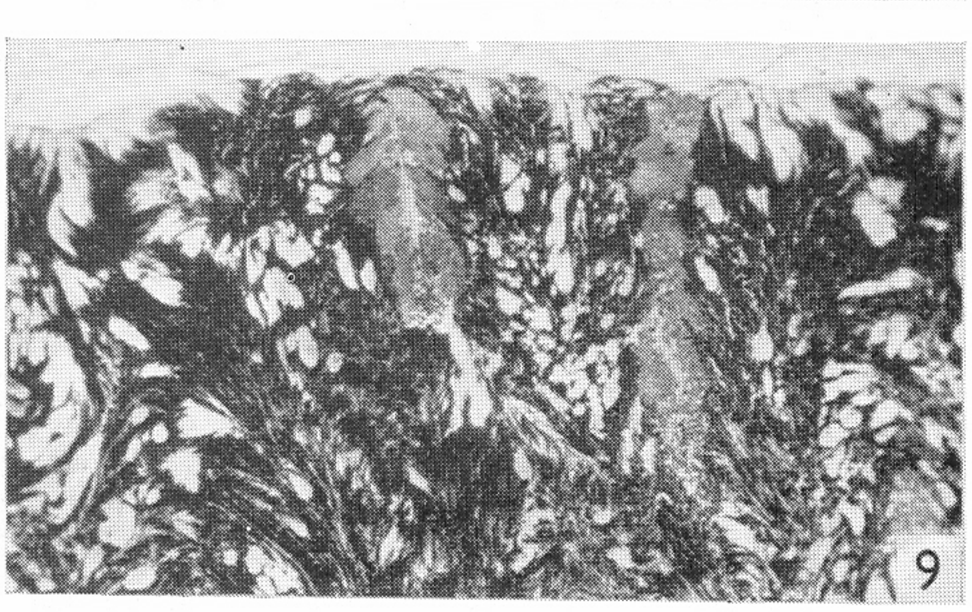


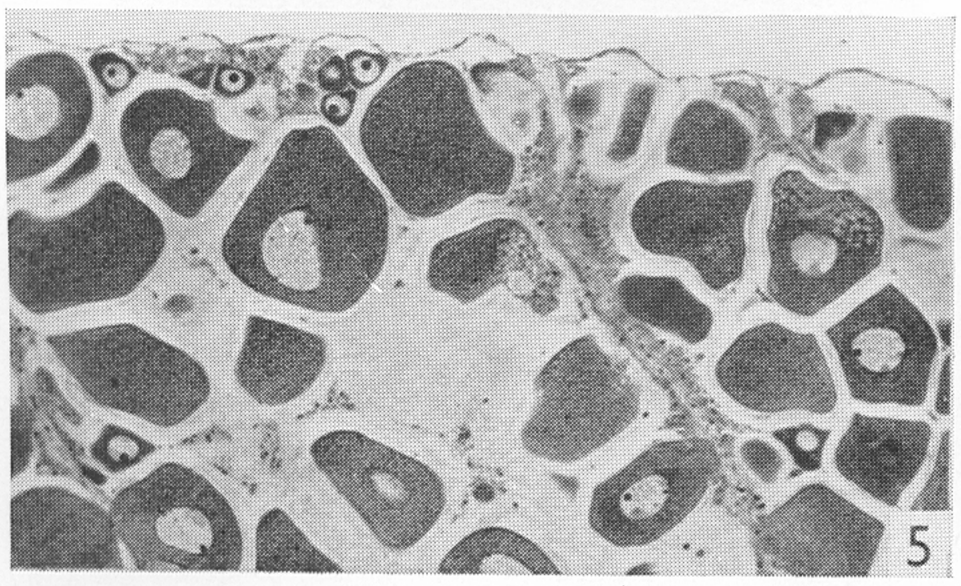


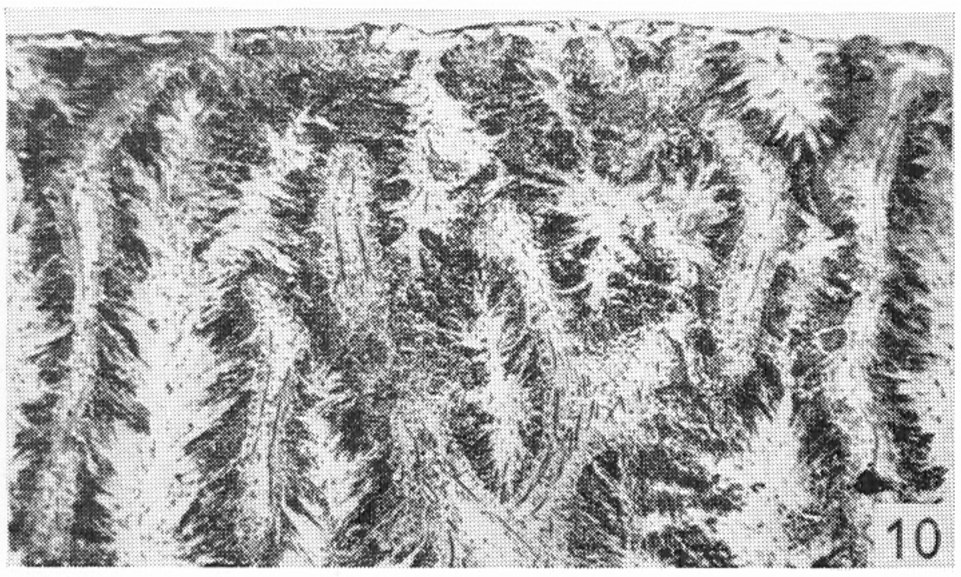












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limit of the rest of the visceral mass. Some extension of the lateral margins of the gonad has also occurred, and most of the radula has now been displaced on to the lateral face of the visceral mass.

(*a*) *Female.* All the eggs visible to the naked eye on the ventral surface of the stage IV gonad are large and uniform. Different individuals show marked differences in colour of the gonad, the commonest colour varieties being bluish green, olive green and olive brown.

Examination of sections shows that the great majority of the surface eggs are now large, though there are a few small oocytes attached to the trabecular tissue. Interstitial tissue is small in amount, being mainly represented by islands of tissue consisting of groups of cells having prominent nuclei, but in which cell boundaries are difficult to identify.

(*b*) *Male.* The colour of the stage IV male gonad is predominantly cream, though there are traces of the orange-brown mottling which represents reserve material in the peripheral regions of the seminiferous tubules.

Microscopic examination shows that fully developed spermatozoa are the most prominent constituent, though a few spermatids and earlier stages are present at the periphery of the tubules.

Stage V Gonad (Pl. I, fig. 5; Pl. II, figs. 4, 9)

The stage V gonad is of such a size that it exerts considerable pressure on the tissues which attach the margin of the visceral mass to the foot, and the operation of removing a fully ripe limpet from the rock is often sufficient to tear foot and visceral mass apart. When the visceral mass is partly removed from the foot and turned forward, the entire structure is seen to be overlaid by gonad. To allow for this great increase in gonad volume, the progressive shrinkage in size of the visceral mass, which is noticeable even in the stage III gonad, now reaches a maximum. Although no detailed observations have been made, it appears that feeding either stops or slows down and the gut virtually empties. In this way, since the gut accounts for a considerable part of the volume of the visceral mass, the gonad is allowed to grow in a space of limited volume. The posterior and lateral extension of the gonad which is also a feature of the earlier stages, is maximal in the stage V gonad.

(*a*) *Female.* The stage V female gonad shows a similar range of coloration to stage IV, from which it differs mainly in size. The surface eggs are large and uniform in size and appear loose and ready for shedding.

Microscopic examination of sections shows that the gonad is an almost uniform mass of large eggs in which only traces of the original trabeculae remain and there are very few developmental stages. Each egg is invested by a very thin 'chorion' which, in most preparations, has become separated from the surface of the egg in consequence of the histological procedures employed. There are evident islands of interstitial tissue consisting of masses of small

cells with densely staining nuclei. These islands are characteristic of all the later developmental stages: their function is unknown.

(*b*) *Male.* The overall colour is whitish cream, the brown mottling being largely masked. Surface patches are commonly found which are white in colour and these are believed to represent areas in which spermatozoa are being liberated prior to shedding.

Microscopic examination shows an almost uniform mass consisting mainly of fully developed spermatozoa. The majority of these are attached to the germinal epithelium. A considerable proportion of spermatids is still present, though earlier developmental stages are uncommon. The peripheral areas of the gonad frequently show irregular lacunae.

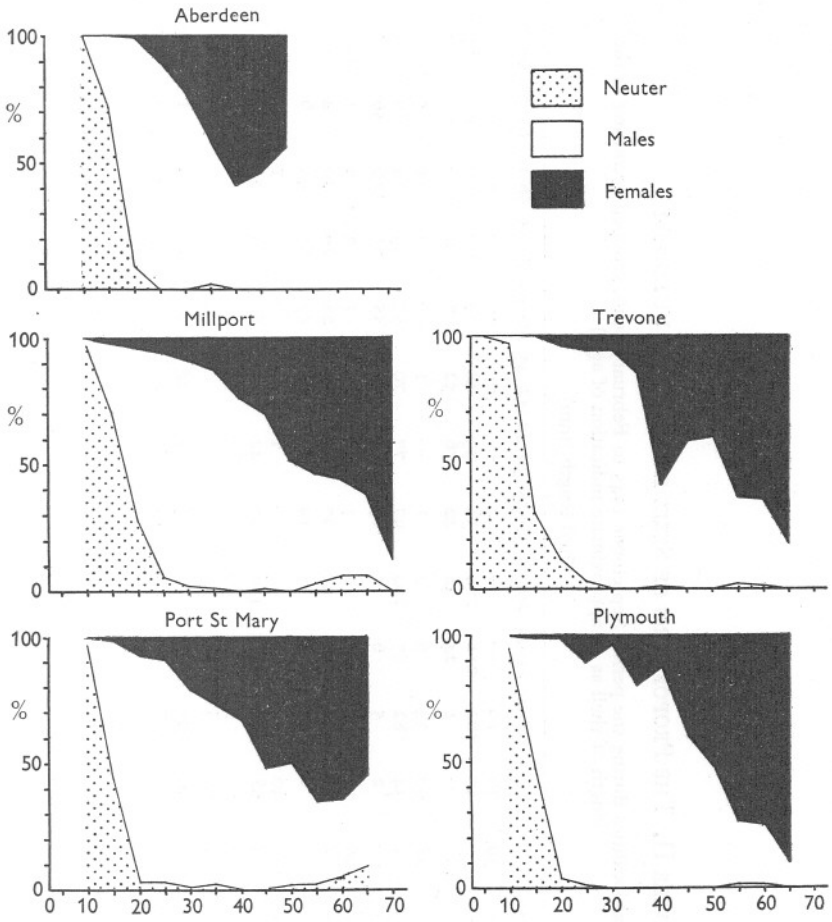
Spawning Stages (Pl. I; Pl. II, figs. 5, 10)

Gonads undergoing regression are subdivided in the same manner as are the progressive developmental stages. Thus a gonad in which spawning has occurred to the extent that it is morphologically similar to developmental stage III is classified as spawning stage III.

Spawning gonads are difficult to distinguish from developing gonads by any morphological criteria. Early spawning stages in females can be distinguished to some extent by the appearance of the surface eggs: as spawning advances they appear much more loosely packed than in the corresponding developmental stages and, in addition, they are more uniformly large. They appear more rounded and seem to be detached from the substratum of the gonad as though ready for shedding. Corresponding stages in development and regression are slightly different in colour, the proportion of the brown non-germinal tissue being higher in the latter. Microscopic examination of sections shows more striking differences, though this method of study would be less practicable for the large-scale investigation of the breeding cycle. The uniformity of the eggs and the pronounced gaps between them, occupied by a loose chromophobe connective tissue, are highly characteristic features (Pl. II, fig. 5). However, considerable numbers of small oocytes are often present in the spawning gonad, since spawning frequently alternates with subsidiary bursts of development. In spawning males, the gonad frequently shows white patches in which the tubules appear to have broken down and, furthermore, the colour of the brown non-germinal tissue changes from orange-brown to a darker brown and becomes progressively greater in relative amount as spawning advances. Sections of male gonads in which spawning is far advanced have a highly characteristic appearance. The main constituent is the chromophobe non-germinal tissue: fully developed spermatozoa are few in number and limited to the free edges of the tubules. Earlier stages in spermatogenesis are practically non-existent.

Thus the criteria that exist for separating spawning individuals from those

which are developing are undoubtedly somewhat subjective and difficult to distinguish and describe: they become recognizable only as a result of considerable experience in examining gonads throughout the breeding season.



Text-fig. 3. *Patella vulgata*: the proportions of sexes at different shell lengths (5 mm groups) in samples from several localities, 1945–6.

Sex Proportions

Approximately 1000 individuals were examined from each of the localities Aberdeen, Millport, Port St Mary, Trevone and Plymouth, between September 1945 and February 1946. As far as possible about 100 specimens were examined in each 5 mm size-group. The actual numbers of males, females, and neuter individuals in each size-group is given in Table II, while their distribution as a percentage of the total number of specimens examined is

TABLE II. THE PROPORTIONS OF SEXES IN *PATELLA VULGATA*

(Samples from several localities during the period September 1945 to February 1946, grouped according to the length of shell as an approximate indication of age.)

		Shell length (mm)														
Locality	Sex	0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	Totals
Aberdeen	Neuter	—	59	76	10	—	—	2	—	—	—	—	—	—	—	147
	Male	—	—	30	102	218	168	58	53	44	9	—	—	—	—	682
	Female	—	—	—	1	24	48	42	76	52	7	2	—	—	—	252
Millport	Neuter	—	28	90	34	6	2	1	—	1	—	3	11	5	—	181
	Male	—	1	34	82	87	104	97	77	70	57	49	74	29	1	762
	Female	—	—	2	5	6	11	15	24	30	55	61	110	56	7	382
Port St Mary	Neuter	—	96	74	6	3	1	1	—	—	1	2	3	1	—	188
	Male	—	3	94	206	76	55	37	22	20	28	26	20	4	—	591
	Female	—	—	1	15	7	15	14	11	22	29	53	41	6	—	214
Trevone	Neuter	3	135	30	28	6	—	—	1	—	—	3	1	—	—	207
	Male	—	4	68	206	164	140	92	42	98	86	56	30	2	—	988
	Female	—	—	—	9	10	9	16	61	71	57	104	66	9	—	412
Plymouth	Neuter	—	101	82	4	—	—	—	—	—	—	1	1	—	—	189
	Male	—	5	90	105	84	115	83	73	28	42	27	32	1	—	685
	Female	—	—	1	1	10	5	21	11	25	46	81	100	9	—	310

shown in Text-fig. 3. As has been known for some time (Orton, 1920, 1928) the smaller sizes of limpet in which sex can be determined are almost entirely male, while the larger sizes are preponderantly female. In the range up to 10 mm shell length practically all specimens examined in the present study were neuter, but between 16 and 25 mm at all localities there were at least 90% males. The two sexes were approximately equal in number in specimens having a shell length around 40 mm, while 60–70% of those specimens having a shell length of 60 mm were female (such large specimens were not found in the Aberdeen samples). In limpets having a shell in excess of 60 mm even higher proportions of females were sometimes found (Millport, Trevone, Plymouth), but this may have been due to the small numbers examined.

These changes in sex proportions with length can be explained in two ways. If one assumes that the rates of growth and development of males and females are similar, and this is certainly true for the gonad, it can be suggested that almost all individuals start their breeding life as males, but that later in life a large proportion of them become female. On the other hand, if one assumes that the rate of growth and development may differ markedly in the two sexes, the preponderance of females in the larger size-groups can be explained by differential mortality, more than 90% of the males dying before they reach a shell length of 60 mm.

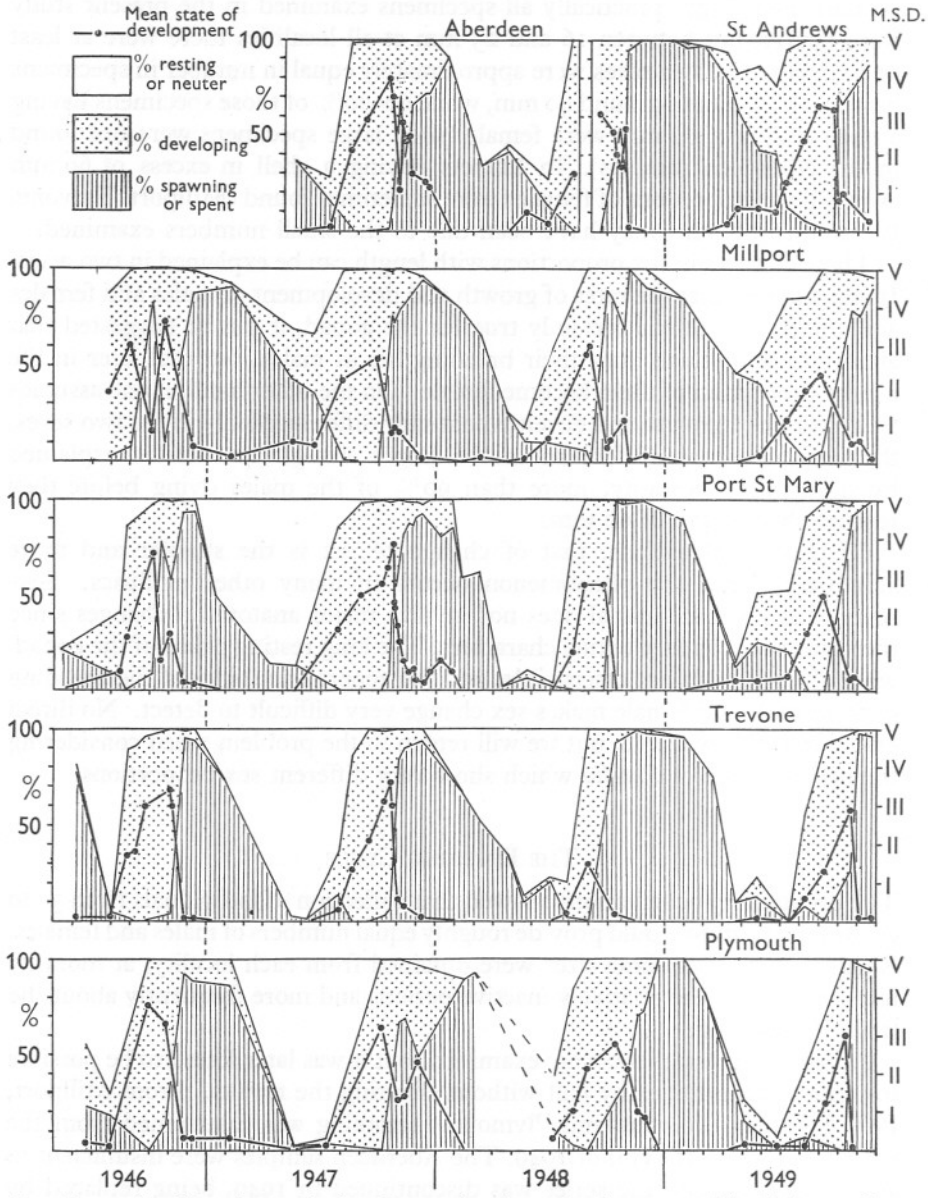
Of these explanations, that of change of sex is the simpler and more acceptable, since the phenomenon occurs in many other molluscs. Sex-reversal in such an animal does not involve major anatomical changes since there are no secondary sexual characters. The long resting phase of the gonad, when the sexes are not discernible, and the general similarity of the breeding cycle in male and female makes sex change very difficult to detect. No direct evidence is yet available, but we will return to the problem when considering the other species of *Patella* which show very different sex proportions.

The Breeding Cycle

The preliminary observations showed that collection of individuals from 30 to 60 mm shell length would provide roughly equal numbers of males and females. Regular samples of these sizes were obtained from each locality, at monthly intervals during the relatively inactive periods and more frequently about the time of spawning.

At first, samples of 100 were examined, but it was later found to be possible to reduce this number by half without affecting the results. From Millport, Port St Mary, Trevone and Plymouth, sampling was maintained from the spring of 1946 to the end of 1949. The Aberdeen samples were insufficient in 1946 and 1947, and the series was discontinued in 1949, being replaced by the St Andrews samples, not begun until late in 1947. The results from these samples are given in Tables IV–IX (Appendix) as numbers of individuals

found in each gonad stage in the separate sexes, and in the neuter or resting stage. The four variables used to define the breeding state of the population (p. 4) have been extracted from these tables, and are shown in Text-fig. 4.



Text-fig. 4. *Patella vulgata*: the breeding cycle of samples from several localities, 1946–9, showing the percentages with gonad resting, developing, and spawning. The thick line gives the mean state of development of the gonad (ordinates on right side).

From the Tables and Text-fig. 4 it can be seen that *P. vulgata* has a long resting phase: during the spring and early summer the animal is effectively neuter. This phase must last up to 9 months in some individuals, and for over 3 months in 50% of the population, except at St Andrews, where it is rather short. The resting phase is followed by a much shorter period of development from June to October, not usually more than 3–4 months, during which the gonad increases steadily in size. There may be a further period of up to 1 month during which little change takes place in the samples. At this stage the gonad is apparently fully mature, and a high proportion of viable larvae may be obtained by artificial fertilization. Some time between the beginning of September and the end of October, according to year and locality, a comparatively rapid change from the developing to the spawning condition takes place. Finally, the proportion of spawning and spent individuals declines slowly and regularly between December and June, as more and more pass into the resting condition.

This cycle is subject to some variation from year to year and variation is also shown between the different localities. Only at the more northerly stations (Aberdeen, St Andrews, Millport; cf. Text-fig. 4) did the spawning and developing phases overlap: in the other localities a higher proportion of the limpets passed into the resting phase together, while the periods of development and spawning were shorter. This suggests that temperature may influence the resting period, and that the emptying of the gonad, and its change to the resting phase is speeded up by the warmer conditions in the south. Temperature differences may also explain the fact that gonad development starts earlier in the more northern localities, in which a longer development period may be necessary because of the lower environmental temperatures. However, temperature differences can hardly account for the variations in the exact time of onset of spawning at the different stations, since, of the years studied, only 1949 and 1952 showed a significant difference between the northerly and southerly localities in this respect.

Spawning

The dates between which spawning started and those on which most (at least 90%) of the limpets in the samples were in the spawning condition are given in Table III. This table includes information for some other stations, in addition to those regularly sampled, and for 1952, when only the spawning period was studied. More precise information for the regular samples will be found in the Appendix Tables IV–IX, while Text-fig. 4 shows diagrammatically the onset of spawning.

In some years, notably 1947 and 1948, spawning seems to have started practically simultaneously at all stations towards the end of October. At Plymouth (both stations) spawning again began in October in 1946, 1949 and

TABLE III. THE SPAWNING PERIOD OF *PATELLA VULGATA* AT SEVERAL BRITISH LOCALITIES IN 1947–49 AND 1952

	1946		1947		1948		1949		1952	
	Dates between which spawning began	Date at which 90% of sample were spawning	Dates between which spawning began	Date at which 90% of sample were spawning	Dates between which spawning began	Date at which 90% of sample were spawning	Dates between which spawning began	Date at which 90% of sample were spawning	Dates between which spawning began	Date at which 90% of sample were spawning
Aberdeen	—	—	9 Sept. to 17 Oct.	19 Jan. 1948	7 Sept. to 10 Oct.	6 Dec.	—	—	—	—
St Andrews	—	—	Before 3 Nov.	5 Nov.	17 Sep. to 18 Oct.	2 Nov.	22 Sept. to 30 Sept.	Not in 1949	21 to 27 Sept.	4 Oct.
Millport	—	—	1 Oct. to 18 Oct.	9 Dec.	30 Sept. to 2 Nov.	2 Nov.	10 Aug. to 3 Sept.	24 Nov.	Before 2 Oct.	Before 2 Oct.
Port St Mary, Isle of Man	21 Aug. to 3 Sept.	25 Nov.	6 Oct. to 22 Oct.	20 Nov.	24 Sept. to 13 Oct.	13 Oct.	5 Sept. to 17 Oct.	23 Oct.	Before 3 Oct.	24 Oct.
Other places on east side of Isle of Man	—	—	1 Oct. to 5 Oct.	21 Nov.	24 Sept. to 25 Sept.	25 Oct.	5 Sept. to 20 Oct.	—	—	—
West side of Isle of Man	—	—	15 Oct. to 28 Oct.	—	6 Sept. to 22 Oct.	22 Oct.	—	—	—	—
Trevone	24 Sept. to 2 Nov.	25 Nov.	17 Sept. to 11 Oct.	14 Nov.	30 Aug. to 13 Oct.	13 Oct.	10 Sept. to 19 Sept.	4 Nov.	Before 3 Oct.	Before 3 Oct.
Plymouth Breakwater	1 Oct. to 28 Oct.	27 Nov.	6 Oct. to 3 Nov.	16 Feb. 1948	1 Sept. to 14 Oct.	21 Dec.	12 Oct. to 27 Oct.	27 Oct.	22 Oct. to 4 Nov.	4 Nov.
Plymouth, Rum Bay	—	—	—	—	1 Sept. to 14 Oct.	23 Nov.	12 Oct. to 4 Nov.	4 Nov.	—	—

1952, but elsewhere occurred up to a month earlier. Practically all stations reached peak spawning (over 90%), in spite of this, some time in November.

Details of spawning can only be inferred from the samples, since actual 'running' specimens were rarely seen, although a slow release of eggs was obtained from later spawning stages in the laboratory. The first indication of spawning in the samples was usually a fairly sudden decline in gonad size, but after this, liberation of eggs and sperm must have continued slowly, for 2–4 weeks passed before the first fully spent (stage I) individuals appeared. Thus the rapid swing from the developing to the spawning condition which frequently occurred must be distinguished from the swift and complete voiding of gametes that can take place in some other organisms. The spawning of the population as a whole was also slow compared with some other organisms, and it was sometimes 6 months or more (10 months in the more northern localities) after the start of spawning before all specimens had passed into the resting or developing phases.

Although the peak of spawning occurred from October to March at all localities, and thus *Patella vulgata* is to be regarded as a winter spawning species in Britain, the tables and figures show that some spawning can occur from September to the following June. In the more northerly stations, in certain years, the spawning season may be very extended. In June 1949, specimens of *P. vulgata* containing ripe genital products were still fairly plentiful at St Andrews (over 40% spawning stages in sample) and an artificial fertilization was made from which larvae were reared through metamorphosis (Dodd, 1956). It must therefore be stressed that gonad examination over a period of more than 1 year is necessary before any decision can be made about the breeding periods of limpets and related gastropods: little reliance can be placed on a few isolated samples. Records of the occurrence of the larvae in the plankton, unless made at frequent intervals over a long period, may also have little value.

Spawning Stimuli

The rapidity of the onset of spawning is to some extent a gauge of the strength of the supposed stimulus that induces spawning. For example, in 1946 at Trevone, in 1947 at Millport, Port St Mary, and Trevone, in 1948 at Millport and Port St Mary, and in 1949 at Plymouth over 90% of the samples were spawning or spent within a month after the first spawning specimens were found. Presumably on these occasions the stimulus to spawn was stronger than at other times. The stimulus can hardly be internal, since in some years the whole population at different localities was involved within a few days, while in other years there were differences between almost adjacent populations (e.g. compare Plymouth breakwater and Plymouth, Rum Bay, in 1948, and the east and west coasts of the Isle of Man in 1947; Table III). At the same time the onset of spawning can hardly be due to some climatic or hydrographic factor

such as temperature or the nature of the water masses, since spawning may start simultaneously in different areas. The spawning stimulus does in fact appear to be capable of over-riding the breeding-cycle; for example, at Trevone in 1948, it appears to have arrived relatively early, for spawning began while the mean state of development of the gonad was low. Quite frequently spawning occurs while this index is at III or IV.

These differences appear to preclude any relation between spawning and the phases of the moon such as may occur in other organisms (e.g. *Ostrea*, Orton, 1926, 1927; *Arenicola*, Newell, 1948). Samples were not taken at sufficiently close intervals for possible lunar periodicity in spawning to be shown with any accuracy, except for the series from Port St Mary and Trevone in 1947. These particular samples show little lunar influence; the main spawning stimulus seems to have arrived between 22 and 25 October, some 12 days behind the full moon, and during a period of neap tides. Samples taken at less frequent intervals in other years, e.g. at Plymouth in 1949 and at Port St Mary in 1948, also suggest that any correlation between the moon and spawning lay with a period of neap tides; but the extent of such correlation was slight.

There is much better indication of a relation between wave-action or on-shore winds and the start of spawning. For example in 1947 spawning began at Trevone between 17 September and 11 October, and continued very weakly (not more than 7% spawners) up to 21 October. During this period some rough seas may have been experienced since on a few days winds at St Eval, North Cornwall, (Air Ministry, 1947) reached 21 knots between south-west and north. A very strong stimulus to spawning appears to have been in operation between 21 October and 4 November, by which date over 80% of the samples were in the spawned condition. This strong stimulus coincided with the occurrence on six of the days of winds of 21 knots and over between south and west at St Eval, and on four of these days the wind reached 30 knots or over. In the same year at Port St Mary the initial stimulus also appears to have been weak, and may have been associated with winds of up to 21 knots (once 27) recorded at the Pt. of Ayre, I.O.M. (Air Ministry, 1947) from south-east to west between 6 and 22 October. The major spawning stimulus seems to have arrived between 22 and 25 October, when the wind (recorded at the Pt. of Ayre) sometimes reached 21 knots from the south-east. This is the critical wind sector for the Port St Mary collecting station, and causes maximum wave action there.

At the other localities in 1947, the onset of spawning was much more gradual.

An extremely rapid swing-over to the spawning condition occurred at Port St Mary in 1948 between 24 September and 13 October, a period of almost continuously high winds and rough seas around the Isle of Man. Thus the winds recorded at the Pt. of Ayre (Air Ministry, 1948) reached 21 knots between south-east and west on 8 days, and over 30 knots on 3 days, while from personal observations the seas at Port St Mary were very rough.

At the other localities conditions were much calmer in 1948. At Plymouth the wind rarely exceeded 16 knots during September and October, and it is therefore interesting that this was a year in which spawning progressed very slowly there.

In 1949 the change to the spawning condition was more rapid at Trevone and Plymouth than at the other stations. At St Andrews and Port St Mary the initial spawning seems to have coincided with the occurrence of winds of up to 21 knots about 21–22 September, from the north-east at Leuchars, near St Andrews (Air Ministry, 1949) and from the south-east at Ronaldsway Airport, near Port St Mary. There was little wind at Plymouth during this period. The maximum change to the spawning condition at Plymouth and Trevone took place from 12 October to the end of the month, and seems to have coincided with winds of 21–30 knots from the south and west between 15 and 22 October, and similar winds from south through west to north between 22 and 26 October, at Plymouth and St Eval (Air Ministry, 1949).

The change to the spawning condition was even more rapid at St Andrews in 1952, between 21 and 27 September; the whole population was spent by 4 October. Winds of over 20 knots, sometimes 30 knots, were recorded practically every day during this period from directions between west and north at Leuchars (Air Ministry, 1952), and rough seas must have been fairly continuous. At Port St Mary spawning appears to have started about the same period, when rough seas were observed practically continuously from 23 to 28 September, and the winds at Ronaldsway Airport exceeded 20 knots, and sometimes reached 30 knots, from directions between south and north-west (Air Ministry, 1952). At Plymouth spawning was later. Rough seas were observed on only 3 isolated days in September, and spawning seems to have corresponded with rough seas on 25 to 27 October, and winds of 22–35 knots from south to west from 25 to 29 October.

Thus in 1947–49 and 1952 the initiation of spawning and periods of rapid changeover to the spawning condition coincided with periods of strong onshore winds and rough onshore seas. On some occasions also, as mentioned above, the greatest changeover to the spawning condition coincided with neap tides. If wave-action or onshore winds are major factors stimulating spawning, their effect would be enhanced during neap tides, when the smaller tidal range and slower movement would prolong their influence on the limpet habitat.

If the phenomena of wave-action or onshore winds and spawning are related, it becomes important to consider how the relationship is mediated. The direct effect of mechanical shock is an obvious factor to consider (cf. Chipperfield, 1953). It might either trigger spawning in the majority of individuals in a population, or else induce it in the ripest members which in turn might stimulate the remainder through a chemical effect of the gametes or other substances released on spawning. It may also be that some chemical or biological factor which induces spawning is brought inshore by turbulence. These and other possibilities have been investigated by an extensive series of laboratory experi-

ments which have failed to show effects of the kind postulated. However, it must be admitted that experiments of this sort are hard to design satisfactorily and extremely difficult to carry out in the laboratory and the negative results must be interpreted accordingly.

The problem of spawning stimuli has been studied in detail in the other species of *Patella*, and will be discussed further in a later paper.

Comparison with other Animals

The breeding cycle which we have described for *Patella vulgata* contrasts markedly with the cycle in the lamellibranch molluscs, the reproductive processes of which proceed more rapidly. For example, in *Ostrea* (Orton, 1926, 1927, 1936), and in *Pecten* (Mason[1]), which breed during the summer in Britain, the gonad empties rapidly, and sometimes two batches of gametes can be matured before the autumn. In the former species, although only one batch of eggs is produced by a female, a high proportion of the females switch rapidly to the male phase and start sperm production immediately after spawning. Even in intertidal *Mytilus* (Chipperfield, 1953) the act of spawning, both of individuals and of the population as a whole, is much more rapid than in *Patella vulgata*. The same applies to most fish with planktonic larvae (e.g. *Clupea*, Orton, 1916), though in some (e.g. *Gadus merlangus*, Bowers, 1954), the spawning period of the population may be as extensive as that of *Patella*.

The resting period in *P. vulgata* seems unduly extended when compared with the duration of the similar stage found in some lamellibranchs (Chipperfield, 1953) and fishes (Orton & Jones, 1940; Jones, 1940). In fact it may be said that the predominant feature of the breeding of *P. vulgata* is the slow progression of the cycle. Since it is possible that this slow progression is connected with winter breeding, it is instructive to compare *Patella* with another intertidal animal which starts to spawn at about the same time of the year, the lugworm *Arenicola marina*. Although the period of development of the eggs and sperm, June to October or November (Newell, 1948; Southward, 1953), is similar to that of *Patella vulgata*, the gametes are voided much more rapidly in *Arenicola*: this means that the resting period is correspondingly longer than in *Patella vulgata*. It may be that animals with a northern distribution, which spawn in late autumn or winter, are characterized by a long resting period.

Some investigations which are being made by one of us (A. J. S.) on the breeding of certain common intertidal top-shells indicate that *Osilinus lineatus* and *Gibbula umbilicalis* have a breeding cycle very similar to that of *Patella vulgata*, although the resting phase may be shorter. As these animals occupy very similar habitats, and have habits similar to those of limpets, it seems

[1] J. Mason. 'Investigations on the scallop (*Pecten maximus* L.) in Manx waters.' Univ. Liverpool, Ph.D. Thesis, 1953.

possible that the slowness of the breeding cycle, and in particular the slow progression of spawning, is coupled with the mode of life. If the rate of growth is any guide (Fischer-Piette, 1941) the vital activities of limpets, especially at the higher levels on the shore, proceed at a very slow rate: one might therefore expect that the breeding cycle in such animals would progress slowly.

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Summary

Observations have been made on the breeding of *Patella vulgata*, by examination of regular samples from six localities in Britain over a period of 5 years.

The gonad stages were examined and grouped according to an arbitrary scheme, the advantages of which are discussed. Criteria are given by which the arbitrary stages can be distinguished, and their anatomy and histology described.

P. vulgata is shown to be a winter breeder in Britain. The gonad develops from June to September, and the peak of spawning occurs between October and December. Subsidiary spawning may take place at any time between September and June. From January to June the gonads pass into a resting phase. The length of the resting phase, and the proportion of individuals resting at any one time may be related to the temperature of the locality.

The main period of spawning varies from year to year and from place to place. It is not obviously related to temperature, tides, nor to the phases of the moon. The only factors with which spawning appears so far to be correlated are rough seas and onshore winds. Spawning may therefore be initiated either by physical shock, or some factor brought inshore from deeper water, but all attempts to investigate the phenomenon experimentally have failed.

In comparison with other molluscs, *P. vulgata* has a much longer resting phase, but it seems possible that this is characteristic of some winter-breeding intertidal animals.

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APPENDIX

TABLE IV. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM ABERDEEN

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1947																				
20 Feb.	116	—	—	—	—	—	—	—	2	23	63	—	—	—	—	—	—	—	—	28
21 May	97	—	—	—	—	—	—	—	—	15	62	—	—	—	—	—	—	—	—	20
12 July	96	10	2	1	—	—	—	—	—	—	68	1	—	—	—	—	—	—	—	14
17 Aug.	113	2	30	17	4	—	—	—	—	—	1	26	31	2	—	—	—	—	—	—
9 Sept.	98	1	6	19	7	—	—	—	—	—	—	7	20	31	7	—	—	—	—	—
17 Oct.	123	—	3	10	25	13	—	1	—	—	1	—	5	13	32	20	—	—	—	—
24 Oct.	118	—	—	6	31	13	1	7	—	—	—	—	—	8	27	13	—	9	3	—
3 Nov.	134	—	2	—	4	1	8	26	26	—	—	—	3	4	18	7	—	3	22	10
8 Nov.	103	—	—	12	22	10	3	11	5	—	—	—	—	—	17	10	1	8	2	2
15 Nov.	110	—	—	4	—	12	9	10	2	2	—	—	2	4	10	26	10	10	8	1
20 Nov.	104	—	—	1	3	15	5	7	4	1	—	—	—	1	1	33	10	13	8	2
24 Nov.	121	—	—	2	1	5	18	21	2	—	1	—	—	—	3	25	11	24	7	1
16 Dec.	101	—	—	2	—	4	7	22	5	—	—	—	1	—	5	16	14	12	12	1
18 Dec.	104	—	1	5	2	5	5	34	5	—	—	—	1	2	5	8	12	9	9	1
1948																				
19 Jan.	98	—	—	—	—	—	—	—	8	35	9	—	—	—	—	—	—	—	—	46
17 Feb.	69	—	—	—	—	—	—	—	—	27	27	—	—	—	—	—	—	—	—	15
13 Mar.	63	—	—	—	—	—	—	—	—	17	41	—	—	—	—	—	—	—	—	5
25 Apr.	62	2	—	—	—	—	—	—	—	13	34	—	—	—	—	—	—	—	—	13
27 May	57	3	—	—	—	—	—	—	—	1	49	—	—	—	—	—	—	—	—	4
27 June	74	10	—	—	—	—	—	—	—	1	54	—	—	—	—	—	—	—	—	9
4 Aug.	62	13	10	1	—	—	—	—	—	—	—	36	2	—	—	—	—	—	—	—
7 Sept.	63	2	16	6	—	—	—	—	—	—	—	12	20	6	1	—	—	—	—	—
10 Oct.	67	—	—	4	2	—	—	14	16	—	—	—	1	4	2	1	1	10	12	—
18 Oct.	56	—	9	9	2	—	—	4	1	—	—	—	5	8	—	—	—	5	12	1
31 Oct.	71	—	3	13	2	—	—	5	18	4	—	—	5	3	1	—	—	8	8	1
27 Nov.	76	—	4	1	1	—	—	—	5	23	—	—	—	2	—	—	—	3	7	30
6 Dec.	64	—	1	1	—	—	—	2	12	18	—	—	—	1	—	—	—	6	4	19
20 Dec.	59	—	—	—	—	—	—	—	—	27	—	—	—	—	—	—	—	—	—	32
1949																				
7 Jan.	71	—	—	—	—	—	—	—	—	34	6	—	—	—	—	—	—	—	—	31
28 Feb.	66	—	—	—	—	—	—	—	2	31	5	—	—	—	—	—	—	—	—	28
24 Apr.	64	—	—	—	—	—	—	—	—	15	20	—	—	—	—	—	—	—	—	29

TABLE V. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM ST ANDREWS

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1947																				
3 Nov.	105	—	1	3	17	10	4	6	2	—	—	—	3	5	29	20	1	1	3	—
5 Nov.	126	—	—	—	—	2*	6	11	24	17	—	—	1	1	4	7	3	4	9	37
18 Dec.	94	—	1	—	—	—	—	8	16	7	—	—	—	1	1	—	3	13	25	19
1948																				
16 Jan.	88	—	1	—	—	—	—	—	7	31	3	—	—	—	—	—	—	1	—	45
11 Feb.	109	—	—	—	—	—	—	—	—	41	24	—	—	—	—	—	—	—	—	44
20 Aug.	105	1	18	38	2	1	—	1	2	—	—	2	29	10	1	—	—	—	—	—
17 Sept.	122	15	40	14	8	—	—	—	—	—	—	—	8	32	5	—	—	—	—	—
8 Oct.	111	—	1	26	22	2	—	18	4	—	—	—	—	2	16	7	4	9	—	—
13 Oct.	120	—	2	30	12	—	—	24	12	—	—	—	1	11	14	—	4	8	2	—
20 Oct.	49	—	—	8	8	—	—	19	—	—	—	—	—	2	5	—	—	5	2	—
29 Oct.	90	—	—	18	20	2	—	13	—	—	—	—	—	4	10	8	10	5	—	—
2 Nov.	128	—	—	4	—	—	—	3	32	40	—	—	—	—	—	—	—	2	5	42
23 Nov.	65	—	—	—	—	—	—	5	18	13	—	—	—	—	—	—	—	—	3	26
24 Dec.	57	—	—	—	—	—	—	—	6	30	—	—	—	—	—	—	—	—	—	21
1949																				
14 Jan.	53	—	—	—	—	—	—	—	6	27	—	—	—	—	—	—	—	—	—	20
4 Feb.	68	—	—	1	—	—	—	—	10	29	2	—	—	—	—	—	—	—	2	24
4 Mar.	65	—	—	—	—	—	—	—	6	41	2	—	—	—	—	—	—	—	—	16
6 Apr.	91	5	6	—	—	—	—	—	—	49	5	2	1	—	—	—	—	—	—	23
26 Apr.	100	4	6	1	—	—	—	—	6	20	17	1	5	3	1	—	—	—	—	36
27 May	57	18	4	—	—	—	—	—	—	11	8	—	1	1	—	—	—	—	—	14
24 June	55	12	5	—	—	—	—	—	—	13	14	3	—	—	—	—	—	—	—	8
9 July	56	10	9	1	—	—	—	—	1	4	3	17	9	—	—	—	—	—	—	2
5 Aug.	69	2	12	12	—	1	—	—	2	—	—	6	20	11	1	—	—	—	2	—
1 Sept.	60	—	1	15	13	3	—	1	—	—	—	—	1	15	7	3	—	1	—	—
22 Sept.	58	—	7	19	8	—	—	—	—	—	—	—	6	9	7	2	—	—	—	—
30 Sept.	62	—	9	6	—	2	—	—	13	—	—	—	2	1	—	—	—	—	26	3
5 Oct.	61	—	2	3	3	1	—	—	24	—	—	—	9	—	2	—	—	4	13	—
19 Nov.	31	—	4	—	—	—	—	—	12	2	—	—	—	—	—	—	—	—	6	7

* Actually stage V+.

TABLE VI. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM MILLPORT

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1946																				
25 July	104	8	33	17	5	—	—	—	—	—	13	7	16	2	—	—	—	—	—	3
29 Aug.	115	—	6	31	28	7	—	—	6	1	1	1	14	8	7	3	—	—	2	—
4 Oct.	92	—	—	—	4	4	—	13	17	—	—	—	—	5	3	1	—	7	31	7
25 Oct.	109	—	—	—	29	6	1	7	—	—	4	—	—	5	41	13	—	2	—	1
9 Dec.	128	—	1	2	7	2	—	26	27	8	2	—	—	2	1	—	—	3	21	26
1947																				
7 Feb.	110	—	2	1	—	—	—	2	17	24	9	—	—	—	—	—	—	2	15	38
15 May	104	—	8	10	1	—	—	—	3	6	32	—	5	2	—	—	—	—	9	28
17 Jun.	106	16	14	1	—	—	—	—	1	3	36	2	—	—	—	—	—	—	4	29
5 July	111	8	29	13	1	—	—	—	—	—	1	23	24	10	1	—	—	—	—	1
1 Oct.	98	1	25	17	—	—	—	—	—	1	—	1	19	33	1	—	—	—	—	—
18 Oct.	104	—	—	5	—	—	—	8	24	—	2	—	—	10	7	1	1	19	27	—
24 Oct.	103	—	—	6	—	—	—	5	22	2	—	—	5	13	4	1	—	12	25	8
4 Nov.	101	—	—	—	3	—	—	7	24	9	—	—	3	8	4	3	1	8	20	11
9 Dec.	111	—	—	1	—	—	—	1	11	29	4	—	—	1	1	—	1	1	12	49
1948																				
11 Feb.	57	—	—	—	—	—	—	—	2	19	12	—	—	—	—	—	—	—	—	24
11 Mar.	54	—	—	—	—	—	—	—	—	15	18	—	—	1	—	—	—	—	—	20
22 Apr.	61	1	—	—	—	—	—	—	—	6	45	—	—	—	—	—	—	—	—	9
18 May	55	4	—	—	—	—	—	—	—	2	45	—	—	—	—	—	—	—	—	4
28 June	59	11	2	1	—	—	—	—	—	1	27	10	2	—	—	—	—	—	—	5
28 Aug.	55	—	8	14	2	—	—	—	3	—	—	3	10	9	5	1	—	—	—	—
3 Sept.	26	—	3	4	1	—	—	—	—	—	—	—	8	7	2	1	—	—	—	—
30 Sept.	52	—	—	—	—	—	—	5	12	4	—	—	1	5	—	—	—	6	13	6
2 Nov.	32	—	—	—	—	—	—	—	11	6	—	—	—	—	—	—	1	1	3	10
1 Dec.	60	—	—	—	—	—	—	1	10	20	—	—	6	—	—	—	—	2	2	19
1949																				
29 Jan.	60	—	—	—	—	—	—	—	—	16	9	—	—	—	—	—	—	—	—	35
2 Mar.	55	—	—	—	—	—	—	—	—	13	18	—	—	—	—	—	—	—	—	24
20 Apr.	54	—	—	—	—	—	—	—	1	13	28	1	—	—	—	—	—	—	—	11
27 May	51	7	—	—	—	—	—	—	—	6	22	1	—	—	—	—	—	—	—	15
9 July	50	5	5	1	—	—	—	—	—	5	8	18	6	—	—	—	—	—	—	2
10 Aug.	54	2	9	2	—	—	—	—	1	—	2	15	20	3	—	—	—	—	—	—
3 Sept.	53	—	10	5	1	—	—	—	2	1	—	3	26	5	—	—	—	—	—	—
20 Oct.	61	—	3	1	—	—	—	4	12	5	1	—	4	3	1	—	—	—	11	16
4 Nov.	29	—	—	—	—	—	—	1	9	1	—	—	5	2	—	—	—	—	4	7
24 Nov.	31	—	—	—	—	—	—	1	6	5	1	—	2	—	—	—	—	—	2	14

TABLE VII. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM KALLOW POINT, PORT ST MARY, ISLE OF MAN

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1946																				
9 May	102	—	—	—	—	—	—	—	—	7	81	—	—	—	—	—	—	—	—	14
15 Aug.	101	10	10	2	—	—	—	—	—	—	60	10	2	—	—	—	—	—	—	7
21 Aug.	100	11	35	8	1	—	—	—	—	—	14	27	4	—	—	—	—	—	—	—
3 Oct.	44	—	—	2	12	2	—	1	2	—	1	—	—	2	18	3	—	—	1	—
17 Oct.	95	—	—	—	6	2	9	22	5	—	—	—	2	7	4	—	—	28	10	—
1 Nov.	94	—	—	8	8	—	7	20	5	—	1	—	—	12	10	—	—	—	20	3
25 Nov.	100	—	—	—	—	—	4	14	14	4	—	—	1	1	5	—	—	9	36	12
13 Dec.	117	1	—	6	—	—	1	29	64	5	—	—	—	—	—	—	—	—	8	3
1947																				
16 Jan.	106	—	—	—	—	—	—	—	16	1	53	—	—	—	—	—	—	—	—	36
9 Apr.	112	—	—	—	—	—	—	—	—	9	96	—	—	—	—	—	—	—	—	7
20 May	115	—	—	—	—	—	—	—	—	9	101	—	—	—	—	—	—	—	—	6
28 July	97	4	20	10	—	—	—	—	—	—	14	25	24	—	—	—	—	—	—	—
4 Sept.	108	2	8	23	7	2	—	—	1	—	1	11	38	14	—	—	—	—	1	—
6 Oct.	110	—	13	27	1	—	—	—	—	—	—	1	30	36	2	—	—	—	—	—
22 Oct.	121	—	—	11	28	6	—	3	—	—	1	—	—	7	39	20	—	6	—	—
25 Oct.	103	—	—	7	15	3	—	21	3	—	—	—	1	19	16	3	—	7	8	—
28 Oct.	111	—	—	3	15	10	13	18	1	—	2	—	—	4	23	6	5	9	2	—
30 Oct.	107	—	—	1	12	3	5	22	6	—	—	—	1	9	12	7	4	18	7	—
3 Nov.	110	—	—	2	12	3	6	32	5	—	—	—	—	2	11	4	7	24	2	—
10 Nov.	103	—	—	—	3	5	4	20	12	5	—	—	—	3	3	4	6	20	8	10
16 Nov.	105	—	—	—	—	1	3	22	18	4	1	—	—	3	4	4	—	21	14	10
25 Nov.	105	—	—	—	—	4	1	17	22	1	1	—	1	1	1	6	6	8	22	14
29 Nov.	101	—	—	—	1	2	5	22	22	4	1	—	—	—	2	3	4	13	15	7
11 Dec.	55	—	—	—	—	—	1	6	19	1	1	—	—	3	—	—	1	8	9	6
22 Dec.	50	—	—	—	—	—	—	9	16	1	—	—	—	—	4	2	5	5	7	1
1948																				
7 Jan.	53	—	—	—	—	—	2	9	12	3	—	—	—	1	—	1	1	8	9	7
28 Jan.	59	—	2	—	—	—	2	6	10	9	—	—	—	4	2	—	1	5	9	9
12 Feb.	56	—	—	—	—	—	—	—	—	11	22	—	—	—	—	—	—	—	—	23
8 Mar.	51	—	—	—	—	—	—	—	—	16	19	—	—	—	—	—	—	—	—	16
16 Apr.	53	—	—	—	—	—	—	—	—	1	52	—	—	—	—	—	—	—	—	—
25 May	53	2	—	—	—	—	—	—	—	1	47	—	—	—	—	—	—	—	—	3
4 July	51	—	—	—	—	—	—	—	—	1	49	1	—	—	—	—	—	—	—	—
5 Aug.	55	17	2	—	—	—	—	—	—	—	9	27	—	—	—	—	—	—	—	—
25 Aug.	129	—	24	27	11	—	—	—	—	—	—	5	35	22	5	—	—	—	—	—
24 Sept.	66	—	9	23	4	—	—	—	—	—	—	—	18	12	—	—	—	—	—	—
13 Oct.	45	—	—	—	—	—	—	1	5	2	—	—	1	—	—	—	—	1	13	22
20 Oct.	46	—	—	—	—	—	—	—	8	12	—	—	1	—	—	—	—	—	9	16
25 Nov.	44	—	—	—	—	—	—	—	1	16	—	—	—	—	—	—	—	—	—	27
1949																				
27 Jan.	48	—	—	—	—	—	—	—	1	20	5	—	—	—	—	—	—	—	1	21
18 Apr.	47	—	—	—	—	—	—	—	—	3	40	1	—	—	—	—	—	—	—	3
25 May	46	10	—	—	—	—	—	—	—	—	22	2	—	—	—	—	—	—	—	12
11 July	45	11	2	—	—	—	—	—	—	3	21	2	—	—	—	—	—	—	—	6
9 Aug.	49	11	14	—	—	—	—	—	—	—	4	14	6	—	—	—	—	—	—	—
5 Sept.	55	—	13	9	—	—	—	—	—	—	—	1	27	5	—	—	—	—	—	—
17 Oct.	48	—	2	—	—	—	—	—	13	10	1	—	5	—	—	—	—	—	—	17
23 Oct.	25	—	2	—	—	—	—	—	8	—	—	—	—	—	—	—	—	—	4	11
24 Nov.	28	—	—	—	—	—	—	—	6	6	—	—	—	—	—	—	—	—	2	14

TABLE VIII. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM TREVONE

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1946																				
17 Jan.	100	—	—	1	1	—	—	5	20	7	—	—	—	—	—	—	—	4	17	45
5 Feb.	43	—	—	—	—	—	—	—	5	22	2	—	—	—	—	—	—	—	—	14
5 June	54	—	—	—	—	—	—	—	13	6	10	—	2	—	—	—	—	—	2	21
29 July	101	4	1	—	—	—	—	—	—	—	95	1	—	—	—	—	—	—	—	—
28 Aug.	105	14	41	14	2	—	—	1	—	—	11	10	10	1	—	—	—	—	—	1
9 Sept.	105	6	22	14	—	—	—	—	—	—	9	16	32	6	—	—	—	—	—	—
24 Sept.	50	—	2	9	5	1	—	—	—	—	2	1	4	19	7	—	—	—	—	—
2 Nov.	104	—	—	8	26	8	—	3	—	—	1	—	4	13	23	16	—	1	—	1
4 Nov.	88	—	2	21	30	3	—	—	—	—	1	—	1	2	6	6	3	11	2	—
28 Nov.	104	—	2	—	—	—	—	3	32	9	1	1	—	—	—	—	—	—	21	35
12 Dec.	112	1	5	1	—	—	—	13	64	18	—	—	—	—	—	—	—	—	3	7
1947																				
31 Jan.	102	—	1	—	—	—	—	—	3	50	26	—	—	—	—	—	—	—	—	22
14 May	105	—	—	—	—	—	—	—	—	—	102	—	—	—	—	—	—	—	—	3
9 June	98	—	—	—	—	—	—	—	—	—	97	—	—	—	—	—	—	—	—	1
29 July	102	19	5	—	—	—	—	—	—	13	64	—	—	—	—	—	—	—	—	1
22 Aug.	104	12	13	—	—	—	—	—	—	—	15	53	11	—	—	—	—	—	—	—
17 Sept.	103	6	29	5	—	—	—	—	—	—	—	26	28	8	1	—	—	—	—	—
11 Oct.	104	—	2	29	7	—	—	1	1	1	1	—	7	40	14	1	—	—	—	—
21 Oct.	99	—	—	4	11	1	1	6	—	1	—	—	—	31	42	2	—	—	—	—
24 Oct.	103	—	—	2	14	4	—	12	3	—	—	—	3	10	35	11	1	4	4	—
4 Nov.	102	—	—	—	—	—	5	35	13	1	—	—	—	8	6	2	—	12	18	2
14 Nov.	104	—	—	—	—	—	—	7	13	12	—	—	2	2	1	5	3	1	11	47
10 Dec.	104	—	5	1	—	—	1	—	11	25	—	—	—	—	—	—	1	1	24	35
1948																				
11 Feb.	52	—	—	—	—	—	—	—	—	13	17	—	—	—	—	—	—	—	—	22
9 Mar.	55	—	—	—	—	—	—	—	—	9	26	—	—	—	—	—	—	—	—	20
24 Apr.	52	—	—	—	—	—	—	—	—	9	34	—	—	—	—	—	—	—	—	9
19 May	52	2	—	—	—	—	—	—	—	1	45	—	—	—	—	—	—	—	—	4
29 June	52	1	—	—	—	—	—	—	—	1	40	—	—	—	—	—	—	—	—	10
27 July	44	3	—	—	—	—	—	—	—	—	35	5	—	—	—	—	—	—	—	1
30 Aug.	54	11	16	—	—	—	—	—	—	—	—	14	13	—	—	—	—	—	—	—
13 Oct.	55	—	—	—	—	—	—	1	13	10	—	—	3	1	—	—	—	1	12	14
14 Nov.	55	—	—	—	—	—	—	—	4	20	—	—	—	—	—	—	—	—	6	25
1 Dec.	55	—	1	—	—	—	—	—	2	31	—	—	—	—	—	—	—	—	1	20
1949																				
31 Jan.	53	—	—	—	—	—	—	—	—	25	2	—	—	—	—	—	—	—	—	26
14 Mar.	52	—	—	—	—	—	—	—	—	23	13	—	—	—	—	—	—	—	—	16
21 Apr.	52	1	—	—	—	—	—	—	—	1	47	1	—	—	—	—	—	—	—	2
27 May	53	4	—	—	—	—	—	—	—	1	41	1	—	—	—	—	—	—	—	6
11 July	53	—	—	—	—	—	—	—	—	1	52	—	—	—	—	—	—	—	—	—
9 Aug.	51	9	1	—	—	—	—	—	—	2	23	13	1	—	—	—	—	—	—	2
10 Sept.	51	10	9	—	—	—	—	—	—	—	4	26	2	—	—	—	—	—	—	—
19 Oct.	54	—	6	16	—	—	—	5	4	—	1	—	3	9	5	—	4	1	—	—
4 Nov.	53	—	1	—	—	—	—	—	17	14	—	—	—	—	—	—	—	—	1	20
24 Nov.	26	1	—	—	—	—	—	—	—	16	—	—	—	—	—	—	—	—	—	9

TABLE IX. DISTRIBUTION OF GONAD STAGES IN SAMPLES OF *PATELLA VULGATA* FROM PLYMOUTH BREAKWATER

		Male									Neuter	Female								
		Developing					Spawning					Developing					Spawning			
Date	No. in sample	I	II	III	IV	V	IV	III	II	I		I	II	III	IV	V	IV	III	II	I
1946																				
18 June	141	10	7	2	—	—	—	—	—	15	91	—	—	—	—	—	—	—	1	15
30 July	137	14	—	—	—	—	—	—	—	—	97	1	—	—	—	—	—	—	—	25
1 Oct.	112	—	—	9	39	6	—	—	—	—	—	—	2	19	31	6	—	—	—	—
28 Oct.	120	—	—	10	15	7	—	1	2	—	—	—	1	25	33	14	—	9	3	—
27 Nov.	134	—	—	3	4	—	1	24	19	1	—	—	1	3	1	—	—	15	54	8
10 Dec.	141	—	9	4	2	—	—	38	38	5	—	—	1	—	—	—	1	6	29	8
1947																				
17 Feb.	154	—	12	3	—	—	—	—	31	31	7	—	—	—	—	—	—	1	6	63
17 May	189	—	—	—	—	—	—	—	—	2	187	—	—	—	—	—	—	—	—	—
11 July	126	4	1	—	—	—	—	—	—	—	118	—	—	—	—	—	—	—	—	3
6 Oct.	126	—	1	30	2	1	—	—	—	—	—	—	5	70	15	2	—	—	—	—
3 Nov.	108	—	2	—	6	1	2	17	12	2	—	—	—	6	16	6	—	9	23	6
11 Nov.	103	—	—	—	1	2	1	15	13	2	—	—	—	4	8	17	1	13	23	3
4 Dec.	100	—	4	2	3	7	2	9	4	—	—	—	—	2	—	33	2	10	22	—
1948																				
16 Feb.	50	—	—	—	—	—	—	—	—	21	5	—	—	—	—	—	—	—	—	24
2 Mar.	15	—	—	—	—	—	—	—	—	4	1	—	—	—	—	—	—	—	—	10
5 July	61	5	—	—	—	—	—	—	—	3	37	12	—	—	—	—	—	—	—	4
7 Aug.	93	17	2	—	—	—	—	—	—	—	14	56	1	—	—	—	—	—	—	3
1 Sept.	71	4	21	2	—	—	—	—	—	—	—	12	32	—	—	—	—	—	—	—
14 Oct.	62	—	—	8	10	—	—	3	—	—	—	—	2	15	8	2	4	10	—	—
2 Nov.	38	—	1	5	—	—	—	—	7	—	—	1	4	10	3	1	—	2	3	1
17 Nov.	100	—	2	3	1	—	—	5	24	7	—	—	5	12	7	—	—	10	5	19
30 Nov.	99	—	—	4	—	—	—	6	21	3	—	—	8	10	4	1	1	13	15	13
21 Dec.	85	—	—	—	—	—	—	—	2	38	—	—	—	—	—	—	—	—	—	45
1949																				
1 Feb.	75	—	—	—	—	—	—	—	—	29	—	—	—	—	—	—	—	—	—	46
2 Mar.	80	—	—	—	—	—	—	—	—	27	16	—	—	—	—	—	—	—	—	37
3 May	82	2	—	—	—	—	—	—	1	6	61	4	3	—	—	—	—	—	—	5
28 June	50	1	—	—	—	—	—	—	—	1	47	1	—	—	—	—	—	—	—	—
9 Aug.	52	4	3	—	—	—	—	—	—	—	30	12	—	—	—	—	—	—	—	3
10 Sept.	66	9	18	—	—	—	—	—	—	—	—	25	13	—	—	—	—	—	—	1
12 Oct.	56	—	4	14	2	—	—	—	—	—	—	—	11	24	1	—	—	—	—	—
27 Oct.	82	—	1	—	—	—	—	—	29	1	—	—	—	—	—	—	—	—	15	36
23 Nov.	40	2	—	—	—	—	—	—	6	10	—	—	—	—	—	—	—	—	1	21

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THE ABNORMAL DEVELOPMENT OF PLAICE EMBRYOS AND LARVAE IN MARINE AQUARIA

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(Plate I and Text-figs. 1–15)

We know that a mature female plaice can liberate many thousands of eggs into the sea during the spring spawning season in the southern North Sea (Simpson, 1951). We also know that a very small percentage indeed of these eggs develops into fish of marketable size. At all stages in its life history a plaice is confronted with environmental hazards, which, if not overcome, may have fatal consequences.

The pelagic egg and subsequent larval phase of marine teleosts appear to be particularly vulnerable to adverse conditions in the sea. Rollefsen (1930, 1932) demonstrated the lethal effect of agitation on early cod eggs and discussed wave-action as a mortality factor. Battle (1944) elaborated this work for other teleost species. Tester (1942) observed a high mortality of herring eggs spawned inshore, on weeds, along the south-east coast of Vancouver Island, and thought this loss was due to respiratory difficulties resulting from low carbon dioxide or high oxygen content of the water, a theory not substantiated by the work of Kelley (1946). There must also be considerable losses of eggs and larvae due to the activity of predators. Spent herring caught in the Southern Bight in early spring are frequently found with stomachs full of plaice eggs.

Undoubtedly, estimates of early mortality in the sea are needed for a better understanding of the population dynamics of a particular fishery. At the same time, rearing experiments in the laboratory, to determine the effect of known environmental changes on the development and survival of pelagic eggs and larvae, should give some knowledge of the possible causes of natural death.

Salt-water fish are notoriously difficult to raise. Petersen (1894), after several abortive attempts to rear plaice from artificially fertilized eggs, was emboldened to say, '. . . at the pelagic stage, after the yolk has been absorbed, it (the plaice post larva) cannot be kept alive in aquaria; at this stage the larva must be caught in open water and this is not always easy'. Cunningham (1894), using circulating water at 12° C in his aquaria, also failed to rear whiting, flounder and plaice eggs to the post-larval stage. Dannevig (1897) succeeded in taking a small proportion of an artificially fertilized batch of plaice eggs through and beyond metamorphosis for the first recorded time.

Since these early attempts, Norwegian workers have had more success with plaice, cod and herring, accounts of which have been given by Rollefsen (1940) and Dannevig & Hansen (1952).

At Lowestoft, during our recent experiments on plaice reared under static sea-water conditions in temperature-controlled aquaria, we encountered the usual difficulties. However, by the close observation of living material under known laboratory conditions, it becomes possible, first of all, to recognize normal healthiness, and then, against this standard, to detect even slight symptoms of abnormality. Knowledge of these symptoms and their causes aids the development of efficient rearing techniques and the interpretation of experimental results.

Some abnormalities of fish larvae in marine aquaria are already known. Dannevig (1897) observed that larvae from newly hatched plaice eggs could readily be divided into two fractions: a minority of healthy, richly pigmented individuals found ranging for food at all levels in the aquarium, and a majority of abnormal specimens which, after early hyperactivity at the surface, soon lost their pigment, and during long quiescent periods were to be found suspended head downwards near the bottom of the jar. Gorham's early work on the gas-bubble disease of adult marine fish (1901) has been followed by observations on the same complaint in larvae with swim-bladders, by Kotthaus (1939), Soleim (1940) and Henly (1952). Plaice larvae are not affected by this disease. Bückmann, Harder & Hempel (1953) found that herring larvae in lighted glass-vessels often sustain deformations of the head by continual collision with the walls of the jars. Attacks by *Vibrio anguillarum* and *Lentospora cerebralis* (Dannevig & Hansen, 1952), and by bacteria, can produce abnormality and death in marine aquaria.

Trout larvae in freshwater aquaria frequently suffer from a fluid inflammation of the yolk-sac, known generally as *Hydrocoele embryonalis*, or as 'Dotterblasenwassersucht' by German workers. Dieterich (1938) proved the offspring of young parents to be more susceptible to 'yolk-sac dropsy' than the issue of older fish, and that offspring of crosses of related species are similarly prone.

The present paper describes a further common, abnormal condition in plaice, and attempts to explain it in terms of what is already known of the structure and physiology of pelagic marine larvae.

Collection of Material and Rearing Methods

Plaice eggs were periodically collected by stramin net in the Southern Bight and off Flamborough, during the spawning season of 1953. Additional supplies of eggs were available from an artificial fertilization carried out in the Irish Sea area. After separation from the rest of the catch, plaice eggs at various stages of development were kept in 7 lb. jars at 6° C with frequent

water changes. On arrival in the laboratory, the eggs were transferred to fresh sea water from the spawning area in glass vessels cooled to 6° C ±0·5° in a large tank. 150–200 eggs were pipetted into each vessel of 1500 ml. capacity. The eggs were gently aerated up to the start of hatching; the emergent larvae were then removed into identical vessels, in batches of fifty. *Artemia salina* nauplii were introduced as food into the aquaria 4 days after hatching.

The shortcomings of this technique are manifold. Temperature alone was adequately controlled. In such small volumes of static sea water there must be inevitable ionic variations with the excretion and accumulation of carbon dioxide and other metabolites. Plaice larvae are able to adapt themselves to these gradual changes, some individuals more completely than others. A gross water renewal invariably kills off the weaker brethren. Ionic changes are also brought about on the addition of living food to the jars, and by the use of air from which the carbon dioxide has been incompletely removed.

Light conditions were uncontrolled; noticeable changes in larval activity were caused by the use of inspection lamps, thus disturbing the natural diurnal cycle of activity as witnessed by their feeding habits (Shelbourne, 1953).

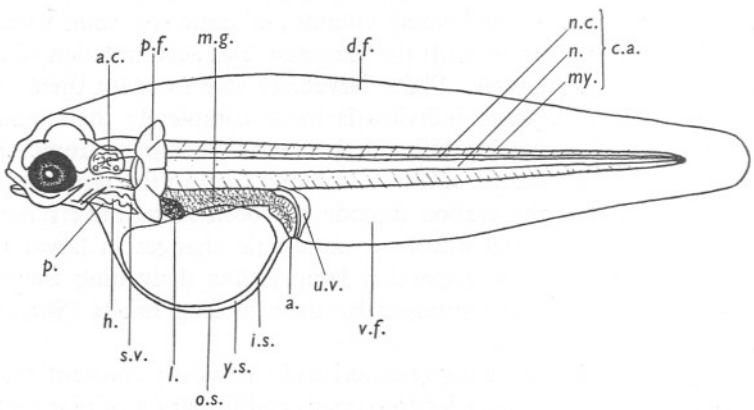
Where a supply of continually renewed and chemically constant sea water is available, many of the obstacles to the successful rearing of marine teleost eggs and larvae are removed. However, it must be recorded that during the 1954 plaice-spawning season, batches of artificially fertilized eggs were taken through to a normal early post-larval stage with no water change and no aeration, under constant temperature and light conditions. On the introduction of washed *Artemia* nauplii into the jars, the majority of the larvae started feeding. Successive additions of food called for frequent water changes; it was under these rapidly changing physico-chemical conditions that the larvae began to sicken.

The Features of a Normal Larva and the Course of its Yolk Loss

Text-fig. 1 is a diagram of a normal newly hatched plaice larva 6·5 mm long. The fore-, mid- and hind-brains are clearly seen through the transparent cartilage of the cranium, whilst the heavily pigmented eyes and expanded auditory capsules are the most conspicuous of the associated sense organs. The nervous tissue of the brain narrows into the nerve cord posteriorly, which, together with the notochord, developing median blood vessels, and investing muscle myotomes, forms the larval axis or trunk.

The angular mandible is normally mobile soon after hatching, its articulation with the upper jaw producing a mouth gape sufficiently wide to accommodate the large, thin-walled diatoms and small zooplankton of northern waters. The future branchial apparatus is as yet incomplete; the gill clefts are

open, but the gill bars lack branchial filaments. Even at this early stage, the simple gut tube is already coiling to the right, and is wedged between the ventral surface of the larval axis and the dorsal side of the underslung yolk-sac. Sphincters divide the alimentary canal into fore-, mid- and hind-gut, opening ventrally at the anus. The liver is a conspicuous gut appendage lying just behind the heart.



Text-fig. 1. Early plaice larva: side view. *a*, anus; *a.c.*, auditory capsule; *d.f.*, dorsal marginal fin; *h.*, heart; *i.s.*, inner yolk-sac; *l.*, liver; *m.g.*, mid-gut; *my.*, myotome; *n.*, notochord; *n.c.*, nerve cord; *o.s.*, outer yolk-sac; *p.*, pericardium; *p.f.*, pectoral fin; *s.v.*, sinus venosus; *u.v.*, urinary vesicle; *v.f.*, ventral marginal fin; *y.s.*, yolk-sac sinus.

In the ventro-lateral tissue of the trunk, above the gut, run the two urinary ducts, each of which connects in front with a pronephros behind the auditory capsules. The two ducts coalesce posteriorly as the urinary vesicle, opening behind the anus.

The heart, in its pericardium, lies underneath the oesophagus, and at hatching is differentiated into a muscular ventricle, an auricle, and a membranous sinus venosus. Anteriorly, the ventricle is twisted upwards and forwards to the ventral aorta, whilst posteriorly the sinus venosus opens directly into the yolk-sac sinus, a space between the membrane of the outer yolk-sac and the periblast layer, or inner sac surrounding the fluid yolk. There are neither cellular structures nor oil globules in the yolk, and it has no direct communication with the gut. The outer yolk-sac is but a ventral extension of the very thin integument completely clothing the larva, except at the gut and renal openings. This membrane forms the large marginal fin extending from the head above the larval axis, round the caudal extremity and back ventrally as far as the anal aperture. The two lamellae of the continuous fin are separated from one another, and supported by turgor in the serous subdermal space between (Text-fig. 4). The paired pectoral fins project outwards and backwards, just behind the auditory vesicles.

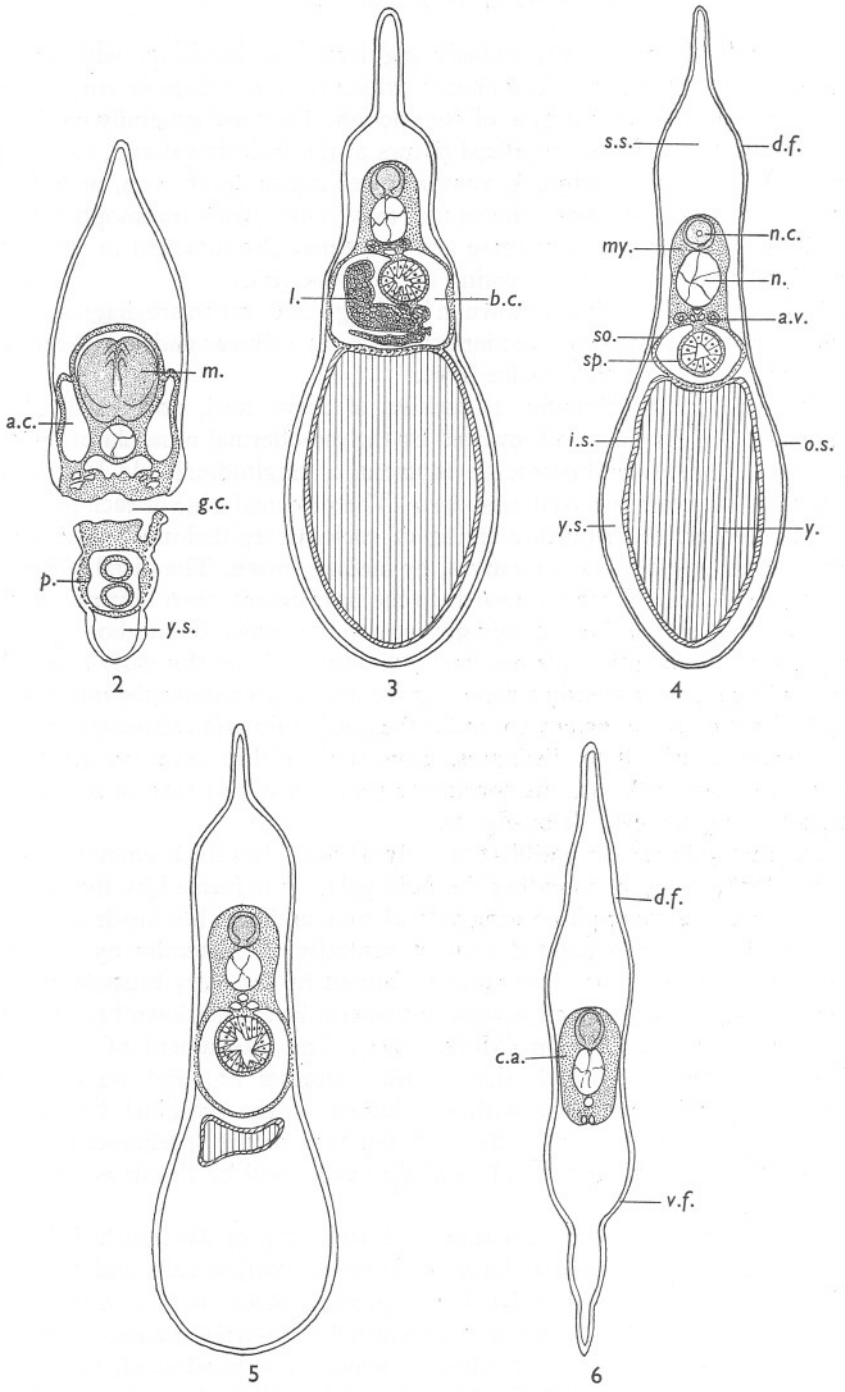
A normal plaice is conspicuously pigmented at hatching, with yellow xanthophores and black melanophores, which have a stellate or corpuscular form, according to their degree of contraction. They are generally confined, in early larval life, to the superficial tissues of the main larval axis, though in good environmental conditions, xanthophores either develop on, or migrate into, the marginal fin above the caudal axis. Only black melanophores are found on the inner yolk-sac; these are sometimes also attached to the inner face of the outer sac, thus traversing the yolk-sac sinus.

Finer structural details are shown in Text-figs. 2–6, which are diagrammatic reconstructions of transverse sections through the yolk-sac and caudal regions of a normal newly hatched plaice larva.

The larval trunk (Text-fig. 4) consists of nerve cord, notochord, blood vessels and urinary ducts, all invested by the mesodermal musculature of the myotomes. During embryonic devlopment, a longitudinal fold has grown down each side from the axial mesoderm. The proximal edge of each fold, the splanchnopleure, contributes a lining of coelomic epithelium to the outer surface of the gut and its derivatives, by median fusion. The distal edges, or somatopleure, are reflected outwards to the integument, then inwards to join under the gut, and will eventually give rise to the ventro-lateral body wall of the post-larva, when the yolk has finally disappeared. At this early stage, the somatopleure is indifferently attached to the integument above the inner yolk-sac, whilst the ventral extension under the gut is thin and attenuated, and in some sections difficult to distinguish from the periblast tissue on the dorsal side of the inner yolk-sac. Its continuity ventrally is best seen in sections of the posterior gut region (Text-fig. 5).

The inner yolk-sac, or periblast of early workers, is a thick syncytial membrane (Meek, 1913) surrounding the fluid yolk. It is formed by the activity of the ventral blastopore lip during gastrulation, and now lies inside the outer ectodermal yolk-sac, separated from it ventrally and laterally by a serous space, the yolk-sac sinus. This sinus is formed by the early breakdown into mesenchyme, of the extra-embryonal mesoderm layer laid down between the two sacs during gastrulation (Meek, 1924). The integument of the outer yolk-sac is continuous with that of the marginal fin, and the yolk-sac sinus has lateral connexions with the lumen of the marginal fin due to the indifferent attachment of the somatopleure to the integument at gut level. The continuity of the yolk and fin spaces will be discussed in more detail later.

The transparent skin is very thin, and according to McIntosh & Prince (1890) consists of an external layer of flattened, corneal cells and an inner Malpighian layer with a very loose cell texture. Meek (1924), on the other hand, states that in the cod the integument has a syncytial nature, reinforced by mesodermal elements. According to which view is adopted, the serous space between the integument and larval axis dorsally and the inner yolk-sac



Text-figs. 2–6. Diagrams of transverse sections through an early plaice larva: through (2) pericardial region, (3) anterior part of yolk-sac, (4) mid-yolk-sac region, (5) posterior part of yolk-sac, and (6) caudal region. *a.c.*, auditory capsule; *a.v.*, axial vessels; *b.c.*, body cavity; *c.a.*, caudal axis; *d.f.*, dorsal marginal fin; *g.c.*, gill cleft; *i.s.*, inner yolk-sac; *l.*, liver; *m.*, medulla; *my.*, myotome; *n.*, notochord; *n.c.*, nerve cord; *o.s.*, outer yolk-sac; *p.*, pericardium; *so.*, somatopleure; *sp.*, splanchnopleure; *s.s.*, subdermal space; *v.f.*, ventral marginal fin; *y.*, fluid yolk; *y.s.*, yolk-sac sinus.

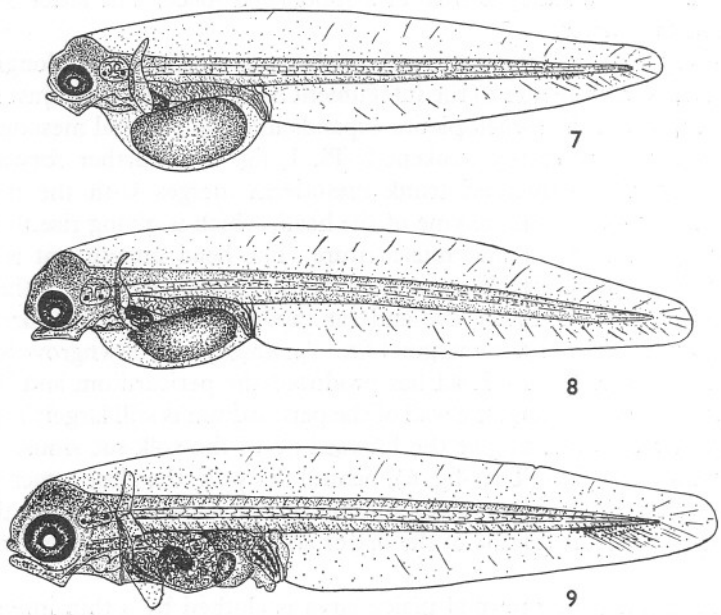
ventrally is either a subepidermal or a subdermal space. The latter term will be used in this paper.

Text-fig. 3 is a transverse section through the liver. The lateral, longitudinal fusion of the somatopleure to the integument is still apparent, but just in front of the liver, where the somatopleure expands into the pectoral mesoderm, the attachment is considerably weakened (Pl. I, fig. 2). Further forward still (Text-fig. 2), the segmented trunk mesoderm merges with the plate-like mesoderm and loose mesenchyme of the head, which is giving rise to skeletal structures, blood vessels and musculature. The head integument is closely integrated with the underlying tissue in front of the optic lobes of the brain; for the most part, the brain, eye and ear capsules lie in a fluid-filled space, continuous with that of the marginal fin. An embryonic downgrowth of two mesoderm folds under the head has produced the pericardium and heart by ventral fusion; at hatching, the wall of the pericardium is still largely separated from the skin beneath, to give the forward tip of the yolk-sac sinus.

In the caudal region (Text-fig. 6), behind the yolk, the close attachment of the integument to the sides of the somites of the larval axis divides the subdermal space into the lumina of the upper and lower portions of the marginal fin.

To sum up, a young normal plaice larva is clothed by a thin integument, which is strongly attached to the mesoderm in the anterior head region and to the sides of the caudal axis; it is indifferently attached above the yolk-sac sinus, particularly in the region of the pectoral fin. The inner yolk-sac is thus a bag of yolk lying in a ventral extension of this skin, its subdermal space (yolk-sac sinus) incompletely roofed over by somatopleural fusion with the integument. It is cut off behind by mes-ectodermal fusion round the rectum and urinary vesicle, and in front by the integration of the integument with the mesoderm on the underside of the head.

Although the normal embryo is coiled inside an egg-shell seldom greater than 2 mm in diameter, the freed larva may be 6 mm long, with straight axial structures and a turgid marginal fin. Shortly before hatching a marked diminution occurs in the volume of the yolk-sac system. The larva emerges with a boat-shaped yolk which continues to diminish smoothly and evenly for 8–10 days at 7° C (Text-figs. 7–9). Towards the end of larval life the small inner yolk-sac becomes pushed up into the arch formed by the looping intestine, so that the ventral surface of the animal, under the gut, is uninterrupted by pendulous projections of any kind. A small spherule of inner yolk-sac tissue may persist in what is now the posterior part of the body cavity for some time after the main mass of fluid yolk is lost. The final disappearance of this yolk vestige marks the end of the arbitrary larval stage and the beginning of the post-larval life of the plaice.



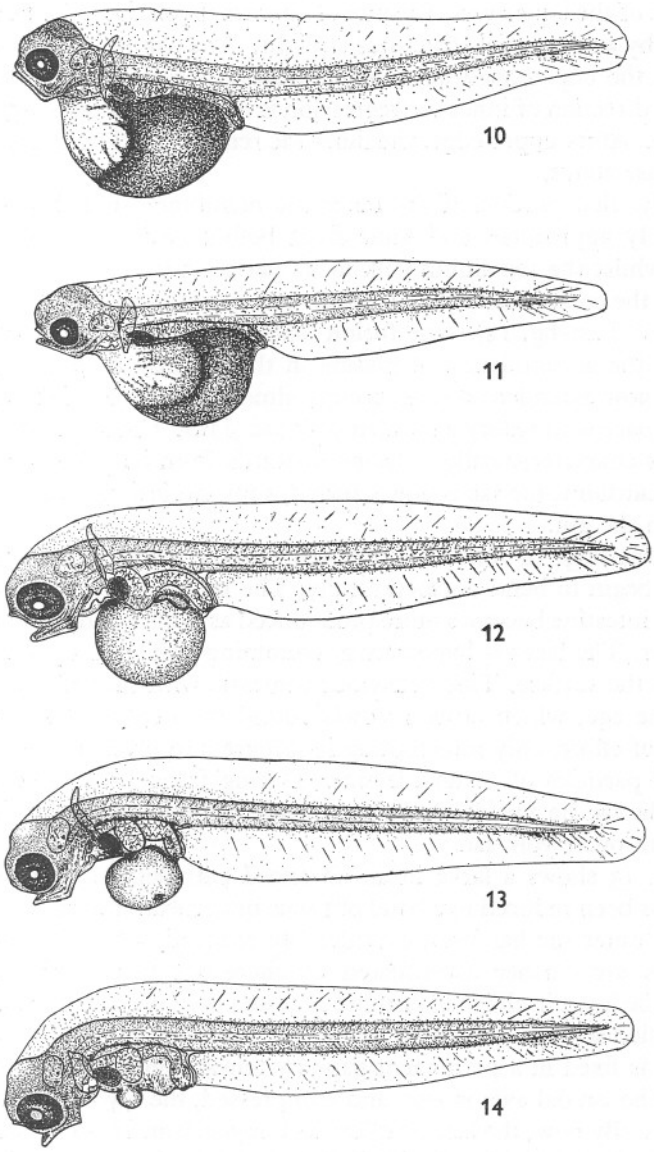
Text-figs. 7–9. Plaice larvae at (7) 24 h, (8) 3–4 days, and (9) 2–3 weeks, showing the normal course of yolk loss during development. Drawings are exact reproductions of preserved larvae. × 13.

Abnormal Yolk Loss and the Development of Axial Bending

In contrast to the normal course of observable yolk loss just described, tank-reared larvae are prone to abnormal and uneven yolk resorption. Towards the end of the larval phase, affected plaice invariably develop a marked dorso-ventral bending of the axial structures above the gut.

Abnormal individuals hatch with an unusually large inner yolk-sac, excavated anteriorly where the sinus venosus opens into the yolk-sac sinus (Text-fig. 10). This anterior dimpling of the yolk is very characteristic and is caused by an accumulation of plasma in the yolk-sac sinus underneath the pericardium. The larval axis may still show signs of embryonic curling as the fish lies at the surface, moving only when touched.

The subpericardial space enlarges as the inner yolk-sac decreases, so that the outer investing sac remains swollen. At the same time, the periblast tissue of the inner sac adheres closely to the inside surface of the outer sac, forming fingers of tissue extending forwards towards the heart (Text-fig. 11). The dimpling of the yolk is usually intensified as the surface area of the outer sac slowly decreases. The yolk mass comes to occupy the posterior part of the outer sac, which, when viewed from above, appears much more bulbous than



Text-figs. 10–14. Plaice larvae showing abnormal course of yolk loss and the gradual bending of the axis above the gut during development: (10) 12 h, (11) 24 h, (12) 8 days, (13) 2 weeks, and (14) 3 weeks. Exact reproductions of preserved larvae. × 13.

the streamlined sac of a normal specimen. The larva is now active at or near the surface of the aquarium. In spite of abnormal yolk loss, the larva appears to be healthy and its axial structures straight. At a slightly later stage in yolk retraction, the cup-shaped anterior excavation becomes obliquely inclined, so that the direction of inner sac regression is now postero-dorsally, instead of posteriorly. At its upper edge, the inner sac remains in close contact with the visceral mass above.

Normally, that portion of the outer sac membrane underlying the heart progressively approaches and knits from before backwards with the pericardium, whilst the remaining yolk in its periblast retracts up into the arch formed by the coiling intestine. In abnormal specimens with a similar degree of yolk loss (Text-fig. 12) some fusion takes place under the heart, but it is limited by the accumulation of plasma in the original subpericardial space, which has now extended so as to occupy almost the whole of the outer yolk-sac. This space is in reality a swollen yolk-sac sinus. The anterior edge of the outer sac is characteristically reflected forwards from its point of attachment to the pericardium; the sac is now a turgid, subspherical, pendulous structure underneath the gut.

It is at this stage of abnormal yolk loss that the axial structures above the coiled gut begin to bend dorso-ventrally. The mouth starts to gape, and the arch of the intestine becomes more pronounced as the head and anus approach one another. The larva is hyperactive, swimming jerkily in a plane obliquely inclined to the surface. This behaviour contrasts with that of a normal larva of the same age, which cruises slowly round the depths of a jar with the minimum of effort, only interrupting its progress to investigate and perhaps catch food particles of a suitable size. During frequent quiescent periods, abnormal larvae lie upside down at the surface, supported by the swollen and buoyant outer yolk-sac.

Text-fig. 13 shows a larva in an advanced pathological state. The inner yolk-sac has been reduced to a band of tissue underneath the arching gut. The still turgid outer sac has been considerably reduced, whilst its subspherical proportions are further accentuated by increased fusion with the tissue beneath. The heart itself is noticeably congested in living specimens. Bending has intensified so as to incline the plane of the pectoral fin to the vertical, and the mouth is fixed in a permanent gape by tension on the mandibular musculature. The caudal axis is thin and compressed, the myotomes losing their distinctness. By now, the larva has lost its buoyancy and is to be found feebly spiralling near the bottom of the jar, showing no interest in offered food particles. In contrast to normal larvae, pigmentation is poor; some black chromatophores may persist, but all the yellow xanthophores have disappeared.

Finally, the outer yolk-sac becomes just visible as a slight ventral protuberance, and the head is bent downwards almost at right angles to the caudal axis (Text-fig. 14). The inner yolk-sac has disappeared, and the larva lies

intermittently vibrating on the bottom of the jar. Dehydration soon follows, marked by the spread of opacity from the anterior and posterior extremities of the animal.

All abnormal larvae reared in artificial conditions do not display the same severe axial bending as the series just described. Generally speaking, the earlier the onset of abnormal symptoms, the greater is the distortion at the end of the larval phase, and the smaller are the chances of survival through to the post-larval stage.

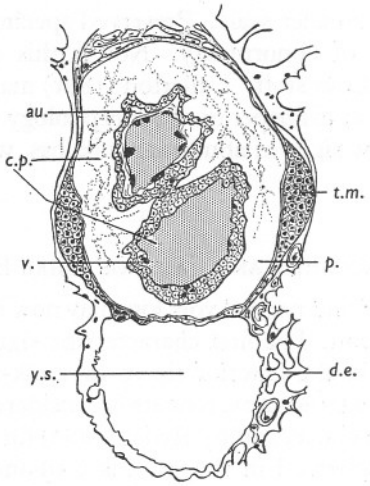
Some idea of the high incidence of this complaint is given by experimental data for 1953. In January, 74% of North Sea plaice eggs used in the laboratory gave rise to severely bent larvae. In February, 66% were abnormal and only 5% of the remainder survived through to the post-larval stage. Bending also occurred in April on a smaller scale. Preserved specimens from earlier years frequently show signs of abnormality. Nor is this complaint confined to plaice-rearing work at Lowestoft. Fullarton (1891) made a series of drawings of whole larvae, as part of his paper on the morphology of plaice development; the younger larvae show signs of abnormal yolk loss, whilst the oldest is very definitely bent.

The Mechanics and Cause of Axial Bending

The course of abnormal and normal yolk loss may now be reconsidered against the structural background. The first characteristic sign of abnormality is the accumulation of fluid in the anterior tip of the yolk-sac sinus, to form the 'subpericardial space', a structure erroneously considered normal by McIntosh & Prince (1890) for the plaice, and by Ryder (1883) for the embryo shad. The space is significantly spherical in shape; it is contained partly by the outer yolk-sac and pericardial wall, and partly by the dimpled membrane of the inner yolk-sac, the tissue of which is left sticking to the inside surface of the outer sac during yolk loss. These indications suggest that the contents of the outer yolk-sac are under a slight pressure. Two conditions are necessary for the development and maintenance of this pressure: first, an increase in the tension of the outer-sac membrane, and secondly, the outer yolk-sac must be a closed system.

During the normal course of larval yolk loss, the surface areas of the inner and outer yolk-sacs rapidly diminish in phase with one another, until finally the inner sac completely disappears and the outer sac becomes the integument covering the thickening mesoderm under the gut. Abnormally, on the other hand, the speed of diminution of the outer sac is much less than that of the inner sac, due to the persisting accumulation of plasma in the sinus between the sacs. Although the outer sac membrane may have elastic properties, there is evidence that it loses surface area by an active process of degeneration and reorganization, rather than one of mechanical reassertion.

Text-fig. 15 is a transverse section of a newly hatched larva, through the front part of the outer sac underneath the heart, showing this active process at work. Before degeneration sets in, the thin membrane is only sparsely sprinkled with nuclei. As degeneration proceeds, the affected area becomes much more richly supplied with vesicles and nucleated structures, some of which may be budded off externally, resembling the process of inner-sac corpusculation described by Ryder (1883) and Williamson (1898). Others open, and presumably discharge their contents at the outer face of the integument, leaving sharply defined depressions in the matrix of the membrane, which is thickened and puckered at this point. These activities can be followed in the living larva.



Text-fig. 15. Transverse section through the pericardium of a young plaice larva, × 150. *au.*, auricle; *c.p.*, coagulated plasma; *d.e.*, degenerating ectoderm; *p.*, *pericardium*; *t.m.*, thickening mesoderm; *v.*, ventricle; *y.s.*, yolk-sac sinus.

Despite corpuscular discharge, the membrane remains imperforate. It is significant that integumental activity occurs for the most part anteriorly, and in close proximity to growing mesoderm. As a result, the two tissues knit closely together from before backwards, the surface area being reduced in the process. The early direction of mes-ectodermal fusion is shown by the receding anterior edge of the outer sac in Pl. I, figs. 1–4; only at a late larval stage does similar activity start in front of the anus, nipping in the posterior edge of the sac. The somatopleural walls each side of the body cavity behind the heart become thickened, and, together with the ectodermal contribution from the degenerating outer yolk-sac, form the ventral body wall of the larva.

When yolk loss is normal, the tension set up in the outer sac, by its fusion with mesoderm under and behind the heart, pushes the remaining yolk in

its periblast up into the arch of the intestine. Abnormal yolk loss does not prevent, but hinders, the trend towards degeneration of the outer sac membrane as it knits with the underlying mesoderm anteriorly; the tension thus generated fulfils the first condition needed to produce a slight pressure in this sac.

The second condition is that the outer sac remain a closed system. It was first shown by Fulton (1898) that a large volume of low density yolk diluent enters the egg at maturation in the ovary, thereby enabling it to float in sea water when liberated. It is clear that the overall volume enclosed by the embryonal integument can only decrease, during incubation, within the limit laid down by the initial reserve buoyancy, if the egg is to remain floating. As soluble nutrients are withdrawn from the dilute yolk during tissue-synthesis and respiration, so the yolk diluent accumulates beneath the integument and separates it from the underlying mesoderm to form the system of subdermal spaces so characteristic of pelagic embryos and larvae. In this way, the embryonal volume is maintained; but equally important is the fact that, as a result of integumental separation, the yolk-sac sinus is put in lateral communication with the lumen of the dorsal marginal fin, so that yolk derivatives have easy access to the growing tissues, without the intervention of the heart and ill-developed vascular system (Shelbourne, 1955).

In abnormal plaice larvae the lateral subintegumental route connecting the yolk sinus with the fin space either fails to develop or becomes occluded. The somatopleure fold, by its non-separation from the integument at gut level and in the region of the pectoral fin, cuts off these two subdermal spaces. As the vascular circuit outside the yolk-sac sinus is simply a by-pass with neglible volume at this early larval stage, the outer sac is virtually a closed system, conforming to the second abnormal condition necessary for the maintenance of a slight internal pressure.

The inner yolk-sac is indistinctly separated from the body cavity by the weak ventral extension of the somatopleure under the gut. During abnormal yolk loss this thin membrane ruptures; the body cavity becomes continuous with the swollen sac underneath and is likewise inflated. The sac and body cavity system then has an hour-glass shape, the waist being an inpushing ring of fusing integument and mesoderm. As this ring closes under slight pressure, so the axial structures above the gut bend in conformity. Further mes-ectodermal fusion under the gut, to complete the ventral body wall, takes place with the axis thus bent, with the result that, when the pendulous sac disappears in anything up to a month, the distortion is maintained.

The cod also has a pelagic embryo, but it is not prone to the distortion already described for the plaice. Pl. I, fig. 1, is a slightly oblique transverse section through the pectoral fin region of a young cod larva, to show the great extent of the subdermal space and the complete separation of the integument from the underlying structures. This separation is visible in the living larva towards the end of the yolk-sac stage. The yolk sinus is continuous with

the lumen of the marginal fin, to give free access to yolk derivatives to all parts of the larva. The cod may be said to be a classical example of the pelagic mode of teleost egg development.

During normal plaice development such gross separation of integument from underlying tissue does not occur; turgor is never as great, and its degree is much more critical. In the static water conditions of our rearing experiments, it is likely that chemical deterioration of the water, with the accumulation of respiratory metabolites, particularly carbon dioxide (Burfield, 1928), can impair the efficiency and impermeability of the embryonic and larval integument. Water will then be lost and salts gained, under the osmotic gradient, and with the reduction in turgor the integument will come to lie closer to the underlying mesoderm. If turgor is sufficiently reduced by poor conditions at an early embryonic stage, the important connexion between the yolk and fin spaces may never develop. Moreover, with loss of water there will be an increase in salt concentration. The internal salt balance must be restored, or the biochemical processes of normal development will be seriously affected. No mechanism of salt regulation for the marine fish embryo is yet known; if salt tolerance is slight, then it is easy to understand why marine larvae are difficult to rear in shore aquaria.

Significant metabolite accumulations are not likely to occur in the sea during the early life of the plaice, since chemical conditions are practically constant. One might therefore expect to find but a slight incidence of axial bending in larvae from plankton hauls. Only a single suspect has been found during the investigation of some thousands of sea larvae, although an adult plaice with a badly bent axis was landed on Lowestoft fish market in the spring of 1954.

My thanks are due to Mr G. T. Thacker of this laboratory, for executing the drawings in this paper; and also to Dr H. A. Cole of the Fisheries Laboratory, Burnham-on-Crouch, and to Mr A. C. Simpson, of the Shellfish Experimental Station, Conway, for their helpful criticisms of the original manuscript.

Summary

In 1953 laboratory attempts to rear plaice post-larvae were marred by the regular occurrence of structural distortion in late larval life. Normally the yolk-sac system diminishes rapidly and evenly during the short larval phase; abnormally the outer integument of the yolk-sac houses a persistent accumulation of plasma.

Under normal circumstances the yolk-sac sinus communicates laterally with the subdermal space of the dorsal marginal fin, providing free access of fluid yolk derivatives to the growing tissues. Abnormally the lateral channels become occluded or fail to develop, and yolk derivatives, under a slight

pressure, are trapped in the outer sac. This prevents the smooth fusion of mesoderm and ectoderm to form the ventral part of the body wall, and ultimately causes structural distortion of the larval axis above the body cavity.

Cod larvae never suffer this distortion, because the turgor within their subdermal spaces is sufficient to separate completely the integument from the somitic mesoderm above the yolk sac. The degree of turgor in plaice embryos and larvae is much more critical, and water loss under the osmotic gradient, in poor tank conditions, may account for the described abnormality.

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EXPLANATION OF PLATE I

⊢, indicates progress of mes-ectodermal fusion, from before backwards.

Fig. 1. Transverse section through the pectoral region of a cod larva; stained haematoxylin.
Fig. 2. Transverse section through the pectoral region of a plaice larva: stained haematoxylin.
Fig. 3. Preserved plaice larva; 1 day old. Early mes-ectodermal fusion anteriorly, under the pericardium.
Fig. 4. Three days old. Mes-ectodermal fusion now extends posteriorly beyond the pericardium.
Fig. 5. Four days old. Inner and outer yolk sacs much reduced. Integument knitted to underlying mesoderm beyond the level of the liver.
Fig. 6. Nine days old. Mes-ectodermal fusion almost complete ventrally.

Figs. 1–2, ×68.
Figs. 3–6, ×23.

Figs. 1–6.

(*Facing p.* 192)

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NEUROHORMONES OF INVERTEBRATES

I. CARDIO-REGULATORS OF *CYPRINA* AND *BUCCINUM*

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(Text-figs. 1–5)

The present study had two aims: first to seek among the molluscs of the Plymouth region, one with a heart suitable for the bioassay of acetylcholine and 5-hydroxytryptamine; and secondly, to examine molluscan nervous tissue for the presence of cardio-regulator substances. Brief reports of some of the results have appeared elsewhere (Welsh, 1953*a*, 1954*a*).

Material and Methods

Following a preliminary study of the hearts of *Pecten maximus* (L.), *Modiolus modiolus* (L.), *Mytilus edulis* L., *Cyprina islandica* (L.), and *Buccinum undatum* L., the last two were considered most promising for purposes of bioassay. The common whelk, *Buccinum*, was available in adequate numbers but the supply of the bivalve, *Cyprina*, was limited.

The *Cyprina* heart was isolated and perfused in the same manner as previously described for the heart of *Venus mercenaria* (Welsh & Taub, 1948). The *Buccinum* heart was exposed and a glass cannula inserted into the ventricle either by way of the auricle or the aorta. When the cannula was inserted via the auricle, two or three separate ligatures of rather coarse thread were used to secure the cannula. This usually prevented the tearing of the delicate auricle from the thick-walled ventricle. The *Buccinum* ventricle was perfused in a manner similar to that used by Alexandrowicz & Carlisle (1953) in perfusing hearts of decapod crustaceans. Sea water was the perfusion fluid.

When examining a tissue for the presence of acetylcholine (or acetylcholine-like substance) an extract was made by grinding with sand in the presence of eserine, 1:10,000, or grinding after heating the tissue at 100° C for a few minutes. When looking for an opposing, heart-exciting substance, a tissue was ground and allowed to stand at room temperature for 30 min before filtering, or, the extract that had been made with eserinized sea water was brought to pH 9–10 with NaOH and boiled for 10 min to hydrolyse the acetylcholine.

Several drugs were used to help in the identification of the suspected cardio-regulator substances. Among them were lysergic acid diethylamide or LSD (supplied by Sandoz Products Ltd., London), 5-hydroxytryptamine (supplied

as serotonin creatinine sulphate by the Abbott Laboratories), and 2:5 bis-(3-diethylaminopropylamino)-benzoquinone bis-benzylchloride or mytolon (supplied by Sterling-Winthrop Research Institute).

Concentrations of drugs are expressed in grams per millilitre of fluid surrounding the heart (*Cyprina*) or passing through the heart (*Buccinum*); thus: 10^{-9} ACh = 10^{-9} g acetylcholine chloride per millilitre of perfusing fluid or 0·001 μg/ml.

Results

The Cyprina *heart*

This heart was tested for its usefulness in the bioassay of acetylcholine and 5-hydroxytryptamine. The threshold to acetylcholine lay between 10^{-10} and 10^{-9} g/ml. (see Fig. 1). Unlike the *Venus* heart, which usually shows a graded slowing and a graded decrease in amplitude of the beat with gradually increasing concentrations of acetylcholine, the *Cyprina* hearts which were tested showed only a slowing. One heart with a normal frequency of 12 beats/min was unaffected by 10^{-10} acetylcholine; at 10^{-9} acetylcholine the frequency was 9·5 beats/min; at $2{\cdot}5 \times 10^{-9}$ acetylcholine, 8 beats/min; at 5×10^{-9} acetylcholine, 6 beats/min; while at 10^{-8} acetylcholine the heart stopped.

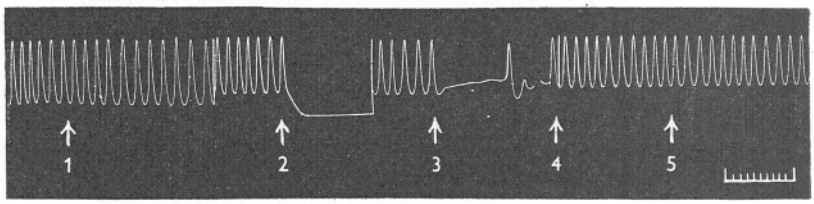

Fig. 1. Records of the action of acetylcholine ACh on the isolated heart of *Cyprina islandica*. (1) ACh 10^{-9} g/ml.; (2) ACh 10^{-8} g/ml.; (3) ACh 5×10^{-9} g/ml.; (4) Mytolon 10^{-6} g/ml. for 10 min, drum stopped; (5) ACh 10^{-8} g/ml. Kymograph stopped and heart washed between each test. Time units = 6 sec and 1 min.

Mytolon, a neuromuscular blocking agent in vertebrates, is the most effective acetylcholine antagonist known for the *Venus* heart (Luduena & Brown, 1952; Welsh & Taub, 1953). It is also highly effective as an antagonist to acetylcholine on the *Cyprina* heart. In Fig. 1 the action of 10^{-8} acetylcholine is recorded before mytolon was added to the bath and again after the heart had been exposed to 10^{-6} mytolon for 5 min. This concentration of mytolon completely antagonized a dose of acetylcholine that earlier had caused diastolic stoppage. Treatment of a heart with mytolon makes it possible to estimate the amount of excitor substance in an extract when acetylcholine is also present.

The *Venus* heart responds to 5-hydroxytryptamine with an increase in both frequency and amplitude (Welsh, 1953*b*). The same is true for the *Cyprina* heart (Fig. 2). Threshold is near 10^{-10} 5-hydroxytryptamine, but in contrast

to acetylcholine action there is a graded response over a wide concentration range. Certain of the ergot alkaloids have a remarkable excitatory action on the *Venus* heart (Welsh & Taub, 1948; Welsh, 1953*b*). LSD is a synthetic derivative of the ergot alkaloids. Since LSD had been shown by Gaddum (1953) to be an effective antagonist for 5-hydroxytryptamine on certain vertebrate smooth muscles it was tested on the *Cyprina* heart. It proved to be a very satisfactory antagonist, as may be seen in Fig. 2.

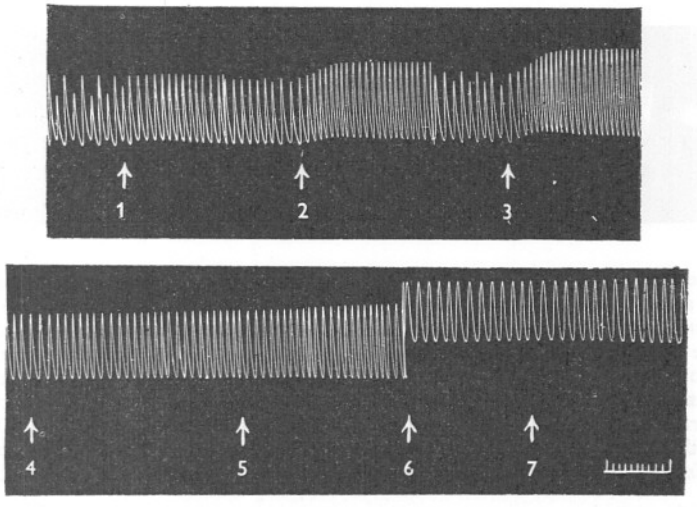

Fig. 2. Records of the action of 5-hydroxytryptamine (5-HT) on the isolated heart of *Cyprina*. (1) 5-HT 5×10^{-9} g/ml.; (2) 5-HT 5×10^{-8} g/ml.; (3) 5-HT 5×10^{-7} g/ml.; (4) LSD 10^{-6} g/ml.; (5) 5-HT 5×10^{-8} g/ml.; (6) LSD 10^{-6} g/ml.; (7) 5-HT 5×10^{-7} g/ml. Heart washed after each test with 5-HT. Time units = 6 sec and 1 min.

From these results, obtained with a limited number of hearts, it is suggested that the *Cyprina* heart (ventricle) should be a useful object for the quantitative estimation of small amounts of acetylcholine and 5-hydroxytryptamine in tissue extracts and biological fluids. Also, it appears probable that the *Cyprina* heart, like the *Venus* heart, is doubly innervated, with the inhibitor nerves releasing an acetylcholine-like substance and the excitor nerves a 5-hydroxytryptamine-like substance.

The Buccinum *heart*

Acetylcholine produces a decrease in amplitude of beat of the *Buccinum* heart with little effect on frequency (Fig. 3). The threshold is below 10^{-9}, and complete inhibition occurs at about 10^{-8} acetylcholine. Mytolon is an effective acetylcholine antagonist (no record of its action is illustrated).

The threshold to 5-hydroxytryptamine is between 10^{-10} and 10^{-9}. A graded response occurs over a wide range of concentrations. The response may be an increase in amplitude with little change in frequency or rise in base-line (Fig. 4), or there may be a marked rise in base-line and increase in frequency as seen in Fig. 5. This variation in response is largely due to the manner of perfusion. A heart from which the perfusion fluid escapes readily is less likely to shorten and beat in a fast, irregular manner, even when strongly excited by 5-hydroxytryptamine.

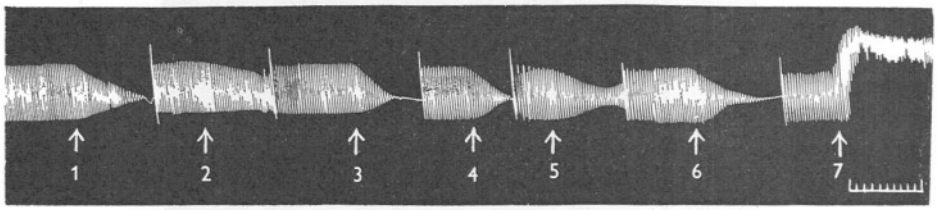

Fig. 3. Records of the actions of extracts of *Buccinum* ganglia and salivary glands compared with acetylcholine (ACh) on the isolated heart (ventricle) of *Buccinum*. (1) Extract of *Buccinum* ganglia made with eserinized sea water. Active material from one milligram of tissue per millilitre of perfusion fluid; (2) ACh 10^{-9} g/ml.; (3) ACh 10^{-8} g/ml.; (4) ACh 5×10^{-9} g/ml.; (5) ACh 2×10^{-9} g/ml.; (6) *Buccinum* ganglion extract as in record 1; (7) extract of salivary glands of *Buccinum* made with sea water (no eserine). Active material from 1 mg tissue/ml. perfusion fluid. Time units = 6 sec and 1 min. (From these records, if the action of the ganglion extract is taken to be equal to 5×10^{-9} AChCl, *Buccinum* ganglia may be considered to contain the equivalent of 5 μg AChCl/g tissue.)

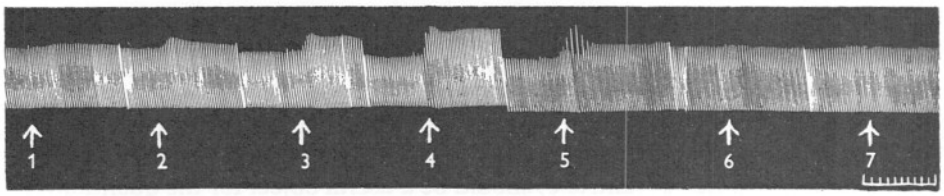

Fig. 4. Records of the action of 5-hydroxytryptamine (5-HT) on the isolated *Buccinum* heart and the blocking action of lysergic acid diethylamide (LSD). (1) 5-HT 5×10^{-9} g/ml.; (2) 5-HT 5×10^{-8} g/ml.; (3) 5-HT 5×10^{-7} g/ml.; (4) 5-HT 5×10^{-6} g/ml.; (5) LSD 10^{-5} g/ml.; (6) 5-HT 5×10^{-7} g/ml.; (7) 5-HT 5×10^{-6} g/ml. Time units = 6 sec and 1 min.

LSD blocks the action of 5-hydroxytryptamine on the *Buccinum* heart. While 5-hydroxytryptamine is readily washed out of the heart, LSD is not. From a concentration of 10^{-5} LSD in the bath, the amount adsorbed by the heart in a period of a few minutes is such that after prolonged washing the excitor action of added 5-hydroxytryptamine is effectively abolished (Fig. 4).

Cardio-excitor and inhibitor substances in molluscan tissues

When the paired, visceral ganglia of *Venus mercenaria* are stimulated electrically, the heart beat is depressed or the heart stops, depending on the voltage and frequency of the stimuli. Prosser (1940) presented evidence that this inhibition of the *Venus* heart resulted from the release of acetylcholine from endings of cardio-inhibitor neurons that had their cell bodies in the visceral ganglia. The results from stimulating isolated hearts led Welsh & Slocombe (1952) to a similar conclusion.

TABLE I. ACETYLCHOLINE CONTENT OF GANGLIA AND HEARTS OF MOLLUSCS

Class of Mollusc	Species	Tissue	ACh μg/g	Reference
Gastropoda	*Helix pomatia*	Ganglion 'cérébroides'	12	Corteggiani (1938)
	Haliotis tuberculata	Ganglion 'cérébroides'	20	Corteggiani (1938)
	Aplysia depilans	Peri-oesophageal	2–3	Bacq (1935)
	Busycon canaliculatum	Pooled ganglia, except visceral	3–9, av. 5·5	Welsh (unpublished)
Lamellibranchiata	*Venus mercenaria*	Pooled ganglia	1–5, av. 2	Welsh (unpublished)
Cephalopoda	*Octopus vulgaris*	Cerebral ganglia	77	Bacq (1935)
	O. vulgaris	Cerebral ganglia	90	Corteggiani (1938)
	Sepia officinalis	Cerebral ganglia	80	Corteggiani (1938)
Gastropoda	*Aplysia depilans*	Heart	0·2*	Vincent & Jullien (1938)
	Helix pomatia	Heart	2·5–5	Vincent & Jullien (1938)
	Limnaea stagnalis	Heart	5·3–5·5	Vincent & Jullien (1938)
	Murex brandaris	Heart	21	Vincent & Jullien (1938)
	M. trunculus	Heart	23–35	Vincent & Jullien (1938)
Lamellibranchiata	*Anodonta cygnea*	Heart	0·3–0·4	Vincent & Jullien (1938)
	Ostrea edulis	Heart	0·7	Vincent & Jullien (1938)
	Mytilus galloprovincialis	Heart	0·12	Vincent & Jullien (1938)
	Venus mercenaria	Heart	0·1	Welsh (unpublished)
Cephalopoda	*Octopus vulgaris*	Heart	0·1	Vincent & Jullien (1938)
	O. vulgaris	Heart	0·2	Bacq (1935)
	Sepia officinalis	Heart	0·1	Vincent & Jullien (1938)

* See Vincent & Jullien (1938) for values from other species and by a different method of extraction.

Nerve tissue from a variety of molluscs has been shown to contain acetylcholine (or a closely related substance). If the mollusc heart is innervated by cholinergic neurons it, too, should yield acetylcholine. This appears to be true. In Table I will be seen some of the published values for acetylcholine equivalents in molluscan nerve tissue and hearts as well as some unpublished values obtained by the author.

When the cerebral ganglionic mass of *Buccinum* was extracted in eserinized sea water, a heart-depressing substance was found to be present (Record 1, Fig. 5). This substance had an action on the *Buccinum* heart resembling that of acetylcholine. When a portion of this same extract was brought to pH 9–10 with NaOH and boiled for a few minutes, its effect on the heart was excitatory (Record 2, Fig. 5). The excitor action of the extract closely resembled that of 5-hydroxytryptamine.

If there are two sets of cardio-regulator nerves innervating the molluscan heart, demonstration of excitor and inhibitor substances in extracts of heart tissue might be possible. Extracts of hearts (ventricles and auricles) of *Buccinum*, *Pecten* and *Mytilus* were found to have excitor or inhibitor actions on the *Buccinum* heart depending on the method of preparing the extract.

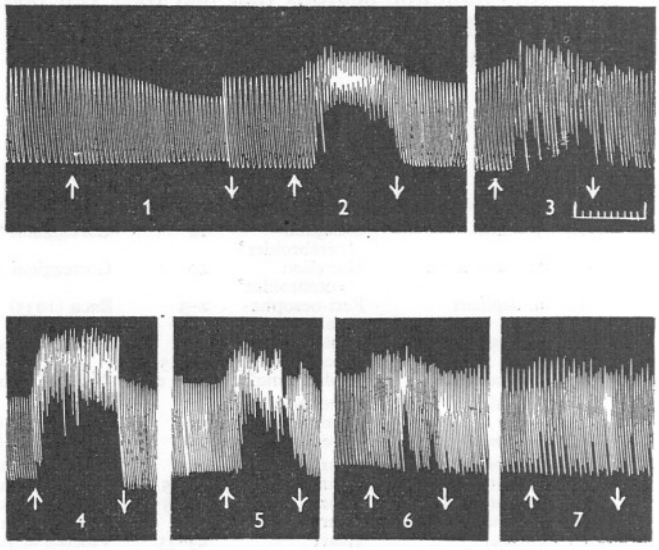

Fig. 5. Records of the action of extracts of *Buccinum* ganglia and salivary glands compared with 5-hydroxytryptamine (5-HT) on the isolated *Buccinum* heart. (1) Extract of ganglia made with eserinized sea water; active material from 1 mg tissue per ml. of perfusion fluid. (2) Part of same extract as in record 1 after bringing to pH 9–10 and boiling. (3) Extract of salivary glands made with sea water (no eserine); active material from 0·1 mg tissue per ml. perfusion fluid. (4) 5-HT 5×10^{-7} g/ml. (5) 5-HT 5×10^{-8} g/ml. (6) 5-HT 5×10^{-9} g/ml. (7) 5-HT 5×10^{-10} g/ml. Time units = 6 sec and 1 min.

5-Hydroxytryptamine occurs in considerable amounts in the posterior salivary glands of *Octopus vulgaris* and *Eledone moschata* (see Erspamer, 1954, for references). When the salivary glands of *Buccinum* were extracted in such a manner that acetylcholine would be destroyed by cholinesterase or by alkaline hydrolysis, the extracts were found to have a marked excitor action on the *Buccinum* heart (Record 3, Fig. 5). The action resembled that of 5-hydroxytryptamine. The excitor activity of a given weight of salivary gland tissue was nearly ten times as great as that of an equal weight of ganglion tissue. Further experiments would be necessary to determine the true nature of the heart-exciting material from the salivary glands of *Buccinum*.

Discussion

It has long been recognized (e.g. Carlson, 1905*a*, *b*) that molluscan hearts are innervated by cardio-regulator nerves. Carlson concluded that the innervation could be single and either excitatory or inhibitory, or, in some species, double with both sets of fibres apparently present. If opposing sets of fibres ran together in the same nerve, as they may in molluscs, it would be difficult to demonstrate double innervation by stimulation experiments. For example, it has long been held that the heart of *Venus mercenaria* is innervated only by inhibitor nerves (Budington, 1904). Actually, following stoppage of the *Venus* heart by electrical stimulation of the visceral ganglia, there is often a much increased frequency and amplitude of heart beat during the recovery period. If an agent is used to block the action of the inhibitor nerve, one then sees only excitation of the *Venus* heart upon stimulation of the visceral ganglia (Welsh, 1953*b*). Such observations have led to the conclusion that the *Venus* heart is doubly innervated and when the mixed nerves to the heart are stimulated the cardio-inhibitory action is normally dominant and tends to mask the simultaneous action of cardio-excitatory neurons. It is quite possible that in some species cardio-excitation is dominant.

When Fredericq in 1947 reviewed the literature on cardio-regulatory nerves in invertebrates, he concluded that the cardio-inhibitor nerves of *Venus* probably released acetylcholine and that the cardio-accelerator nerves of *Aplysia* and of cephalopods probably released adrenaline. He further concluded that the cardio-moderator nerves of cephalopods might possibly act through the release of tyramine. From the present study, incomplete as it is, it appears probable that there are cholinergic inhibitor fibres to the heart of *Buccinum* and perhaps *Cyprina* as well. On the basis of this study it can only be said that possibly the cardio-excitor neurons of *Buccinum* and *Cyprina* act through the release of 5-hydroxytryptamine. The fact that this indole amine has been identified in nervous tissue of *Venus mercenaria* and the whelk, *Busycon canaliculatum* (Welsh, 1954*b*), lends support to this view. Twarog (1954) has also shown that 5-hydroxytryptamine is present in the anterior byssus retractor muscle (nerve endings?) of *Mytilus edulis*, where it perhaps acts in the mediation of inhibitory nerve impulses to this muscle.

The true nature and role of the cardio-excitor substance from the salivary glands of *Buccinum* remain to be determined. Since it acts like 5-hydroxytryptamine and since 5-hydroxytryptamine relaxes certain non-cardiac smooth muscle in molluscs (cf. Twarog, 1954), it is possible that the salivary secretion of *Buccinum* acts, in part, to relax its molluscan prey. The posterior salivary glands of *Octopus vulgaris* contain very large quantities of 5-hydroxytryptamine (Erspamer & Asero, 1952; Bacq, Fischer & Ghiretti, 1952), which is responsible for some of the toxic properties of the saliva of this species.

This paper is based on studies made at the Plymouth Laboratory while the author held a fellowship from the John Simon Guggenheim Memorial Foundation. I wish to express my deep appreciation to the Director and Staff of the Plymouth Laboratory for the excellent facilities placed at my disposal and for their kind hospitality and co-operation.

SUMMARY

Among the larger molluscs available in the Plymouth region, *Cyprina islandica* and *Buccinum undatum* have hearts which, when isolated, are suitable for the bioassay of acetylcholine and 5-hydroxytryptamine.

Mytolon, a vertebrate neuro-muscular blocking agent, is a highly effective antagonist of acetylcholine in the hearts of *Cyprina* and *Buccinum*. Lysergic acid diethylamide (LSD), a synthetic ergot alkaloid derivative, is an effective antagonist of 5-hydroxytryptamine in these hearts.

The nervous system of *Buccinum* contains a cardio-inhibitor material that resembles acetylcholine in its action on the *Buccinum* heart. It also contains a cardio-excitor material whose action on the heart resembles 5-hydroxytryptamine.

Hearts of *Pecten*, *Mytilus* and *Buccinum* also yield (from nerve endings?) similar cardio-excitor and inhibitor principles.

Salivary glands of *Buccinum* contain large amounts of a substance that has an action on the *Buccinum* heart resembling that of 5-hydroxytryptamine.

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THE ACCUMULATION OF RADIOACTIVE IODINE BY *AMPHIOXUS*

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(Text-figs. 1–4)

The endostyle of *Amphioxus* has for a long time been regarded as homologous with the subpharyngeal gland of the cyclostome ammocoete larva. This view was promulgated by Müller (1873) and by Dohrn (1886), and has been supported by many workers, for example Leach (1944). The metamorphosis of portions of the subpharyngeal gland to the thyroid gland of the adult lamprey was followed by Marine (1913), and uptake of radioactive iodine by certain cell elements of the subpharyngeal gland was demonstrated by Gorbman & Creaser (1942). At the same time the latter workers failed to establish the presence of iodine in the endostyle of *Amphioxus* by autoradiographic techniques. Gorbman (1941), however, showed that the ascidian *Perophora annectans* could accumulate radioactive iodine in its pharyngeal stolon but not in its endostyle. The part of the pharyngeal stolon involved is derived from the endostylar region of the pharynx. Recently, Sembrat (1953) has shown that endostyles of *Amphioxus* implanted into larval axolotls accelerated their metamorphosis, and concludes from this that the organs contain an 'active substance (or substances) which may evoke amphibian metamorphosis similarly to the active hormone of the thyroid gland. It is probable that the substance is not identical with thyroxin which may be inferred from the fact that the endostyle of *Branchiostoma* does not accumulate radioactive iodine (Gorbman & Creaser, 1942) as well as from its different and tolerably little efficacious influence on the amphibian metamorphosis as compared with typical metamorphic symptoms induced by the thyroid gland.' The work about to be described has demonstrated the presence of iodine in the endostyle, and suggests, further, that it is present in the form of a 'thyroid hormone-like' substance, thereby supporting Sembrat's general conclusion.

This work was done at the Laboratory of the Marine Biological Association at Plymouth. The author's thanks are due to the Director for making table space and equipment available and to the staff for many kindnesses. His thanks are especially due to Dr D. B. Carlisle for healthy criticism and comment on the work throughout its progress. Miss E. A. Robson of the Zoology Department of the University of Cambridge has assisted greatly with the photographing of the autoradiographs.

Material and Methods

Amphioxus (*Branchiostoma*) *lanceolatus* (Pallas) used in this work were dredged from the Eddystone Grounds off Plymouth. They were kept in circulating sea water in their native shell gravel at the Laboratory of the Marine Biological Association at Plymouth, and maintained thus for at least 1 week, usually several weeks, before being subjected to treatment with radioactive iodine. In all the experiments to be described, carrier-free ^{131}I (as sodium iodide) was used at a concentration of 2 μc/ml. for 15–17 h, at temperatures between 5 and 10° C. The animals were fixed in Bouin-in-seawater, washed thoroughly to remove excess radioactive material, dehydrated, embedded in paraffin wax and sectioned at 10 μ. Sections were mounted on gelatinized slides according to the technique of Doniach & Pelc (1950), dried, washed in xylol to remove the wax, hydrated and covered with Kodak Autoradiographic Stripping Emulsion. The normal exposure time was 14 days. Slides were developed in Kodak D 19*b* developer, fixed and washed in the usual way. Some slides were stained, either with haemalum or Ehrlich's haematoxylin through the gelatin layer of the emulsion. D.P.X. was used as a mounting medium.

Experimental Results

No uptake of ^{131}I has been noted in the prepharyngeal part of the body, in the velum or in the peripharyngeal bands. In the endostyle two longitudinal bands of activity appear, extending from within a millimetre of its anterior extremity to its posterior extremity. In transverse sections they are seen to be situated near the periphery of and slightly lateral to the lateral series of mucous glands (Fig. 1). The two median series of mucous glands show no activity at all. In some sections of the endostyle, these peripheral centres can be seen to consist of two regions, one of which is fairly compact and flattened, outside, but closely applied to its wall, while the other is more diffuse and lies immediately inside the endostylar wall. The two regions are connected by a 'neck' which lies within the cell walls (Fig. 1). The outer region may be extended along the surface of the endostyle laterally on to the lowest gill bar. This activity is undoubtedly associated with mucus secreted by the lateral mucous glands. The inner region, immediately inside the cell walls of the endostyle, is slightly lateral to the lateral mucous glands. In some sections (Fig. 2) several spots of activity can be seen within the lateral mucous glands, near their bases and their borders with the layer of non-mucous cells which separate them from the median mucous glands.

Radio-activity in the secreted mucus can frequently be traced, especially in the posterior part of the pharynx, up the pharyngeal wall and into the epipharyngeal groove. From thence it can be followed backwards into the oesophagus, the mid-gut (Fig. 3) and the hind-gut. The radioactive mucus does not, however, form a continuous cord throughout the length of the gut,

but appears in smaller or larger isolated patches. Food particles in the gut, too, having taken up some of the ^{131}I, often form autoradiographic images. No radioactivity either in mucus or in food particles has been found in the lumen of the mid-gut diverticulum.

Radioactive iodine has also been located in the walls of the alimentary canal. The oesophagus may show very slight traces of uptake, but in the mid-gut, mid-gut diverticulum and hind-gut, uptake is much stronger, though

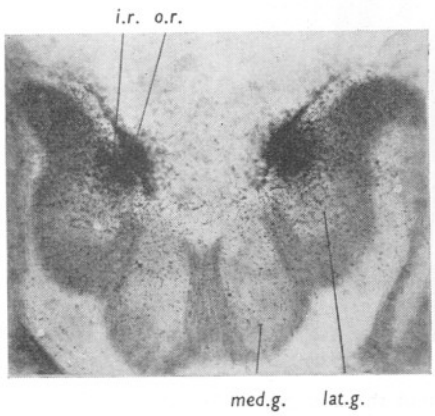

Fig. 1.

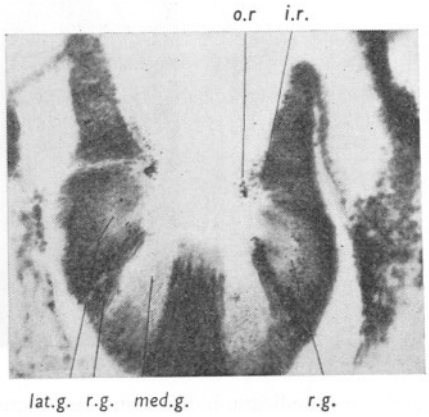

Fig. 2.

Fig. 1. Autoradiograph of a transverse section of the endostyle of *Amphioxus* stained with haemalum; ×300. *lat.g*, lateral mucous gland; *med.g.*, median mucous gland; *o.r.*, outer region of iodine uptake; *i.r.*, inner region of iodine uptake.

Fig. 2. Autoradiograph of transverse section of the endostyle of *Amphioxus* stained with Ehrlich's haematoxylin; ×300. *r.g.*, iodine uptake in body of lateral mucous gland; *lat.g.*, lateral mucous gland; *med.g.*, median mucous gland; *o.r.*, outer region of iodine uptake: *i.r.*, inner region of iodine uptake.

never as intense as it is in the endostyle. In the wall of the mid-gut (Fig. 3) and hind-gut it forms a fairly well-defined band near the lumen but separated from the luminar wall by a clear space. This band, in control specimens not treated with ^{131}I, is frequently pigmented yellow or brown and may contain food particles. In the mid-gut diverticulum (Fig. 4) radioactivity in treated specimens is confined to the walls and is found throughout the length of the organ, though less strongly in the anterior part. Within the cells it is distributed mainly in their distal halves. In the ventral wall the activity tends to be concentrated in the middle, and near the distal border of each cell, but in the lateral and dorsal walls it is more evenly distributed, with a slight concentration near the distal border. The reason for the accumulation of iodine in the diverticulum is obscure, unless it is either associated with the mucus of that organ or unless the diverticulum is an additional region for assimilation of the

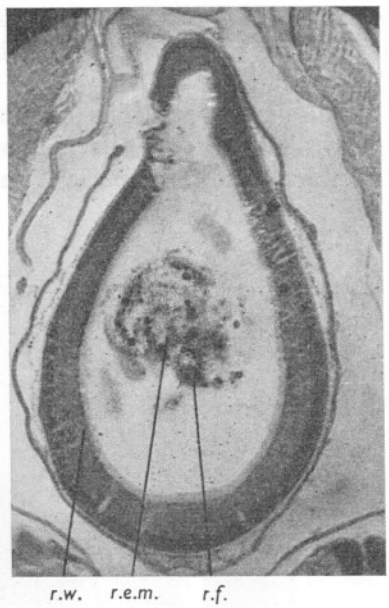

Fig. 3. Autoradiograph of a transverse section of the mid-gut of *Amphioxus* stained with haemalum; ×60. *r.f.*, radioactive iodine associated with food particle; *r.e.m.*, radioactive iodine associated with endostylar mucus; *r.w.*, radioactive iodine in wall of mid-gut.

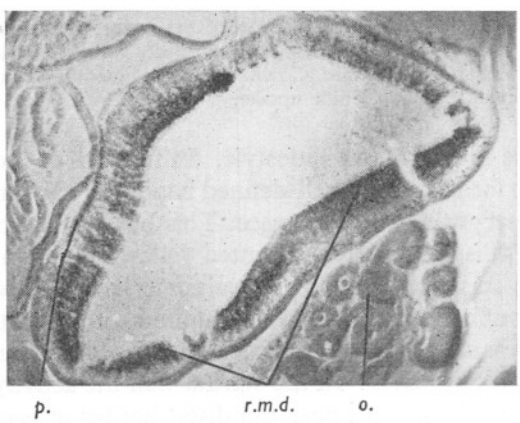

Fig. 4. Autoradiograph of a transverse section of the mid-gut diverticulum of *Amphioxus* unstained; ×84. *r.m.d.*, radioactive iodine in wall of mid-gut diverticulum; *o.*, ovary; *p.*, pigment granules in basal membrane of mid-gut diverticulum.

element. If the latter supposition is true, the iodine must be assimilated directly from the sea water as, according to Barrington (1937), very few food particles find their way into the diverticulum, and those which do are usually quickly swept out again by strong ciliary currents.

A further region of the body where iodine uptake has been observed is in the ventral epithelium of the body wall between the metapleural folds and occasionally extending on to the folds themselves. Here radioactivity is weak and diffuse though it is confined within the limits of the epidermal cells. Van Weel (1937) has observed that cells in this region may have an excretory function. Carbon and melanin particles with which he fed the animals were taken up by amoebocytes in the region of the gut, and then carried by these cells through the blood stream or the coelome to the ventral body wall and deposited in the epithelium or in its underlying connective tissue. From here they were presumably released from the body. The radioactivity in this region is limited to the epithelium. None has been observed in the connective tissue in the vicinity.

Effect of Goitrogens on the Distribution of Radioactive Iodine

The most obvious centre of radioactivity in ^{131}I-treated animals is in the mucus of the lateral mucous glands of the endostyle. The possibility of its being merely adsorbed as inorganic iodine on to the mucus was considered, and to check this point specimens were treated with thiourea or thiouracil simultaneously with their treatment with ^{131}I. Thiourea was used in a concentration of 0·01% and thiouracil in a concentration of 0·04%. In the higher vertebrates these and some other similar substances are known to inhibit the formation of the thyroid hormone.

In the concentrations used, thiourea and thiouracil were found to be in no way detrimental to the animals in the period of 15–17 h during which they were subjected to it. Both goitrogens had the same effect. In animals so treated, no radioactivity appeared in the endostyle, in the mucus in the lumen of the gut or in the ventral epithelium. It was still apparent, however, in food particles in the gut and in the walls of the mid-gut, the mid-gut diverticulum and the hind-gut. Its distribution in these organs was the same as in control specimens without goitrogens.

Discussion and Conclusions

Experiments with goitrogens indicate that the sites for assimilation of iodine are in the walls of the mid-gut, the hind-gut and possibly the mid-gut diverticulum. Here it might be taken in either directly from the water in the gut or in association with food particles as intracellular digestion has been postulated by Barrington (1937) and others. Barrington also assumes, as the result of feeding experiments with minute particles of gold, carmine and indian ink,

the transference of food particles by the blood stream from the gut to the diverticulum. Iodine might be transferred in the same way. The volume of sea water which passes into the gut is probably small, and as the mucus in the gut of animals treated with goitrogens appears to be free of iodine it follows that most of the iodine entering the gut wall does so in association with food particles. In higher vertebrates iodine taken in through the gut walls is transferred by the blood stream to the thyroid gland, so it is reasonable to assume a similar transference from the gut to the endostyle in *Amphioxus*. This view is supported by the experiments with goitrogens which inhibit its appearance in the endostyle but which do not interfere with its occurrence in the gut wall.

In the endostyle 'fixation' of the iodine occurs in the lateral mucous glands near their inner borders and their bases (Fig. 2), to form a 'thyroid hormone-like substance'. This is associated with the mucus produced by these glands and its formation is prevented by goitrogens. This substance is then transferred to the periphery of the gland and secreted with the mucus. At this point, then, the iodine would be most concentrated, as is apparent from the autoradiographic images. The mucus, once secreted, would become diluted by imbibition of water. No function can at present be ascribed to this hypothetical 'thyroid hormone-like substance', though it may be significant that *Amphioxus* undergoes a marked metamorphosis involving alteration from a very assymmetrical larva to an externally symmetrical adult. All the specimens of *Amphioxus* studied have been fully adult and sexually mature. No work has yet been done on larval forms.

The absence of iodine in the ventral epithelium of specimens treated with goitrogens suggests that the iodine normally located in those cells is derived from iodine 'fixed' in the endostyle. Van Weel (1937) has described the accumulation of excretory material in the ventral epithelium, and assumes that waste food matter can be disposed of by this means. Unless the radioactive iodine remained attached to food particles throughout the digestive processes it is difficult to account for the iodine associated with food appearing in the ventral epithelium. The concentration of ^{131}I used in these experiments (2 μc/ml.) is low, being only about one-twentieth the concentration of the stable isotope normally present in sea water. The animals were thus not subjected to abnormally high concentrations of iodine (^{131}I plus ^{127}I) during the experiments such as might bring about the excretion of abnormally large quantities through the ventral epithelium.

In this connexion an observation made during the course of the experimental work is worthy of note. It was found that animals subjected to treatment with radioactive iodine within 24 h after being caught took up no detectable quantity of iodine. When kept in the circulating water of the laboratory, the amount taken up increased to reach a maximum in about 7 to 10 days. Unfortunately no figures are available for the iodine content of Plymouth Laboratory circulating water, nor for Plymouth coastal waters; but

the former value is likely to be low, and a continued sojourn in the Laboratory water might deplete *Amphioxus* of its iodine and make it more ready to take up ^{131}I when that becomes available. *Amphioxus* has been kept alive in circulating water for more than 2 months, however, without apparent detriment. This observation might account for the failure of Gorbman & Creaser (1942) to locate radioactive iodine in *Amphioxus* if they worked with freshly caught material or if their animals were kept in the laboratory in normal sea water before being subjected to iodine treatment.

Two points of interest arise from the results noted in this work. First, the association of iodine with the endostylar mucus, which, of course, passes directly into the gut, may link up with the fact that the thyroid hormone in the higher vertebrates is the only hormone which retains a high degree of effectiveness when administered orally. The secretions of the gonad and the adrenal cortex have a much reduced effectiveness when given by mouth, while the secretions of the remaining endocrine glands are quite ineffective when administered by this path.

The second point concerns the 'colloid substance' which is contained in the alveoli of the thyroid gland of vertebrates. This homogeneous, viscid fluid is the stored secretion of the gland and thus contains the bulk of the iodine present. This colloid substance is, in the author's opinion, the direct evolutionary successor to the endostylar mucus of a protochordate ancestor.

Summary

The localization of iodine has been studied in *Amphioxus* using radioactive iodine and autoradiographic techniques. The most intense concentration was found near the lateral mucous glands of the endostyle where an iodine-containing mucus is secreted into the pharynx. Iodine was also found in the bodies of the lateral mucous glands, in the walls of the alimentary canal and mid-gut diverticulum and in some cells of the ventral body wall. It was found associated with food particles and with mucus in the lumen of the alimentary canal but not in the lumen of the mid-gut diverticulum.

Thiourea and thiouracil inhibit the appearance of iodine in the endostyle, in mucus in the gut, and in the ventral body wall, but do not prevent its appearance in the walls of the alimentary canal and mid-gut diverticulum. The significance of these results is discussed.

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FLUCTUATIONS IN THE DISTRIBUTION AND ABUNDANCE OF INTERTIDAL BARNACLES

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(Text-fig. 1)

It is well known that towards the limits of an animal's geographical range both its abundance and exact boundaries may fluctuate from time to time. These fluctuations can sometimes be correlated with environmental changes, and thus may help to assess the relative importance of the factors that control distribution. For example the distribution of the common intertidal barnacles *Chthamalus stellatus* (Poli) and *Balanus balanoides* (Linnaeus) underwent changes which were attributed to a general rise in temperature over several years (Southward & Crisp, 1954*a*). The abundance of these barnacles has fluctuated further since 1951–52, and we are now able to analyse more closely the relation between the population changes and environmental variations.

Geographical Distribution

The present distribution of *Chthamalus stellatus* and *Balanus balanoides* illustrates very well Darwin's (1872) contention that where two allied species of different range have similar habits and habitats, the territory common to both is small compared with the areas occupied exclusively by either species. Thus, while on the eastern side of the Atlantic *Chthamalus* extends to the tropics and *B. balanoides* penetrates well inside the Arctic circle, the two occur side by side only in the British Isles and northern France. In the course of a general survey aimed at establishing on a quantitative basis the present distribution of some common intertidal animals, we have during the past few years determined carefully the distribution and boundaries of these barnacles in the area common to both. We give here (Fig. 1) the present boundaries of these species in Europe, with our observations grouped into three classes, based on the numerical abundance of the animals: more detailed information has been, and will be, given elsewhere (Southward & Crisp, 1954*b*).

The advantage of demonstrating the limits of a species in terms of objective criteria of abundance rather than by mere records must be stressed. Scattered records of occurrence beyond the margin of measurable decline in population supply little useful information, even when reliable. In the past, too great a reliance has often been placed upon such records, and too often unreliable

information (particularly misidentifications and mislabelled museum specimens) has been quoted by several authors in succession without attempt at verification. The number of authors referring to a record is no guide to its value.

Lack of quantitative information has been particularly confusing in the case of *Chthamalus* and *B. balanoides*, which are often mistaken for each other (cf. Darwin, 1854). For example, there appears to be little factual basis for the reported occurrence of *Chthamalus* at Heligoland. The earliest records, as far as can be ascertained from the description (Frey & Leuckart, 1847), refer to

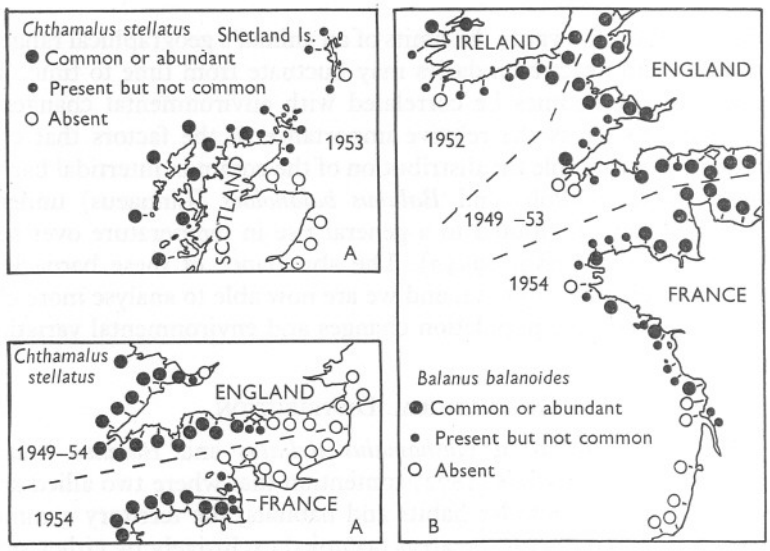

Fig. 1A. The northern and eastern boundaries of *C. stellatus* in Europe. B: the known southern and western limits of *Balanus balanoides* in Europe. In each species, the dividing line between the present and common categories corresponds to a density of approximately 0·5 individuals per square centimetre.

B. balanoides, while Weltner's (1897) record is based on museum specimens on a mussel-shell, a most unlikely habitat for the barnacle at its limits. The supposed occurrence of *B. balanoides* in the Azores appears to be another example of confusion of these species; Barrois' (1888) account of the Crustacea and Nobre's fauna list (1924) are of dubious value since *Chthamalus* is not recorded at all, while Weltner (1900) gives neither locality nor authority in stating that *B. balanoides* is found in the Azores and Portugal. We have recently examined material collected in the Azores by Dr Chapman, to whom we are indebted for this kindness, and find all to be *Chthamalus stellatus*.

Even when comparing the present distribution of these barnacles with previous genuine records there is the disadvantage, already mentioned, that

with notable exceptions the latter are non-quantitative. However, both Fischer-Piette (1936) and Moore & Kitching (1939) give quantitative evidence from which, by comparison with our observations, the following changes in boundaries may be inferred:

(*a*) Between the 'thirties and 1951 *Chthamalus* advanced eastwards in the English Channel from St Alban's Head and Swanage to the Isle of Wight. The most easterly record is now at Ventnor, while at St Catherine's Point it occurs at a density of 20–30/m^2 at high-water mark. There does not seem to have been any corresponding extension along the coast of France.

(*b*) Between 1936 and 1953 *Chthamalus* spread southwards along the east coast of Scotland from Nybster to Wick, Lybster and Latheron.

(*c*) Between the 'thirties and 1949–51 *B. balanoides* retreated from the south coast of Cornwall. The previous evidence is insufficient to show any changes in the position of the limits elsewhere.

Relative Abundance of the Species

By the end of 1952, as reported in Southward & Crisp (1954*a*), considerable evidence was available that in many parts of Britain there had been a change in the relative abundance of *Chthamalus* and *B. balanoides* since the 'thirties. It now appears that the increases of *Chthamalus* and the decreases of *B. balanoides* reached their extreme in 1951–52, and are at present undergoing reversal. The changes have been followed most closely in Devon and Cornwall, where they have been most obvious, but corroborative evidence is available from other places.

Brixham, South Devon

The densities of the two barnacles at Shoalstone Beach, Brixham, in 1949 and 1952, and each year since 1952 are given in Table I (Appendix). The changes are most clearly brought out by comparing the percentage of *B. balanoides* out of the total population of *Chthamalus* and *B. balanoides*:

	1949	1952	1953	1954	1955
H.W.N.	38	4	5	16	10
M.T.L.	43	14	13	33	25
L.W.N.	93	14	32	43	50

The changes are most marked at L.W.N., where the rate of growth and the mortality are such that both species reach maturity in 1 year, and rarely survive more than 2 years. The evidence demonstrates that although the decline in *B. balanoides* was arrested, and then reversed, from 1953 onwards, the relative proportions of the two species are still different from those existing in 1949. The failure of *B. balanoides* to recover its previous abundance, in spite of some good settlements since 1952, may be accounted for by the continued success of *Chthamalus* in maintaining a population between M.T. level and low water, the intertidal range most favoured by *B. balanoides*.

The Plymouth Area

The results of the observations around Plymouth, at those stations originally chosen by Moore (1936), and at some other places, will be found in Table II (Appendix). In this area the decline in *B. balanoides* up to 1951 was more marked, compared with the 1934 survey, than that which took place at Brixham in 1949–52. The resurgence of the species in the Plymouth area has at some stations been correspondingly obvious, as the following figures (percentages *B. balanoides* out of total *Chthamalus* plus *B. balanoides*) for the estuary of the River Yealm show:

	1934*	1951	1953	1954
H.W.N.	16	<1	<1	1
M.T.L.	87	<1	31	40
L.W.N.	100	<1	9	33

* From Moore (1936).

However, the proportion of *B. balanoides* in the barnacle population of the Plymouth area has not yet reached the level that existed in 1934: at Brixham, where there are no data for the 'thirties, the present proportions of *B. balanoides* are now approaching those of the original survey in 1949.

At some stations in the Plymouth area, e.g. Tinside and Amory Bight, *B. balanoides* is still quite rare. In the Tamar Estuary *Elminius modestus* has remained the dominant barnacle, but at a few other sheltered sites where, for some reason, *Elminius* has always been less common, *B. balanoides* is now equal in number to, or outnumbers, *Chthamalus* at certain levels—e.g. the Breakwater jetty, and Baring Point in the River Yealm.

A most interesting point about the return of *B. balanoides* to Plymouth is that the species reappeared in some wave-beaten sites before other sheltered places. At Church Reef, Wembury, *B. balanoides* settled quite heavily, and survived, on the outermost reefs, as these figures (percentages *B. balanoides* out of total *Chthamalus* plus *B. balanoides*) show:

	1951	1954
H.W.N.	<1	4
M.T.L.	<1	29
L.W.N.	<1	45

while at the shoreward end of the reef it was still as scarce as in 1950–51. Similarly, in 1954, there was a greater density of *B. balanoides* at Amory Bight and on the south side of the Breakwater, than at Tinside on south-facing surfaces which are more sheltered from wave-action. Perhaps more rapid growth and higher mortality at wave-beaten sites causes a greater turnover of established individuals, and hence allows a change in the proportions of the two species to appear more quickly. Alternatively, during a period of increasing abundance larvae may come from elsewhere along the coast, and may therefore settle on the outer reefs first. It is possible that the Yealm Estuary

provided a source of such larvae since it retained significant populations throughout the decline (cf. Table II, Baring Point) and was less heavily populated by *Elminius* than other local estuaries.

North Devon and Cornwall

On the northern coast of the Devon and Cornwall peninsula the present distribution of *B. balanoides* is irregular, and our observations are therefore not easy to interpret. An island of fairly high density exists at, and west of, St Ives nearly to Cape Cornwall. East of this *B. balanoides* is mainly restricted to parts of the coast in the neighbourhood of estuaries, for example at Padstow, Boscastle and Bude. Elsewhere it is often uncommon or rare.

Changes in the density of *B. balanoides* have been noticed only in those areas where the species is relatively sparse, and, in common with the changes observed at Plymouth and Brixham, they show a decrease up to about 1951 and an increase since then. For example in 1950–51 at Westward Ho!, Croyde Bay and Trevone, as reported in Southward & Crisp (1954*a*), *B. balanoides* was practically absent, though it was known to have been present at these places in earlier years. Between 1952 and 1954 some settlements took place and at all three stations the species reappeared at low water in small numbers. As elsewhere, however, it is still less common than reported in the 'thirties by Moore & Kitching (1939). At Millook Haven, for example, Moore reported the proportion of 12% *B. balanoides* to 88% *Chthamalus*, whereas in 1955 only 6% *B. balanoides* was found.

In the St Ives–Zennor region investigations were made in 1950 and 1955, and the population was found to be substantially the same. The density of *B. balanoides* was approximately half that recorded by Fischer-Piette (1936).

South Cornwall

B. balanoides was extremely rare to the west of Plymouth from 1950 to 1955. Only sporadic records of isolated individuals have therefore been obtained, and the evidence shows no trend.

Scotland

Since we reported the general increase in *Chthamalus* in the British Isles as a whole we have obtained more definite information on the changes in the Scottish area since the 1930's. Our survey of the Scottish coast was undertaken in 1953, after the reversal of the previous trend, hence the maximum extent of the increase in *Chthamalus* is not known. Table III (Appendix) shows, however, that some increase in *Chthamalus* had occurred between 1934 and 1953, taking Moore & Kitching's observations for the former period as a basis, and expressing ours as nearly as possible in the same form. For many stations, particularly those in Argyll and the Islands, no comparison

is possible as the previous observations were insufficiently quantitative. For example, Kitching (1935) reports *Chthamalus* as 'plentiful' at two stations in Mull, one exposed and one sheltered, where we found it to constitute respectively 60 and 7% of the total barnacle population. At Millport, Cumbrae, the density of *Chthamalus* is given by Moore & Kitching as 480/m², which is not reconcilable with the figure of 7·5% by weight reported by the same authors for the whole intertidal zone.

In general, the main increase in numbers of *Chthamalus* in Scotland appears to have taken place on west- and south-facing shores.

Irish Sea

Observations on the coasts of the Irish Sea agree generally with those in south-west England. In the Isle of Man, the previous decrease in *B. balanoides* was reversed in 1952, and continued so during 1953–55, although no corresponding decline in *Chthamalus* was noted. At St John's Point, Co. Down, good settlements of *B. balanoides* were noticed on the outer, wave-beaten, reefs in 1953, although in greater shelter *Chthamalus* was still as dominant as it was in 1950 (percentages *B. balanoides* out of total *Chthamalus* plus *B. balanoides*):

	1950	1953
H.W.N.	< 1	59
M.T.L.	38	95
L.W.N.	—	83

Along the Lancashire coast, at Blackpool and Rossall, *Chthamalus* has become less common than it was in 1950, but *B. balanoides* is not noticeably more abundant. *Elminius*, on the other hand, has increased considerably and is abundant even at high-water mark. This increase may well have contributed directly to the reduction in *Chthamalus*. At Blackpool in 1953 we found old individuals of the latter being overgrown by younger specimens of *Elminius*. Changes in the foreshore in this area as a result of storm damage and scour may also have had an adverse effect on a species which had only a precarious foothold in the locality.

Relation to Environmental Changes

In our previous paper (Southward & Crisp, 1954*a*) we suggested that the changes in the barnacle populations up to 1951–52 were related to the higher sea and air temperatures then prevailing, the southern barnacle being favoured at the expense of the northern species. The recent increase of *B. balanoides* following a period of somewhat lower temperatures (see Tables IV–IX, Appendix) appears to support this view. However, the suggestion of Moore & Kitching (1939) that the distribution of one of these barnacles, *C. stellatus*, was connected with the beneficial effect of water masses of Atlantic origin, has recently been revived by Powell (1954), with reference to its distribution in

the north of Scotland. The most marked changes in the barnacle populations have occurred in the south-west of England, where *Chthamalus* is still much more abundant than it was in the 'thirties: yet in this area the proportion of western water, or water of mixed Atlantic and coastal origin, such as had been supposed to favour *Chthamalus*, appears to have continued at the reduced level of the 'thirties (e.g. see Corbin, 1950). The 'Atlantic Water' theory therefore becomes untenable when applied to account for the changes in distribution and abundance.

We shall now consider in more detail how temperature may influence the relative abundance of these two species. In *Chthamalus*, both at Brixham and the Isle of Man, fertilized egg masses, which released nauplii when ripe, were found in the mantle cavity during the breeding period—roughly from May to October—in all the years when observations were made. Therefore temperatures were always sufficiently high to allow the production of young. Nevertheless, summer temperature levels may have some influence on the reproduction. Like *Elminius* and other warm water forms, *Chthamalus* can produce more than one brood of nauplii (Crisp, 1950). It is possible that, as has been demonstrated in *Elminius* (Crisp & Davies, 1955), the interval between broods is shorter at higher temperatures. Thus during a warm summer the number of broods and the larval output would increase directly. Further advantages to the planktonic larvae, the cyprid and the spat would probably result from a warm season, when development and growth would be more rapid, and temperatures closer to those normally experienced by the species at the centre of its geographical range.

Owing to the relatively long period during which *Chthamalus* produces successive broods in the south-west, the developmental stages in the plankton overlap with each other and with the growth of young spat. It is not therefore possible to separate these phases in the life history and to correlate them with temperature anomalies month by month. In 1949 and 1950 (Table IV), however, there was better correlation between annual spatfall and monthly temperature for the months of July–October, than with the months of May and June. This suggests that the temperature requirements of embryonic development, which alone takes place in May and early June, are less exacting than the requirements of planktonic development and the growth of spat.

For the Isle of Man, the evidence is a little more decisive, for the settlement is confined to high water and is restricted to a later period of the year. (Settlement at high water, as distinct from settlement in lower parts of the intertidal zone, occurs later in the south-west also.) There is a good correlation between positive temperature anomalies for any or all months from September to December and good settlements of *Chthamalus* (Table V), rather than for the months from May to August. Taken together, the evidence from Brixham and the Isle of Man points to the need for warmth during the later larval stages and at the time of settlement, and possibly thereafter, rather than

during gonad building and embryonic development. However, information over longer periods is desirable.

In the light of the above analysis we may examine mean sea and air temperatures for the months of July to December only, in order to attempt an explanation of the changes in the proportions of *Chthamalus*. At Torquay, from 1946 to the end of 1949, conditions were generally warm, with anomalies above the period mean (Table VI). From 1950 to 1954, there were 2 years, 1950 and 1952, when the temperatures during these 5 months were consistently below the mean, but in the remaining years, the monthly means of sea or air temperature from July till December have been nearly always above the period means. During the same period at Plymouth (Table VII), except for 1950 and 1952, the temperatures were again consistently above the long period mean. Therefore, at these localities, and probably elsewhere in Britain, late summer and autumn temperatures, for 7 out of the 9 years 1946–54, were at a level that should have favoured good settlements of *Chthamalus*. Moreover, the 2 poor years, 1950 and 1952, were separated by a relatively better year. This temperature trend could therefore explain quite well the increase in the species and the continued maintenance of high densities in the south-west, even at low water. On this basis, it could be predicted that there will be no reduction in *Chthamalus* to its former proportions unless there is a succession of colder autumns.

Observations on the settlement of *B. balanoides* at Brixham show no close correlation with winter temperatures as a whole, nor with any particular month (Table VIII). However, over the period 1948–50, when the species was declining, temperatures were generally above the period mean. Since then, with certain exceptions, either sea or air temperatures (or both) have been below the period mean, and settlements, except in 1951, have correspondingly shown a marked increase. There would thus be some correlation of the recent more successful settlements of *B. balanoides* with lower winter temperatures, if some explanation could be given for the anomaly in 1951. It must be remembered that at this time stocks of the species were probably low generally in the south-west, as they were known to be at Plymouth (Table II). Few young individuals were present at this time, for the spatfalls of 1948–50 appear to have failed to survive in any numbers. The old individuals had a very high rate of infection by the castrating parasite *Hemioniscus* (Southward & Crisp, 1954*a*). If the generally colder conditions in the early part of 1951 allowed the survival of the spatfall, small though it was, these younger individuals with, presumably, a lower infection rate of the parasite, would augment the spatfall the following year. A lag of this sort in the recovery of the species would not be surprising in view of its precarious position in the south-west.

At Plymouth the decline of *B. balanoides* during the warm period ending in 1950 reached a much lower level than at Brixham, and it would be corres-

pondingly more difficult for the species to return. Moreover, although since 1951 temperatures below the long period average have occurred in January and February, in March and April, the months when settlement takes place, temperatures have been above the period mean.

The main part of this paper was prepared during the winter and spring of 1954–55. On the basis of the above discussion it was predicted that the persistent cold weather that occurred in the early part of 1955 might favour good settlements of *B. balanoides* in the south-west. The meteorological observations now available show that during the months of January to June 1955, apart from air temperatures for April, temperatures were consistently below the period means (Table X). Sea temperatures were especially low in March and April, when the major (or only) settlement of *B. balanoides* takes place. It is therefore interesting to report that quite a good settlement of *B. balanoides* occurred in south Devon in the spring of 1955. Comparatively heavy spatfalls (1–2/cm^2) were seen at Wembury, the Erme Estuary, and at Prawle Point in April and May. Good settlements were probably general along the whole of the south coast since Stubbings (private communication) reports exceptionally heavy and prolonged spatfalls of *B. balanoides* in the Portsmouth area. In the Menai Straits the settlement was heavier and more prolonged than had been experienced during the past 4 years.

Counts were made in the Plymouth and Brixham area in August 1955, when the spat had reached almost adult size, and these observations showed that increases in the relative proportions of the species had occurred at Brixham and in the Yealm Estuary, though no change was discernible at Plymouth, Tinside (Table X). At Wembury, although the relative proportions of *B. balanoides* and *C. stellatus* on the outer, wave-beaten, reefs were the same as in 1954, settlement had spread to the shoreward reefs from which the species had been completely absent for at least 5 years. Thus at three out of four localities, *B. balanoides* showed an increase in number or range during the cold spring of 1955.

Influence of Temperature

Darwin (1872) cautioned against attributing the distribution of an organism directly to temperature differences in the environment. He pointed out that competition between species would probably be the most exacting factor, but that this would be influenced by conditions of temperature or climate, which favoured one or other of the competitors.

There is little doubt that in south-west Britain, *C. stellatus* and *B. balanoides* are in direct competition over the greater part of the intertidal zone, for the total numbers supported by the environment have remained fairly constant in spite of changes in the proportions of each species. How, then, can temperature influence this competition?

We have already pointed out how warm summer temperatures may increase

directly the fecundity and survival of *Chthamalus*, and support of this view is afforded by the heavy spatfalls in certain areas such as Loch Sween, Dundrum Bay, etc. (Southward & Crisp, 1954*b*). Increased fecundity will ultimately lead to increased intertidal cover by the species, and so deny rock space to its competitor. However, no similar beneficial effect of low winter temperature can increase the fecundity of *B. balanoides*, for the eggs are spawned by mid-winter, before the sea and air temperatures reach their minima, and breeding can occur only once a year. Hence, then, low winter temperature can directly favour *B. balanoides* only by delaying embryonic development to a point where the larvae may benefit more from the spring phytoplankton, or, more dubiously, by some effect on the survival or vigour of the larvae or spat.

On the other hand, competition between the species for food is operating continuously and must be especially severe on young spat surrounded by cirral nets of older individuals. Moreover, barnacles will readily feed on newly hatched nauplii of their own or other species (unpublished observations). The rate of feeding of each species, in addition to its direct influence on its own growth and fecundity, will therefore influence competing species both by removal of food and by ingestion of their larvae.

It has been shown that the frequency of cirral beat in each species has an optimal temperature range; in *B. balanoides* it lies between 0 and 18° C, in *Chthamalus* between 5 and 30° C (Southward, 1955). Outside these ranges the species will not be able to feed efficiently. Results of frequency determinations show that after allowing for much individual variation, *B. balanoides* is the more efficient below about 15° C, *Chthamalus* above (Southward, 1955). Therefore the lower the temperature at which the spat of *B. balanoides* are growing, the less the competition to which they will be exposed. On the other hand, above 17° C *B. balanoides* shows reduced activity, while *Chthamalus* shows a rapidly increasing beat. High summer and autumn temperatures should therefore favour the survival of the spat of *Chthamalus*. On this basis the balance between the two species will depend on the temperature variation throughout the year, and their distribution limits would not necessarily correspond with temperature extremes.

Moore & Kitching (1939) minimized the significance of competition as a limiting factor because the zones of adult *Balanus* and of adult *Chthamalus* were occasionally distinct. Our observations agree with most of theirs in that almost everywhere the zones interpenetrate, if only to a slight extent, with no space between them. The sharpness of separation of the two zones where it occurs could be an indication of extreme interspecific competition within each zone; it would also be enhanced by gregariousness on the part of the cyprid larvae (Knight-Jones, 1953), for *Chthamalus* has no appreciable effect in stimulating the setting of *Balanus* (Knight-Jones, 1955).

The view that competition, modified by temperature, is the main factor limiting each species however suggests possible explanations to problems

which have been posed by previous authors. It explains the gradual restriction of *Chthamalus*, both in its northern and estuarine limits, to the upper zones where it suffers less competition from *Balanus*. The restriction of *Chthamalus* at its northern limits to very exposed headlands may not be due entirely to the suitability of exposed areas to *Chthamalus*; it may survive there because it is relatively better equipped to withstand severe exposure than is *Balanus*, and so benefits from lack of competition. The nature of the microhabitat, in which it is found under such conditions (viz. slightly sheltered cracks in the rock), suggests that the exposure is not optimal, but almost as severe as the species can endure. In more sheltered localities in most parts of Britain *Balanus* normally dominates completely, but it may be significant that wherever *Chthamalus* is found abundantly in shelter, other than in a narrow zone at high water, *B. balanoides* is scarce. For example, in harbours west of Plymouth, where *Chthamalus* occurs at all levels, *B. balanoides* is scarcely represented. In Loch Sween, *B. balanoides* is absent in the areas of abnormal abundance of *Chthamalus* (cf. Southward & Crisp, 1954*b*). In a similar way, the presence of *Balanus balanoides* in creeks and estuaries in south-west England, and its absence from the open coast, may be attributable to the adverse effect of estuarine conditions on *Chthamalus stellatus*.

We conclude that the distribution of these two species, with their similar habitat and habits, cannot be adequately explained by the action of the physical environment on either of them alone. We suggest, instead, a dynamic concept with the two competing species in a state of equilibrium. Even quite minor changes in the physical environment, favouring one or the other of the species, will, if sufficiently prolonged, cause the boundaries of distribution to alter.

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Summary

Recent observations on the distribution and abundance of the commoner intertidal barnacles indicate that, following a decline up to 1950–51, the northern *Balanus balanoides* is now increasing in abundance. The influence of environmental variations, particularly temperature, is discussed.

It is suggested that the distribution of the two competing species *B. balanoides* and *Chthamalus stellatus*, and the fluctuations in their abundance, are best explained dynamically. The equilibrium existing between them is

altered by changes in the environment, which give one species an advantage over the other. Temperature, which affects the cirral activity of the two species differently, has probably the most important influence on their relative abundance.

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APPENDIX

TABLE I. THE DISTRIBUTION AND ABUNDANCE OF CERTAIN BARNACLES AT SHOALSTONE BEACH, BRIXHAM, 1949–55

(The levels are only approximate. The density is given in numbers per square centimetre, based on counts at several different places at each level. Some of the counts made within 5 months of the larval settlement show the numbers of adults and spat separately.)

	March 1949		February 1952		May 1953		April 1954		January 1955	
Level	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*	*C. stellatus*	*B. balanoides*
H.W.N.	2·76	1·69	5·49	0·25	9·5	0·55+0·40	4·55+7·75	0·85+0·70	3·02+0·28	0·34
M.T.L.	2·64	1·98	2·80	0·45	6·8	1·05+0·80	2·20+4·15	1·10+1·95	3·14+0·74	1·06
L.W.N.	0·15	2·00	2·10	0·35	4·8	2·25+1·60	1·60+2·70	1·20+2·70	0·88+0·32	0·87

TABLE II. THE DISTRIBUTION AND ABUNDANCE OF CERTAIN BARNACLES IN PLYMOUTH AREA, 1951–54

(To be compared also with tables in Moore, 1935. The numbers are given as the average per square metre, based on 2–5 counts of areas of 25–100 cm² at each level; the levels at Tinside were referred to the nearest bench mark of the Ordnance Survey, and those on the Breakwater obtained from Southward & Orton (1954), but at the other stations the levels were estimated by reference to the predicted height of the tide at the time; counts of *Chthamalus* made later in the year than September give adults and young specimens separately.)

(*a*) Amory Bight, between Rame Head and Penlee Point

	September 1951	December 1954		
Level in metres, relative to O.D.	*C. stellatus*	*C. stellatus*		*B. balanoides*
		Adults	Spat	
3·9	450	—	—	—
3·3	2,200	2,200	0	0
2·7	67,600	—	—	—
2·3	—	12,400 +	1,000	0
2·1	75,200	—	—	—
1·5	73,600	41,600 +	1,400	100
0·9	78,200	—	—	—
0·6	—	40,000 +	8,800	1,600
0·2	44,400	—	—	—
−0·2	—	41,600 +	56,000	800
−0·4	23,400	—	—	—
−1·0	15,500	28,800 +	32,000	200
−1·6	9,600	12,000 +	4,000	0

(TABLE II—*continued*)

(*b*) Plymouth Breakwater, vertical pile of jetty on north side

Level	September 1951			October 1954		
	C. stellatus	*B. balanoides*	*E. modestus*	*C. stellatus*	*B. balanoides*	*E. modestus*
M.T.L.	44,800	1,000	1,100	16,000	16,000	1,200

(*c*) Plymouth Breakwater, south side, Traverse B of Southward & Orton, 1954

Level (m)	September 1951		October 1954		
	C. stellatus	*B. balanoides*	*C. stellatus*		*B. balanoides*
			Adults	Spat	
3·0	28	0	570	0	0
2·5	12,200	0	33,600 +	8,000	0
2·0	9,500	0	35,200 +	12,400	0
1·5	46,400	0	48,000 +	17,600	0
1·0	37,600	0	37,600 +	38,000	2
0·5	30,600	0	38,400 +	24,000	80
O.D.	40,200	200	24,600 +	36,000	1,100
−0·5	33,200	0	20,800 +	22,800	2,800
−1·0	9,400	2	11,600 +	8,400	500

(*d*) Tinside, below the laboratory

Level (m)	September 1951		October 1953			December 1954		
	C. stellatus	*B. balanoides*	*C. stellatus*		*B. balanoides*	*C. stellatus*		*B. balanoides*
			Adults	Spat		Adults	Spat	
2·28	500	0	800	0	0	500	0	0
1·88	—	—	—	—	—	4,800 +	300	0
1·67	28,840	0	26,000 +	6,000	0	—	—	—
1·38	—	—	—	—	—	24,000 +	8,000	0
1·06	32,400	0	24,800 +	32,000	0	—	—	—
0·88	—	—	—	—	—	34,800 +	16,000	0
0·45	30,800	0	27,200 +	32,000	0	—	—	—
0·38	—	—	—	—	—	24,000 +	27,200	0
−0·16	24,000	0	21,200 +	24,000	60	34,800 +	21,600	200
−0·62	—	—	—	—	—	24,000 +	16,800	0
−0·77	19,600	0	—	—	—	—	—	—
−1·12	—	—	—	—	—	2,800 +	12,400	0
−1·38	1,150	0	1,350 +	500	0	500 +	1,600	0
Shaded surfaces at −1·3	—	400	—	—	—	—	—	2,500

(*e*) Henn Point, Tamar Estury

Level (m)	September 1951			October 1953			December 1954		
	C. stellatus	*B. balanoides*	*E. modestus*	*C. stellatus*	*B. balanoides*	*E. modestus*	*C. stellatus*	*B. balanoides*	*E. modestus*
1·1	550	—	—	400	—	225	500	—	300
0·4	400	66	2,300	100	100	800	0	800	9,600
0·2	0	500	25,600	0	?	9,200	0	500	18,000
O.D.	0	75	28,200	0	0	16,000	0	?	5,200
−0·2	0	100	9,600	0	0	9,600	—	—	—
−0·7	0	0	8,800	0	0	18,400	0	700	20,800
−1·9	0	0	18,800	—	—	—	0	800	16,800

(TABLE II—*continued*)

(*f*) Misery Point—Cellar Beach, Yealm Estuary

	September 1951		October 1953			December 1954		
	C. stellatus	*B. balanoides*	*C. stellatus*		*B. balanoides*	*C. stellatus*		*B. balanoides*
Level (m)			Adults	Spat		Adults	Spat	
3·0	—	—	1,200	0	0	—	—	—
2·7	4,500	0	—	—	—	1,600	0	0
2·4	—	—	20,000+	2,000	0	—	—	—
2·1	46,800	0	—	—	—	80,000+	2,000	1,200
1·5	41,200	100	48,000+11,200		0	68,000+24,000		6,000
1·2	—	—	60,000+13,600		0	—	—	—
0·9	73,600	0	—	—	—	26,400+27,200		4,800
0·6	—	—	16,000+18,000		5,500	—	—	—
0·3	30,000	0	—	—	—	—	—	—
O.D.	—	—	16,000+32,000		7,00	12,000+32,000		8,200
−0·3	21,200	0	—	—	—	24,000+16,000		5,600
−0·6	—	—	29,600+40,000		1,600	3,200		1,600
−0·9	766	0	4,000		400	—		—
−1·5	0	0	—		—	400		800

(*g*) Baring Point, Yealm Estuary

	September 1951		October 1953		December 1954		
	C. stellatus	*B. balanoides*	*C. stellatus*	*B. balanoides*	*C. stellatus*		*B. balanoides*
Level					Adults	Spat	
H.W.N.	3,700	200	?	?	8,000+2,000		400
M.T.L.	2,500	1,000	?	2,600	2,000+4,000		13,600

TABLE III. ABUNDANCE* OF *C. STELLATUS* IN SCOTLAND

Place	In 1934–36 (from Kitching, 1935; Moore & Kitching, 1939)	In 1953
Nybster, Caithness	Rare—2 found in 15 min	No change, but 5/m² at Wick
Dunnet Head, Caithness	Rare—6 seen in 10 min	0·2% (mean of observations on both sides of headland)
Skullomie, Sutherland	0·6%	0·8% (but much commoner at Farr Bay, to the east)
Geodha Chobhair, Sutherland	6·4%	29%
Seana Chamas, Ross	0·6%	3% (on shores to north and south, between 20 and 40%)
Elgol, Skye	10%	Not visited; corresponding area on Sleat, 56%
Easdale, Seil	Scarce	11·4%
Loch Melfort, Argyll	Scarce	30%
Ardnoe Point, Argyll	Rather sparse	700/m²
Southend, Kintyre	Absent	7%

* As numbers found at H.W.N. in a certain time, or numbers per unit area of rock at H.W., or as percentage of total barnacle population over whole intertidal zone.

TABLE IV. SETTLEMENT OF *C. STELLATUS* AT SHOALSTONE BEACH, BRIXHAM, IN RELATION TO TEMPERATURES AT TORQUAY NEARBY

		1949		1950	
Numbers per sq. cm. at end of main settlement (July–October)	H.W.N.	12		5	
	M.T.L.	14		5	
	L.W.N.	28		11	
		Air	Sea	Air	Sea
Monthly anomalies of mean temperature	July	+1·1	+2·2	−0·3	0
	Aug.	+0·5	+0·8	−0·7	−0·1
	Sept.	+2·0	+2·2	−0·5	−0·5
	Oct.	+1·6	+2·1	−0·6	−0·6

Note. Anomalies of sea temperature are relative to the means for the period 1946–54; anomalies of air temperature are relative to the means for the period 1906–35. In this and subsequent tables the anomalies are based on sea and air temperatures supplied by the following authorities: Borough Meteorologist, Torquay; Director, Marine Biological Station, Port Erin; Port Health Officer, Plymouth. Additional values of temperature, including the means of air temperature for the period 1906–35, were obtained from the following: Air Ministry, 1936; Air Ministry, 1946–55: Southward, 1953.

TABLE V. SETTLEMENT OF *C. STELLATUS* AT KALLOW POINT, PORT ST MARY, ISLE OF MAN, IN RELATION TO LOCAL SEA AND AIR TEMPERATURES

		1949		1950		1951		1952		1953	
Numbers per sq. m at 1 ft. above H.W.N. the following spring		24		4		30		0		23	
		Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Monthly anomalies of mean temperature	Sept.	+1·9	+1·1	−0·9	0	+0·3	−0·2	−0·9	−0·9	+0·4	+0·5
	Oct.	+1·3	+1·0	−0·3	−0·4	+0·6	+0·1	−2·0	−1·2	+0·4	+0·6
	Nov.	+0·3	+0·5	−1·5	−0·9	+0·9	+0·7	−2·7	−1·0	+2·4	+0·7
	Dec.	+0·6	+0·2	−3·0	−1·2	+0·6	+1·0	−1·3	−1·3	+2·0	+1·2

Note. The anomalies are relative to the means for the period 1947–53.

Table VI. Anomalies of Mean Air,* and Mean Sea† Temperatures at Torquay

	1946		1947		1948		1949		1950		1951		1952		1953		1954	
	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Jan.	−0·4	0·2	−1·7	−0·6	1·1	0·9	0·8	0·6	0·1	0·3	0	−0·3	−1·2	0	−1·6	−1·3	−1·0	0·4
Feb.	1·9	1·8	−5·7	−3·0	0·1	0·9	1·2	1·1	1·4	1·1	−0·4	0·2	−1·0	−0·2	−0·9	−0·4	−1·1	−0·9
Mar.	0·6	−0·2	−0·9	−2·3	2·1	0·7	0	0·3	2·2	1·3	−0·9	−0·5	1·1	0·6	−0·7	−0·3	−0·4	0·4
Apr.	1·0	0·8	−0·4	−0·8	0·5	0·3	1·3	0·7	0	0·4	−0·9	−0·5	0·6	−0·6	−0·4	−0·6	−0·5	0·3
May	−0·9	0·2	0	0·6	0·1	0·8	0·1	0·5	0·1	0·4	−1·7	−1·1	0·5	0·3	0·5	0	−0·6	−0·6
June	−1·3	−0·8	0	−0·3	−0·1	−0·5	−0·1	1·2	1·4	0·9	−0·2	−0·3	0	0·9	0·3	−0·5	−0·7	−0·9
July	−0·4	−0·3	−0·1	−0·4	−0·5	−0·2	1·1	2·2	−0·3	0	0·1	0	0·1	0·7	−0·1	−0·4	−2·0	−1·2
Aug.	−0·1	−1·1	2·3	1·9	−0·9	−0·1	0·5	0·8	−0·7	−0·1	−0·7	−0·4	0·3	0·4	0	0	−1·6	−1·3
Sept.	−0·3	−1·0	0·9	1·4	−0·3	0	2·0	2·2	−0·5	−0·5	0·1	−0·5	−2·5	−0·9	−0·3	0	−0·8	−0·6
Oct.	0·5	−0·4	0·6	0·8	0	−0·1	1·6	2·1	−0·6	−0·6	−0·3	−0·2	−1·3	−1·4	−0·3	0·1	1·3	0
Nov.	1·4	−0·3	1·3	1·1	1·9	0·1	0	0·2	−0·4	−1·1	1·4	0·4	−1·7	−0·9	1·4	0·7	0·3	0·2
Dec.	2·1	−0·7	0·1	−0·2	1·1	1·1	0·5	0·5	−3·3	−2·1	1·0	0·6	−1·3	−1·6	2·4	1·8	1·7	0·2
Year	0·1	−0·2	−0·2	−0·3	0·4	0·3	0·8	1·0	0	0	−0·2	−0·3	−0·5	−0·3	0	−0·1	−0·4	−0·3

* Relative to the means for the period 1906–35.

† Relative to the means for the period 1946–54.

Table VII. Anomalies of Mean Air Temperature,* Plymouth Hoe, and Mean Sea Temperature,† Plymouth Tinside

	1946		1947		1948		1949		1950		1951		1952		1953		1954	
	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Jan.	−0·3	0·6	−1·9	−0·6	1·0	0·7	1·3	0·7	−0·1	0·6	0	−0·6	−1·3	−0·2	−1·3	−0·3	−1·0	0·1
Feb.	1·8	1·0	−5·7	−2·7	0	0·5	1·3	1·2	1·6	1·3	−0·3	−0·2	−1·1	0·1	−0·9	−0·2	−1·1	−0·4
Mar.	0	0	−0·7	−1·9	3·1	0·1	0·6	0·9	2·4	1·1	−0·8	−0·2	1·0	0·1	0·2	0·3	0·8	0·3
Apr.	1·9	0·9	0·7	0·1	1·4	0·4	1·9	0·9	0·6	0·6	−0·5	−0·5	1·2	0·3	0·6	0·1	−0·3	0·6
May	0	0·3	0·8	−0·2	1·2	0·9	0	0·7	1·2	0·8	−1·0	−0·7	1·5	1·1	1·2	0·5	0	0·1
June	−1·1	0·3	0·5	−0·2	0	0·2	1·9	0·8	1·9	1·3	0·2	−0·1	0	0·4	0·6	−0·6	−0·5	0
July	−0·2	0·4	0·3	−0·4	0	−0·6	2·2	2·0	−0·1	0·4	0·5	−0·1	0·7	0·4	−0·3	−0·3	−1·9	−1·6
Aug.	1·2	−0·5	3·7	1·5	0	0	1·6	0·9	−0·3	0·3	−1·1	−0·2	0	0·4	0·5	0	−0·7	−1·7
Sept.	0	−0·2	1·4	1·6	0·3	−0·2	3·2	1·9	−0·4	0·1	0·1	−0·4	−2·0	−0·4	0·5	−0·2	−0·7	−0·4
Oct.	0·8	0	0·9	1·3	0·4	0·3	2·4	1·3	−0·2	−0·1	0·2	0·1	−0·4	−0·6	0·3	0·5	1·3	0·3
Nov.	2·0	0·3	1·7	1·5	2·2	0·6	0·5	0·7	−0·3	−0·3	1·7	0·4	−1·3	−0·2	2·0	0·8	0·6	0·2
Dec.	−1·6	−0·2	0·2	1·0	1·1	0·8	0·8	−0·4	−3·0	−0·6	0·9	0·6	−0·9	−0·9	2·6	2·1	1·8	0·9
Year	0·1	0·3	0·1	0	0·9	0·3	1·3	1·1	0·3	0·4	0	−0·1	−0·2	−0·1	0·5	0·2	−0·4	−0·1

* Relative to the means for the period 1906–35.

† Relative to the means for the period 1893–1948.

Table VIII. The Settlements of *B. balanoides* at Shoalstone Beach, Brixham, Compared with some Winter Temperatures Experienced at Torquay nearby

	1946		1947		1948		1949		1950		1951		1952		1953		1954	
Maximum numbers per sq.cm of rock at end of settlement (March–April)	12		10		8		7·5		3·6		< 1		10		6		12	
	*	†	*	†	*	†	*	†	*	†	*	†	*	†	*	†	*	†
Anomalies of temperature:	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea	Air	Sea
Previous December	—	—	2·1	−0·7	0·1	−0·2	1·1	1·1	0·5	0·5	−3·3	−2·1	1·0	0·6	−1·3	−1·6	2·4	1·8
January	−0·4	0·2	−1·7	−0·6	1·1	0·9	0·8	0·6	0·1	0·3	0	−0·3	−1·2	0	−1·6	−1·3	−1·0	0·4
February	1·9	1·8	−5·7	−3·0	0·1	0·9	1·2	1·1	1·4	1·1	−0·4	0·2	−1·0	−0·2	−0·9	−0·4	−1·1	−0·9
March	0·6	−0·2	−0·9	−2·3	2·1	0·7	0	0·3	2·2	1·3	−0·9	−0·5	1·1	0·6	−0·7	−0·3	−0·4	0·4
April	1·0	0·8	−0·4	−0·8	0·5	0·3	1·3	0·7	0	0·4	−0·9	−0·5	0·6	−0·6	−0·4	−0·6	−0·5	0·3

* Relative to the period means 1906–35. † Relative to the period means 1946–54.

Table IX. Anomalies of Mean Annual Air Temperature* at Douglas, and Mean Annual Sea Temperature† at Port Erin, Isle of Man

	1946	1947	1948	1949	1950	1951	1952	1953
Air	0	−0·2	0·2	0·4	−0·2	−0·3	−0·4	0·6
Sea	0·3	−0·7	0	0·7	0·3	−0·2	−0·2	0·2

* Relative to the grand mean for 1906–35.
† Relative to the grand mean for 1903–51.

TABLE X. THE ABUNDANCE OF *B. BALANOIDES* AT CERTAIN LOCALITIES IN SOUTH DEVON, AUGUST 1955, AS NUMBERS PER SQUARE CENTIMETRE OF ROCK, SHOWING THE RELATION OF THE SPRING SETTLEMENT OF SPAT TO SEA AND AIR TEMPERATURES

	Plymouth, Tinside	Wembury, outer reefs		River Yealm, Misery Point		Brixham, Shoalstone	
		Adults	Spat	Adults	Spat	Adults	Spat
H.W.N.	Nil on usual sampling area, up to 0·2 at L.W. in places	0	0·1	0·28	0·54	0·16	0·18
		(2)		(11)*		(12)	
M.T.L.		0·1	1·22	0·48	1·08	0·15	0·94
		(24)		(22)		(45)*	
L.W.N.		0·2	1·4	0	1·13	0·01	1·30
		(43)		(66)*		(89)*	

	Plymouth Hoe temperatures, anomalies of		Torquay temperatures, anomalies of	
	Mean sea† (° C)	Mean air‡ (° C)	Mean sea§ (° C)	Mean air‡ (° C)
January	−0·3	−0·2	−0·4	−0·2
February	−0·1	−2·4	−0·3	−3·0
March	−1·3	−2·2	−2·6	−2·1
April	−1·4	+1·2	−1·0	+0·8
May	−1·2	−1·3	−0·9	−1·9
June	−0·6	+0·2	−0·2	−0·2

The figures in brackets show the percentage of *B. balanoides* out of the total *B. balanoides* and *Chthamalus stellatus* populations.

* Indicates an increase in this percentage compared with 1954.

† Relative to the grand means for the period 1900–55.

‡ Relative to the grand means for the period 1906–35.

§ Relative to the grand means for the period 1947–54.

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A RECORD OF PLANKTON ON THE ECHO-SOUNDER

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(Text-figs. 1–3)

A number of records from echo-sounders have been loosely attributed to the presence of plankton. Only two records have been adequately identified as having been produced by plankton; first, the traces of fish larvae, or shallow scattering layers (Burd & Lee, 1951); and secondly, the echo layer, at the depth of the temperature discontinuity or thermocline, which is sometimes associated with plankton animals or plants (Cushing, Lee & Richardson, in press). A third type of record, that from the 'deep scattering layer', has been associated with the presence of euphausiids (Hersey & Moore, 1948; Moore, 1950; Boden, 1950): an equally plausible association with the presence of fish has been made by Marshall (1951), Tucker (1951) and Hersey & Backus (1954). A fourth type of echo record, a 'noisy' record, will be described and will show that it is probably attributable to plankton organisms, consisting, in one instance, of euphausiids.

Material and Method

During the work to be described the amplifier was kept at known settings, engines had no effect on our echo-sounders and we carried out most of our work in flat calm weather. We sought to identify a factor in 'water noise' by studying the plankton content of the water, at the time when records of noise were being made.

There are three groups of observations, the first in Windermere, followed by two in the North Sea.

Results at Windermere

When we were working on Windermere, the lake was flat and calm. During the daytime, noise appeared suddenly at a high level of amplification, as a black band right across the paper. On one night, however, at a lower level of amplification, noise appeared diffusely from the transmission mark downwards, rather as it does in the sea. But the diffuseness faded with depth, which was not characteristic of noise traces that had been taken at sea. The actual trace is shown in Fig. 1.

Because the diffuse noisy trace that faded with depth was only found at night, it was thought to be associated with the presence of plankton animals that had migrated to the surface at night. The phytoplankton makes no such migration, and although present in the lake in dense numbers, was consequently disregarded.

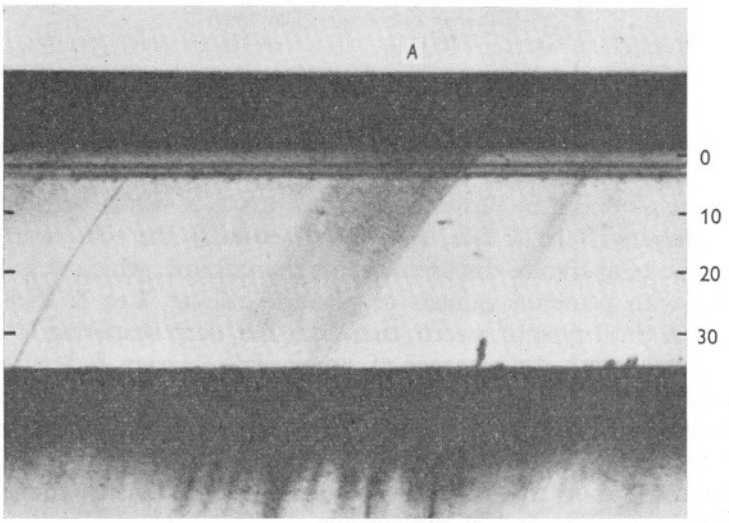

Fig. 1. The 'noisy' trace at night in Windermere. Depth in metres shown on right. The diffuse shadow at A, which appeared when the gain control was turned up, is the noisy trace.

TABLE I. ABUNDANCE AT NIGHT OF *DIAPTOMUS GRACILIS* SARS., AND *DAPHNIA HYALINA* LEYDIG

(Expressed as number per 5 l. of water at Station RNB 5.)

Depth (m)	*Diaptomus gracilis*	*Daphnia hyalina*	Total
0	131	1	132
5	47	21	68
12	31	2	33
16	6	3	9
25	—	2	2

When the diffuse trace was observed, water samples were immediately taken with a 5 l. Rodhe bottle at 0, 5, 12, 16 and 25 m. The depths were chosen according to the appearance of the trace, the record having faded at 16 m. The counts of plankton animals are given in Table I.

During the daytime, samples were taken chiefly from the depths of the thermocline, at about 10 m. When the animals were concentrated near the thermocline, their numbers reached 28/l. and a trace was recorded at that

depth. When the animals were not concentrated at the thermocline, their numbers at 0, 5, 10 and 12 m were not greater than 2 or 4/l.

There are three points of evidence associating this trace with the presence of plankton animals. First, the fact that it appeared only at night, when the animals had migrated to the surface. Secondly, the trace faded at 16 m in depth, which is the lower limit to the distribution in depth of the animals; however, if the animals were uniformly distributed, the trace would be of this character because of the dissipation of transmitted energy with depth and the fading at 16 m could be a coincidence. Lastly, the density of animals that presumably produced the trace, when diffusely spread at the surface, was the same as the density that produced an echo when packed against the thermocline at 10 m, i.e. 28/l. (Cushing *et al.* in press).

Results from the North Sea

In May and June 1953 two gears were being tested, in the North Sea, off North Shields. The first was an echo-sounder, with oscillators mounted on the keel to transmit horizontally. The second was a high-speed plankton net, which was being towed just below the surface.

The horizontal echo-sounder—pulse length, 1 ms (millisecond)—which was a development project of Messrs Kelvin and Hughes Ltd., had two ranges, 150 and 1500 fm (274 and 2742 m); the gain control of the amplifier could be conveniently divided into six points, dividing the degree of amplification into seven levels, 0, $\frac{1}{6}$, $\frac{1}{3}$, $\frac{1}{2}$, $\frac{2}{3}$, $\frac{5}{6}$ and full. The cruise was primarily a survey with an ordinary vertically transmitting echo-sounder; at stations, 10 miles apart, plankton nets were hauled from the bottom to the surface.

At each station, the following procedure was adopted for very roughly estimating the noise and reverberation level. The amplifier was set at 0, $\frac{1}{6}$, $\frac{1}{3}$, $\frac{1}{2}$, $\frac{2}{3}$, $\frac{5}{6}$ and full, successively and at each setting, the machine was allowed to make a few transmissions on the 150 fm range, when the ship was hove to on station. At each level of amplification, the paper record was marked to a certain distance in mm from the transmission mark; these distances were taken as indices of noise and reverberation. Each record was marked for measurement, when wet, so that there would then be no question of differential fading on different records. A typical record is shown in Fig. 2, giving three separate series of measurements, at 150 fm range with the ship stopped, at 1500 fm range with the ship stopped, and again at 150 fm range, when the ship was under way. Thus each record gives a measure of the noise in mm, when the sounder is transmitting horizontally beneath the sea surface.

The plankton at the surface was estimated with the use of the tin tow-net, or high-speed tow-net, hauled just below the surface for 10 min as the ship steamed away from the station. This model (described by Gehringer, 1952) stands 7 ft (213 cm.) high, has a mouth opening of 40 cm in diameter, and has

a metal net of 40 meshes to the linear inch. It was towed from a bracket on its dorsal surface with a light Larsson trawl warp from the boom; a Larsson depressor was slung underneath to stabilize it. Used in this way, it was exceedingly stable at all speeds.

The net was shot from the quarter as the ship steamed away from a station; as the ship accelerated, a little warp was let out until the net was visible between 5 fm (9·1 m) and the surface. After 10 min the net was hauled in at full speed with no difficulty; the catch was washed out with a hose, bottled and preserved.

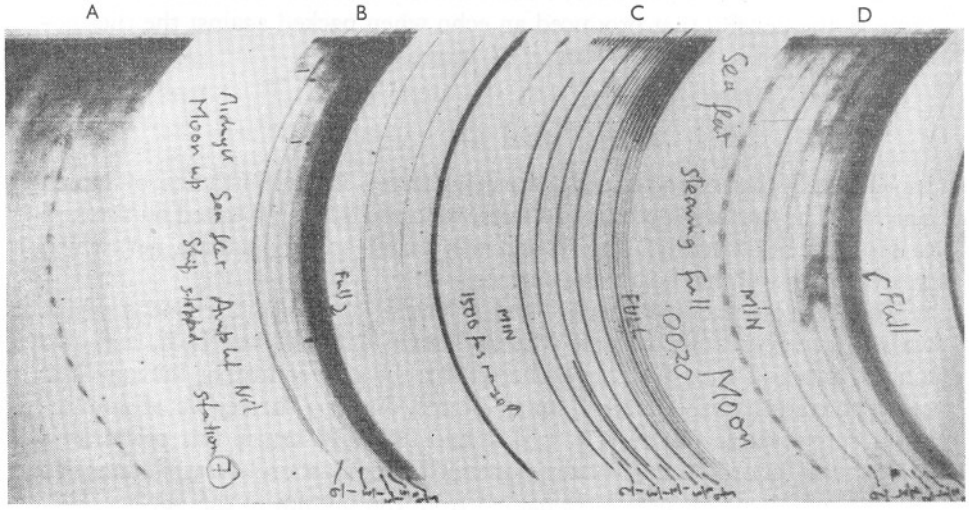

Fig. 2. An illustration of the methods used in estimating the noise levels on *Platessa* Cruise VIII, 1953. A: part of the horizontal sounder trace, as the ship approached station 7, amplifier setting $\frac{1}{3}$. B: transmisson records made at various amplifier settings, using the 150 fm range scale. C: transmission records made at various amplifier settings, using 1500 fm range scale. D: transmission records made at various amplifier settings, using the 150 fm range scale, as *Platessa* was steaming away from the station. The echo at about 80 fm in section D is from the ship's wake.

The plankton animals caught were identified as copepods, sagittae, euphausiids (*Nyctiphanes couchii* Bell), and fish larvae, and were expressed as numbers per 10 min haul; the numbers of animals were multiplied by the cubes of their lengths to give an estimate of volume. A long series of measurements of the lengths of adults and copepodite stages of *Calanus finmarchicus* Gunner, *Pseudocalanus elongatus* Boeck, *Paracalanus parvus* Claus, and *Temora longicornis* Müller, was used for this purpose (Cushing, in press). Additional length measurements of *Acartia*, *Oithona*, *Evadne*, *Sagitta*, euphausiid furcilia, and smaller larvae were also made. The results were expressed as volumes of total zooplankton in mm^3 and as volumes of euphausiids in mm^3.

Six observations were made on *Platessa* Cruise VIII 1953, during flat calm weather at night and during the day. Fifteen observations were made on *Platessa* Cruise IX 1953, under a variety of conditions. The summarized results are set out in Table II, showing volumes of zooplankton in mm^3 and the indices of noise in mm; the state of the sea is also recorded. On Cruise VIII, the degree of amplification was measured as described above, but on Cruise IX, it was placed at a constant low setting, which was unrecorded.

TABLE II. VOLUMES OF ZOOPLANKTON, IN MM^3, PER 10 MIN HAUL AND INDICES OF NOISE, IN MM

Platessa Cruise VIII, 1953

Station	Zooplankton ($mm^3 \times 10^6$)	Euphausiids ($mm^3 \times 10^6$)	Noise, in mm gain $\frac{2}{3}$ 1500 fm range	State of sea
8	1·82	0·02	6	0
6	7·35	1·75	27	0
7	4·55	3·13	21	0
30	1·90	0·07	3·5	0
8*a*	0·41	0·002	5	0
14	0·33	0·02	5	0

Platessa Cruise IX, 1953

Station	Zooplankton ($mm^3 \times 10^6$)	Euphausiids ($mm^3 \times 10^6$)	Noise in mm	State of sea
50	0·81	0·002	6·5–7	0
50*a*	0·003	0·0	5–5·5	0
51	0·68	0·001	7–7·5	0
51*a*	1·10	0·01	5·5	0
52*a*	0·80	0·002	7–7·5	2
54	0·02	0·0	5–5·5	0
54*a*	1·47	0·04	2·5–3	1–2
55	1·25	0·07	1·5	0
56	1·09	0·01	4·5	0
61	1·71	0·10	4·5–5	0
62	0·60	0·17	4	0
63	0·21	0·12	6·5	2
64	1·03	0·01	5·5–6	3
65	1·30	0·02	5·5–6	3–4
66	0·08	0·001	6·5	3–4

On Cruise VIII the highest noise indices occurred when euphausiids were present in large numbers, dominating the whole plankton catch. On Cruise IX, when the euphausiids, in general, comprised only a small part of the plankton catch, the noise indices were always about the same, there being no violent differences as there were on the previous cruise; in fact, the noise did not increase noticeably when the state of sea increased. Again the average volume of euphausiids on Cruise VIII was about twenty times that found on Cruise IX.

Fig. 3 illustrates the traces at stations 3 and 6 on Cruise VIII. Fig. 3A shows the clear trace at station 3 on the 150 fm range, with the amplifier set at $\frac{1}{3}$. Fig. 3B shows the trace at station 6 on the 150 fm range, with the amplifier set at $\frac{1}{3}$. The remarkable difference is attributed to a marked difference in quantity of zooplankton, in this case, mainly euphausiids.

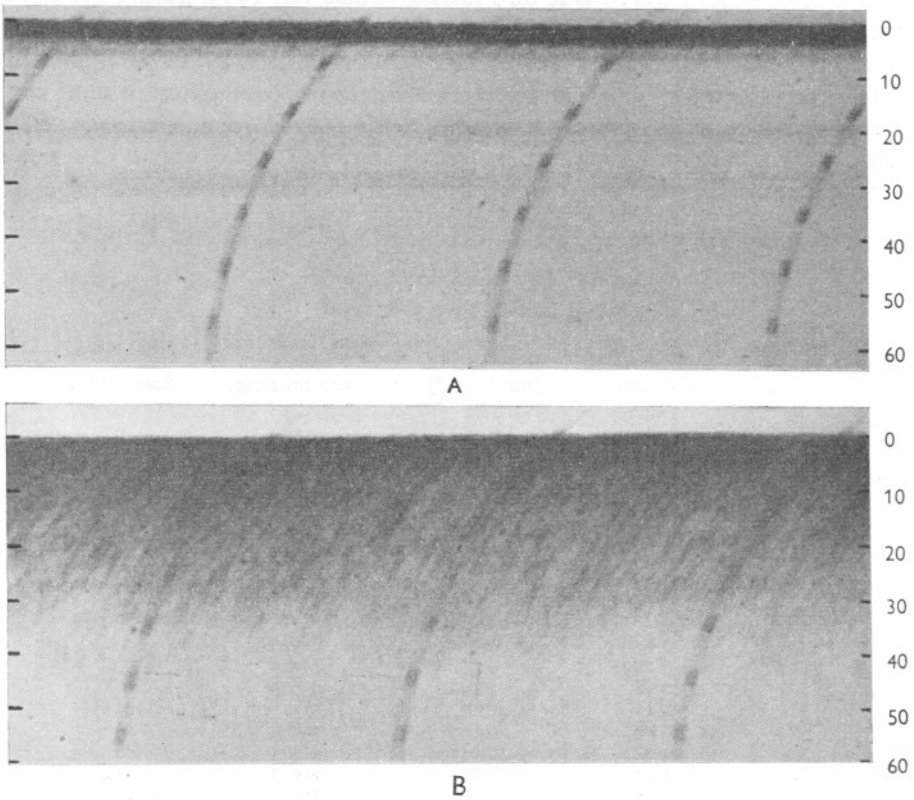

Fig. 3. The 'noisy' trace at night in the North Sea. Range in fathoms shown on right. A: part of the horizontal sounder trace observed 30 miles away from the plankton patches, in full daylight, at an amplifier setting of $\frac{1}{4}$. B: part of the horizontal sounder trace observed in the plankton patch at night, at an amplifier setting of $\frac{1}{4}$. Note the constant decrease in signal strength from the transmission outwards, with increasing range.

Discussion

The traces shown in Fig. 1 and in Fig. 3 are of a particular type. To describe it we must distinguish between noise and an echo. If an echo-sounder is switched on, but is not transmitting, the receiver is open to receive any sound of the frequency to which it is tuned; this is water noise. Again if the echo-sounder is transmitting, part of the transmitted energy is scattered by small particles, by irregularities in the temperature structure of the water and by irregularities on the bottom; some of this randomly scattered energy reaches the receiver as noise. This reverberation, as the scattering is sometimes called, is like the water noise in that it is random in character and appears as a record that is dirty all over.

Now, when the amplifier of an echo-sounder is turned up progressively, the paper becomes marked irregularly all over, with water noise, reverberation and lastly with instrument noise. An echo is distinguished from noise by its consistent appearance at about the same depth or range in successive transmissions. It is only the successive transmissions that establish the identity of an echo, as opposed to noise. This is why a paper recorder is necessary for establishing the presence of signals from fish shoals on a CRT, because, on the screen, noise and echoes cannot readily be compared with signals of previous transmissions.

The traces shown in Figs. 1 and 3 have three characters: first they are horizontally extensive, secondly they are irregularly diffuse, and lastly, when the amplifier is turned up, they show increased range. If the amplifier was turned up whilst a fish trace was being observed, the trace darkened with increased intensity of received signal, but did not increase its apparent range. Again, a fish trace nearly always has a discrete character and is rarely diffuse (however, traces of herring at Sandettié sometimes appear somewhat diffuse at night).

When the traces of euphausiids in the North Sea are compared with those of copepods and cladocerans in Windermere, it is found that the quantity of material was not so very different. At stations 6 and 7 on Cruise VIII, there were between 1·75 and $3{\cdot}13 \times 10^6$ mm^3 of euphausiids per 10 min haul with the tin townet. If we assume that the net filters 233 m^3 in 10 min when towed at an average speed of 6 knots, then the comparable quantity of zooplankton in Windermere would have amounted to $6{\cdot}5 \times 10^6$ mm^3 per 233 m^3. Here we have assumed that each copepod and cladoceran (some of which were small juveniles) occupied an average volume of 1 mm^3 (the length of *Diatopmus gracilis* was 1·128 mm, and that of adult of *Daphnia hyalina* was 1·733 mm—the more common juveniles were 1·2 mm; each value is the mean of fifty measurements). In the North Sea and in Windermere, the average plankton volume was 10 mm^3/l. in the former and 28 mm^3/l. in the latter.

Another similarity between the two traces is that both are continuous from the transmission mark outwards. This is not in itself unusual, but the only other diffuse and extensive trace, the shallow scattering layer, composed of fish larvae, appears to stay at night far enough from the surface, to allow a clear gap to appear between the transmission mark and the trace itself. The density of pilchard larvae in the shallow scattering layer in the Western Channel amounted to 0·11/m^3 as caught (or between 0·5 and 1/m^3 allowing for escape from the Petersen young-fish trawl: J. P. Bridger, private communication). If each fish larva was 8–12 mm long, there would be about 1 mm^3/l. The difference of 10 to 28 times between this estimate and that for the noisy trace is attributed to the presence of air bladders (which were perhaps resonating) in the pilchard larvae, which would scatter sound much more efficiently than would plankton animals or bladderless fish larvae.

When working in Windermere we were primarily interested in the thin echo layer associated with the thermocline. From our observations we associated the echo layer with the presence of plankton animals (*Diaptomus gracilis* and *Daphnia hyalina*) packed against it (Cushing *et al.*, in press). When the echo layer occurred at 10 m, the signal was rather weak but quite discernible, and there were 28 animals/l., which was the same density as that found by night at the surface in Windermere. Between the two signals there was probably not a great deal of difference in strength. The transmitted signal at the depth of the plankton animals was reduced by many times, through being transmitted through 10 m of water; hence, to produce a signal on the echo-sounder at all comparable, the animals must have been packed considerably more tightly than appears from the estimate of 28/l. Since our samples were taken with a Rodhe bottle about $\frac{1}{2}$ m long, one of them could easily be composed of a thin layer of animals at a density of 140/l.

There are three types of echo trace that are perhaps associated with plankton: first, the shallow scattering layer of fish larvae; secondly, the echo layer at the thermocline; and thirdly, the noisy trace. The fourth type of record that has been associated with plankton is that from the deep scattering layer, which has been attributed partly to the presence of euphausiids. The noisy trace that was found in the North Sea is interesting from this point of view, in that it was possibly composed of euphausiids.

Before we discuss the noisy trace in relation to the deep scattering layer, it would be well to consider the numbers of euphausiids caught at Stations 6 and 7 on *Platessa* Cruise VIII, 1953. To obtain a minimum estimate, it will be assumed that the tin townet filtered the water efficiently. The numbers of adult euphausiids caught in 10 min at Stations 6 and 7 were 15,900 and 28,500 respectively, which makes 68 and 122/m^3, if an average speed of 6 knots was maintained during the haul. If the net was only working at 20% efficiency, these figures must be raised by five times. Other reported catches are:

(1) Mackintosh (1934). The average numbers of *Euphausia superba* per haul is 1,000, which is 1–5/m^3, assuming 20% efficiency. The highest catch was 190–950/m^3.

(2) Hardy & Gunther (1935), figs. 90, 92, 95, table 52. The highest numbers of *Euphausia frigida*, *E. superba* and *Thysanoëssa* spp. were up to 150/m^3, assuming 20% efficiency.

(3) Einarsson (1945). Assuming 20% efficiency, the highest catch of *Thysanoëssa longicaudata* was 4–12·5/m^3.

(4) Moore (1950). *Euphausia brevis*. 1·5/m^3; by inference from luminescence observations at night, 7–15/m^3.

(5) Boden (1950). *E. pacifica*; assuming 20% efficiency, the highest catch was 2·5/m^3.

(6) Glover (1952). *E. krohnii*, *Meganyctiphanes norvegica*, *Nyctiphanes couchii*, *Thysanoëssa inermis*, *T. longicaudata*; assuming perfect filtration with the plankton recorder, there were 1–3/m^3 (or 5–15/m^3, assuming 20% efficiency).

It follows that the catches made in the North Sea in May were amongst the highest recorded. This might have been associated in part with the moon-light drawing the euphausiids to the surface, but it is also believed that an important factor was the use of the high-speed tin townet, which has a wide enough mouth to make escape at a towing speed of 6–8 knots (309–412 cm/sec) nearly impossible.

During these trials, euphausiids were found at a density in numbers of perhaps 120/m^3, yet signals in the noisy trace were not recorded from beyond 30–80 fm (55–146 m) range; the nature of the trace and the character of our observations, continuously for 3 h over 20 miles of sea, do not lead us to suspect that there was a boundary to euphausiid distribution running parallel to the ship's course at a range of 80 fm (146 m).

In our North Sea observations euphausiids did not give a signal beyond 80 fm (146 m) depth, whereas the deep scattering layer was recorded down to 450 fm (823 m). There is evidently a difference here of a large order of magnitude, which might be either instrumental or biological. It is difficult to believe that our machine was very much less sensitive than those used in studies on the deep scattering layer because the relatively high figure of 1 kw was put into the transducer although the pulse length was short; so the signal-to-noise ratio was lower than if a longer pulse had been used. To our minds, our observations present yet another reason for doubting that that layer is due to euphausiids.

Summary

An echo trace attributable to plankton organisms has been described. Its diagnostic character is that when the amplifier of the echo-sounder is turned up, the trace extends in range; a fish trace would not extend in range but would darken with increased intensity of signal.

This trace was observed at night in Windermere where it was possibly composed of copepods and cladocerans at a density of about 28 animals/l.; it was also observed at night in the North Sea, where it was probably composed of euphausiids at a density of 0·12 animals/l.

The euphausiids were caught with a high-speed townet, with a mouth opening of 40 cm diameter. A very rough comparison was made between the North Sea trace of euphausiids and the signal that comes from the deep scattering layer, which may be partly composed of euphausiids.

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FILTERING, FEEDING, AND DIGESTION IN THE LAMELLIBRANCH *LASAEA RUBRA*

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(Text-figs. 1–11)

Lasaea rubra is the smallest and commonest of Plymouth bivalves. It is intertidal on rocky shores, often in immense numbers, in crevices and other protected places, and in *Pygmaea pumila* (Colman, 1940). This bivalve is an excellent laboratory animal. It remains fully active for several days under laboratory conditions, and a large number of animals can be used in a single experiment, eliminating the variations in activity found when dealing with a few, or single specimens of larger animals. Because of the relatively high position *Lasaea rubra* may occupy on the shore its feeding cycle is broken by regular dry periods at each tide. Thus, for experiments relating to periodicity in feeding, we have an animal whose times of feeding can easily be ascertained and experimentally varied over a wide range. Lastly, in making serial sections and in examining total gut contents, in work on the digestive system, the small size of the animal is of obvious advantage.

The present paper is devoted to an account of filtering rates with various food organisms, variations in filtering activity and the relation of filtering to feeding and digestion. Further work has been completed by one of us (J. E. M.) on the mechanism of digestion and the cycle of activity of the digestive gland, and experiments are in progress on respiration during submersion and exposure.

We record our special thanks to Dr Mary Parke, botanist at the Plymouth Laboratory, for making available the unialgal cultures of the organisms used, and for her invariable kindness and sound advice. One of us (J. E. M.) has been the holder of a University of London Table at the Plymouth Laboratory, and both of us are grateful to the Director and staff of the Laboratory for many kindnesses. In particular, Dr H. W. Harvey, F.R.S., allowed us the frequent use of his absorptiometer, and gave us great encouragement throughout the work, while Dr B. C. Abbott gave valued assistance in discussion and evaluation of results.

Methods

Specimens of *Lasaea rubra* were collected on the day of each experiment from the limestone rocks below the Hoe bathing pool, in front of the Plymouth Laboratory. To ensure the sampling of a constant population, collections were made, except where otherwise stated, from as nearly as possible the same group of crevices on an outcrop of rocks 18 in. to 2 ft. below the upper limit of *Chthamalus stellatus*. The animals were used as soon as possible after collection. In experiments on filtering rates a group of twenty animals was selected for each experiment and placed in cultures of micro-organisms, in optical glass cuvettes supplied by the Tintometer Co. Ltd., Salisbury. Six such vessels were used in sets of replicate experiments, each containing 3·5 ml., with an optical depth of 20 mm. The unialgal cultures employed as food were grown in Erdschreiber (enriched sea water) medium, and it was found that *Lasaea* rapidly resume normal behaviour in this medium, aggregating in a cluster at the bottom of the cuvette and attaching to each other by byssus threads, with foot and siphon extended (Fig. 1). At this stage normal filtering activity was assumed to have begun. With practice in selection, the eye could pick out samples of *Lasaea rubra* conforming closely to a standard size distribution (Fig. 2A).

Filtering rates were determined by the measurement of the optical density of the medium by means of a Harvey absorptiometer, constructed as described by Harvey (1948). The chief modifications introduced were those outlined by Spencer (1954) for use with micro-organisms. After a series of density-dilution comparisons (see Fig. 2B) the concentration of organisms was taken to be directly proportional to log optical density. Experiments were continued for periods of 6–8 h, and readings of the optical density of the cultures were taken at intervals. An adaptation period of the first hour was allowed, and the rate of filtration was then calculated from the first to the fourth hours. In the experiments using *Arenicola* haemoglobin, measurements were made on the Unicam spectrophotometer.

In experiments where filtering, feeding and digestion were compared it was not possible to allow the animals to adapt themselves to experimental conditions for the first hour. In these experiments measurements were made of the amount of *Phaeodactylum tricornutum* filtered, the amount extruded as pseudofaeces, the amount ingested and the amount digested. In order to do this four experiments were set up and one was discontinued every half hour. After the period of filtration, optical density was measured in order to calculate the quantity of *Phaeodactylum* filtered, 3 ml. of the supernatant culture was then removed, and the remaining 0·5 ml. containing the pseudofaeces was then brought up to the original volume by the addition of 3 ml. of filtered sea water. A second reading of optical density was then made, which—after correction for the 0·5 ml. of supernatant culture left—gave the quantity of

Phaeodactylum in the pseudofaeces. In each experiment the difference between the amount filtered and the amount recovered as pseudofaeces was taken to represent the fraction removed by ingestion. This difference was found in practice to be too small to measure accurately by the absorptiometric method. Gut contents were therefore measured directly by the quick dissection of two

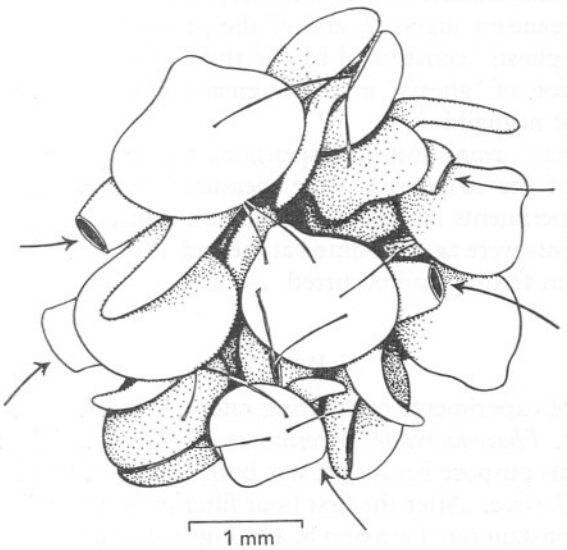

Fig. 1. A group of *Lasaea rubra*, attached together by byssus threads in the normal manner. The inhalant siphon and the foot are extended, and the arrows show the directions of inhalant water currents.

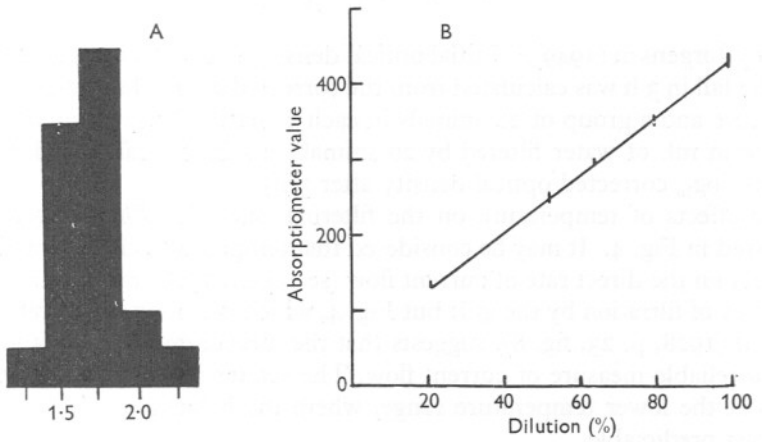

Fig. 2. A: size distribution in mm of a typical group of 20 *Lasaea rubra*, selected in experiments on filtering rates. B: the relation between dilutions of a culture of *Phaeodactylum tricornutum* and the scale of optical density on the Harvey absorptiometer.

animals from each cuvette, on dry slides under a binocular microscope. The full stomach, and as much of the intestine as contained faeces was isolated and opened, so that the total contents were shed upon the slide. Remains of tissue were as far as possible removed and the total gut contents taken up in 0·2 ml. of filtered sea water. The concentration of *Phaeodactylum* cells was then estimated by haemacytometer counts, a measure being thus obtained of the total number of organisms ingested, and of the proportion of intact, undigested cells, and of 'ghosts' constituted by the thin walls of empty, digested cells. (The proportion of 'ghosts' in the original culture was found for present purposes to be negligible.)

In experiments using mixed suspensions the proportions of the various constituents of the suspension were measured by haemacytometer counts before the experiments and either the supernatant culture, pseudofaeces or stomach contents were again counted at the end, in order to determine whether any selection in feeding had occurred.

Results

In the series of experiments on filtering rates a large number of results were obtained with *Phaeodactylum tricornutum* (Table I). *Phaeodactylum* was selected for this purpose because it was both readily available in culture and acceptable to *Lasaea*. After the first hour filtering was usually continued at a more or less constant rate for 6 or 7 h, and Fig. 3 shows curves for a typical set of six experiments. Filtering rates were calculated from optical density by applying the formula

$$P_t = P_0 \exp\left[-\frac{m}{M}t\right],$$

used by Jørgensen (1949).[1] Initial optical density at 1 h was corrected to 500, and the fall in 3 h was calculated from the corrected 4 h reading, using 3·5 ml. of culture and a group of 20 animals in each cuvette. Thus, m, equalling the volume in ml. of water filtered by 20 animals in 1 h, was calculated as 2·684 (2·699 $-\log_{10}$ corrected optical density after 3 h).

The effects of temperature on the filtering rate with *Phaeodactylum* are expressed in Fig. 4. It may be considered that temperature changes will exert an effect on the direct rate of current flow (see Gray, 1928) rather than on the efficiency of filtration by the gill; but Fig. 4, which is closely comparable with Galtsoff (1928, p. 23, fig. 8), suggests that the measurement of filtration rate gives a reliable measure of current flow. The scatter is widest in the experiments at the lower temperature range, where the behaviour of the bivalves was least predictable.

[1] M = quantity of water in cuvette; m = quantity of water filtered; P_t and P_0 are the concentrations of suspended material at times t and 0 respectively.

TABLE I. FILTERING RATES OF *LASAEA RUBRA* WITH *PHAEODACTYLUM TRICORNUTUM*

(Each value represents the volume filtered in ml./h, over 3 h by 20 animals, at room temperature.)

Set of experiments	Filtering rates for 20 animals
1	0·403, 0·681, 0·513, 0·786
2	0·9162, 0·2203, 0·6764, 0·8758, 0·450, 0·952, 0·543, 0·471, 0·550, 0·339, 0·963, 0·698, 0·380, 0·311, 0·212, 0·339, 0·260, 0·499, 0·189
3	0·565, 0·565, 0·963, 0·612, 0·680, 0·754, 0·456, 0·745, 0·804, 0·499, 0·492, 0·488, 0·715, 0·664, 0·644, 0·506, 0·496, 0·715, 0·685, 0·676
4	0·364, 0·308, 0·419
5	0·406, 0·536, 0·399
6	0·4744, 0·9212, 0·809, 1·435, 0·628, 0·847
7	0·795, 0·754, 0·524, 0·577, 1·469, 0·8179
8	0·3672, 0·6396, 0·9162

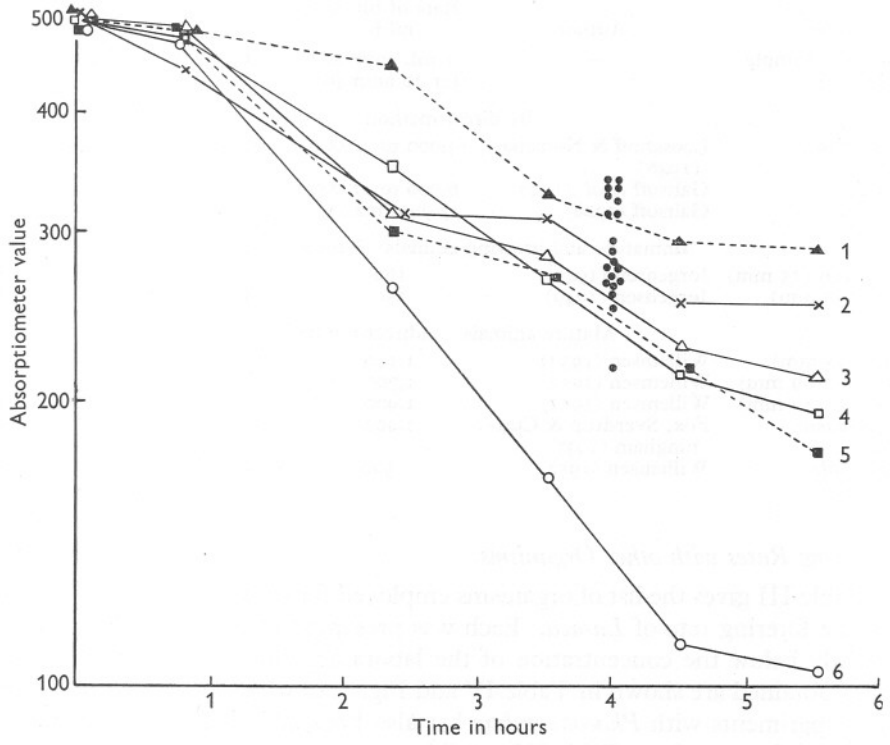

Fig. 3. Six curves showing the filtering rates of sets of 20 *Lasaea rubra* in *Phaeodactylum*, in a typical series of experiments over 5½ h. It is suggested that the highest rate (Expt. 6) approaches the value likely to be achieved by continuous filtering by all the animals during the whole experimental period. The scatter points show the end values of optical density in a further series of twenty experiments, all values being determined with reference to an initial base line of 500 absorptiometer units.

Comparison with other Lamellibranchs

A pumping rate of 1 ml./h/20 animals at 15° C is cited in Table II for *Lasaea rubra* as being typical of the higher rates obtained with groups of mainly adult animals, sampled as in Fig. 2A. Table II summarizes the information on the filtering rates for other lamellibranchs. Verwey (1952, pp. 193–4) gives a useful review of what has already been published on this subject. Filtering rates are produced by comparison of the volume of water filtered by one animal with the volume by displacement of the total animal with shell closed.

TABLE II. COMPARISON OF THE FILTERING RATE OF *LASAEA RUBRA* WITH OTHER LAMELLIBRANCHS

Species	Author	Rate of filtering (ml/h)	Estimated mean volume of single animal	Vol. water filtered in 1 h/volume of animal	Temp. (° C)
Lasaea rubra (sample as in Fig. 2A)	—	1 ml. approx. (for 20 animals)	1·2 mm^3	42	15–16
		By direct method			
Ostrea virginica	Loosanoff & Nomejko (1946)	13,000 to 20,000	100 cm^3	130–200	—
O. virginica	Galtsoff *et al.* (1947)	6,000 to 12,000	100 cm^3	60–120	—
O. virginica	Galtsoff (1928)	3,900 (max.)	100 cm^3	39	—
		Immature and growing animals—indirect method			
Mytilus edulis (15 mm)	Jørgensen (1949)	160	0·8 cm^3	200	17–20
M. edulis (30 mm)	Jørgensen (1949)	750	5 cm^3	150	17–20
		Mature animals—indirect method			
M. edulis (48 mm)	Willemsen (1952)	1,100	19 cm^3	58	12–15
M. edulis (67–69 mm)	Willemsen (1952)	1,700	30 cm^3	57	12–15
M. edulis (77–80 mm)	Willemsen (1952)	1,900	40 cm^3	47	12–15
M. californianus (74 mm)	Fox, Sverdrup & Cunningham (1937)	1,400	38 cm^3	35	20–23
Cardium edule (30–40 mm)	Willemsen (1952)	500	*c.*23 cm^3	22	17·3–19·5

Filtering Rates with other Organisms

Table III gives the list of organisms employed for comparative experiments on the filtering rate of *Lasaea*. Each was presented in unialgal culture at or slightly below the concentration of the laboratory culture, and the filtering rates obtained are shown in Table IV and Fig. 5, to which the range of values for experiments with *Phaeodactylum* has also been added for comparison.

As can be seen from Table IV a striking variation appeared in the filtering rates with various organisms, in particular with *Gymnodinium veneficum*, in which no sustained filtering is observed at all, and which has been shown to be toxic to fish, other molluscs (Ballantine, unpublished) and *Hemimysis lamornae* (Bainbridge, 1953, p. 393, as *Gymnodinium* II). Experiments were

therefore carried out on the effect of pre-treatment with *G. veneficum* on the subsequent filtering rate in *Phaeodactylum*. When placed in the toxic culture the *Lasaea* fail to aggregate or to put out the foot and siphon, but in spite of this shell-closure some of the toxin (which is present both in the cells and the

TABLE III. PHYTOPLANKTONIC ORGANISMS USED IN EXPERIMENTS ON FILTERING RATES OF *LASAEA RUBRA*

Plymouth culture no.	Organism	Classification	Cell size (μ)
85	*Chlorella stigmatophora* Butcher	Chlorophyceae	2·5–4·5
90	*Chromulina pusilla* Butcher	Chrysophyceae	1–2
B	*Dicrateria inornata* Parke	Chrysophyceae	3–5·5
28	*Exuviaella baltica* Lohm.	Dinophyceae	9–15
103	*Gymnodinium veneficum* nom. prov. Ballantine	Dinophyceae	9–19
102	*G. vitiligo* nom. prov. Ballantine	Dinophyceae	7–18
I	*Isochrysis galbana* Parke	Chrysophyceae	5–6
87	*Nannochloris atomus* Butcher	Chlorophyceae	2–3
104	*Peridinium trochoideum* (Stein) Lemm.	Dinophyceae	20–30
100	*Phaeodactylum tricornutum* Bohlin (=*Nitzschia closterium* f. *minutissima* of Allan & Nelson)	Chrysophyceae	20–40
97	*Prorocentrum micans* Ehr.	Dinophyceae	30–40

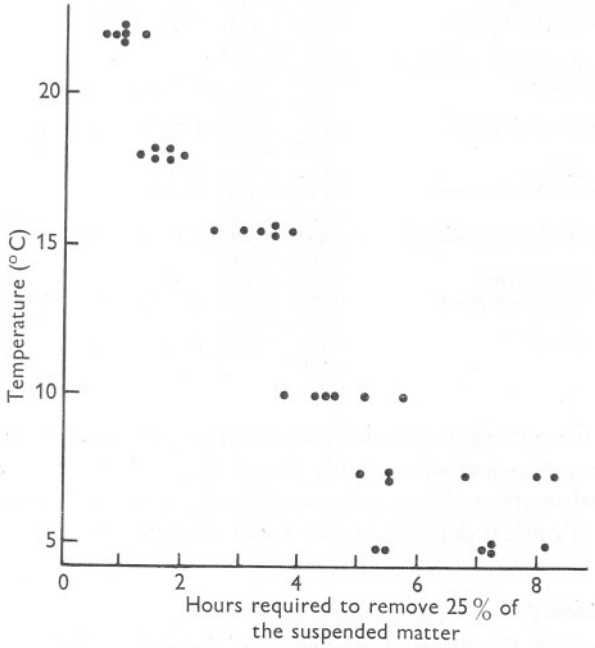

Fig. 4. The relation between filtering rate of *Lasaea* in *Phaeodactylum* and temperature. Filtering rate is expressed inversely as the time required (in hours) to remove 25 % of the suspended food under the experimental conditions. (Cf. Galtsoff, 1928, fig. 8.)

supernatant water) must enter the mantle cavity, and the extent of the retardation of subsequent filtering is roughly proportional to the duration of contact with the toxin (eventually nearly all animals appeared to recover). On removal from the toxic culture the experimental animals were washed for 30 min in several changes of filtered sea water, and full activity of foot and siphon was observed before placing them in *Phaeodactylum* culture. Control

TABLE IV. FILTERING RATES WITH VARIOUS ORGANISMS CALCULATED OVER A 3 H PERIOD

		(Expressed as ml./h/20 animals)	
Expt. no.	Organism (see Table III)	Rates for separate experiments	Average rate, calculated from mean density decrease
1–6	*Chlorella stigmatophora*	0·481, 0·426, 0·377, 0·326, 0·263, 0·226	0·346
7–12	*Chromulina pusilla*	2·120, 1·515, 1·490, 1·490, 0·910, 0·888	1·027
13–18	*C. pusilla*	0·860, 0·840, 0·766, 0·550, 0·487, 0·403	
19–24	*Dicrateria inornata*	0·449, 0·416, 0·294, 0·246, 0·270, 0·246	0·443
25–28	*D. inornata*	0·880, 0·643, 0·502, 0·491	
29–34	*Exuviaella baltica*	1·184, 0·994, 0·870, 0·860, 0·814, 0·814	0·902
35–40	*Gymnodinium veneficum*	No filtering	—
41–46	*G. vitiligo*	0·606, 0·426, 0·374, 0·370, 0·366, 0·223	0·384
47–52	*Isochrysis galbana*	2·181, 1·562, 1·338, 1·298, 0·688, 0·595	1·171
53–55	*I. galbana*	1·150, 0·941, 0·785	
56–61	*Nannochloris atomus*	0·436, 0·374, 0·354, 0·314, 0·297, 0·296	0·343
62–67	*Peridinium trochoideum*	0·492, 0·320, 0·192, 0·139, 0·176, 0·097	0·293
68–70	*P. trochoideum*	0·492, 0·387, 0·342	
71–76	*Prorocentrum micans*	1·129, 1·129, 1·129, 1·068, 0·860, 0·524	1·079
77–81	*P. micans*	1·365, 1·359, 1·230, 1·198, 0·780	

experiments were carried out with animals in sea water for a period equal to the exposure to *Gymnodinium*. Table V and Fig. 6 show the results obtained in these experiments, and Fig. 7 shows the end-points, referred to a base-line of 500 units of optical density, in two series of experiments and controls.

Filtering Efficiency

In dealing with the filtering efficiency of the gill, and the problem of the presence of a mucous sheet over the gill aiding in the retention of minute particles, several lines of approach were used. In sections of *Lasaea rubra*

stained with Alcian blue (see Steedman, 1950), or with mucicarmine or azan, mucous glands could not be detected in the epithelium of the filaments. This evidence is admittedly open to the objection that the contents of glands actually present might have been discharged during feeding immediately prior to fixation, but even on the gills of animals fixed while actually

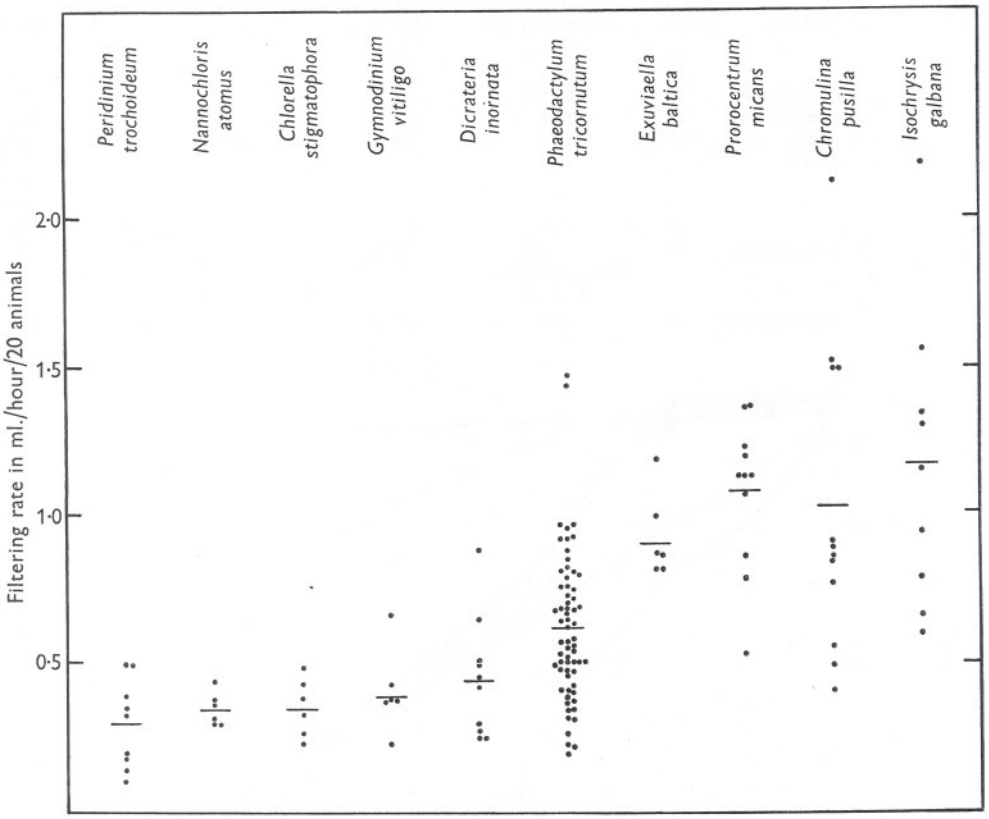

Fig. 5. The range of filtering rates exhibited by *Lasaea rubra* in all experiments with *Phaeodactylum* (Table I), and with the various organisms listed in Table III. The horizontal line on each range of values indicates the average filtering rate with that organism. *Gymnodinium veneficum* is omitted, as showing no measurable filtering rate in any experiment.

filtering 'Aquadag' no mucous covering could be detected by these staining methods.

Lasaea has also been shown to filter organisms and particles of size ranging from 1 to 50 μ (i.e. from *Chromulina pusilla*, marine bacteria and 'Aquadag' to particles of 'Kieselguhr'). In general, the size of the organism, like the concentration of the suspension, appeared to have no correlatable effect on the

TABLE V. EFFECT OF PRETREATMENT WITH *GYMNODINIUM VENEFICUM*

(Filtering rates of *Lasaea rubra* in *Phaeodactylum tricornutum*.)

Group no.	Pre-treatment	No. of expts.	Mean decrease in density in 3 h	95% confidence limits	Filtering rate (ml./h/20 animals): Filtering rate calculated from mean density decrease	Filtering rate (ml./h/20 animals): Extreme variations of filtering rate: Highest	Filtering rate (ml./h/20 animals): Extreme variations of filtering rate: Lowest
1	None	20	218·5	16·02	0·67	0·966	0·457
	None	23	223	28·11	0·69	1·436	0·314
	5 h in *Gymnodinium*	20	147·8	15·2	0·411	0·644	0·243
	5 h in *Gymnodinium*	20	143	27·71	0·396	0·937	0·113
	Variable time in *Gymnodinium*	18	151	28·6	0·42	0·921 (2 h in *Gymnodinium*)	0·065 (10 h in *Gymnodinium*)
2	None	19	169	31·6	0·48	0·963	0·189
	5 h in *Gymnodinium*	18	72·3	15·89	0·182	0·351	0·06
			Mean decrease in 10 h				
3	10 h in *Gymnodinium*	6	189	41·43	0·166	0·189	0·104

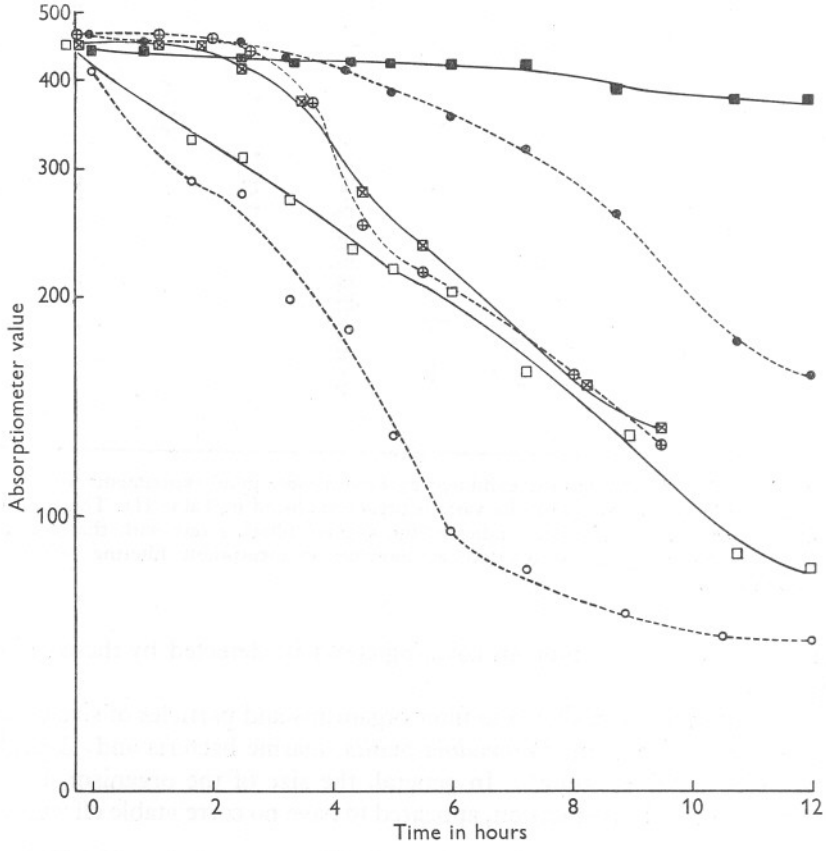

Fig. 6. The effect of pretreatment with *Gymnodinium veneficum* on the subsequent filtering rate of *Lasaea* in *Phaeodactylum*. □——□ and ○- - -○ are curves for control experiments; ⊠——⊠ and ⊕- - -⊕ for animals pretreated for 2 h in *Gymnodinium*; and ■——■ and ●- - -● for animals pretreated for 10 h.

filtration. This would indicate a high degree of filtering efficiency of particles down to 1 μ size.

With marine bacteria suspensions (Fig. 8) some filtration occurred in the first 20 min, but later ceased, probably because of lack of oxygen, and the possible toxicity of the bacteria themselves.

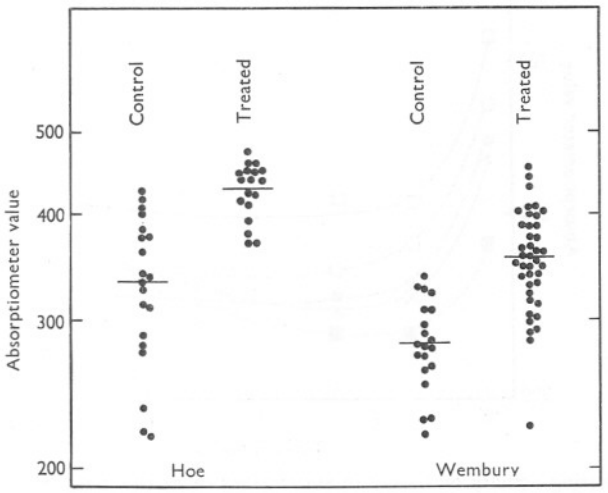

Fig. 7. The fall in optical density in 3 h of *Phaeodactylum* culture (from an initial base level of 500 absorptiometer units) in experiments using *Lasaea rubra* pretreated for 5 h with *Gymnodinium veneficum*, as compared with controls using *Lasaea rubra* from sea water. The horizontal line on each range is the average for that range. Two sets of experiments are recorded, and the higher level of the values obtained from Wembury is to be attributed to the use of animals larger than the average experimental size illustrated in Fig. 2A.

In addition to experiments using colloidal graphite ('Aquadag' 'c' and Graphite 's'), which gave rather variable results, work was carried out based on the results of Jørgensen & Goldberg (1953) on the removal from suspensions, by the gill of *Mytilus* and *Ostrea*, of large protein molecules of vertebrate haemoglobin, and *Haliotis* and crab haemocyanin. The greatest retention (25%) was obtained by Jørgensen & Goldberg with crab haemocyanin, with less than 10% retention in the other experiments. It was suggested that filtration by, or adsorption on, a mucous sheet was responsible for the uptake of these large molecules. Parallel cases are the reports of the survival of clams for periods of several months in filtered beef-tea, and the known ability of lamellibranchs to live for long periods in particle-free solutions of organic nutrients. A series of experiments was carried out on *Lasaea*, using sets of 20 animals in 5 ml. of a solution of *Arenicola marina* haemoglobin in sea water (Table VI). It is perhaps necessary to point out that the percentages quoted

in these results are not directly comparable with those of Jørgensen & Goldberg, as experimental conditions varied in ways not ascertainable from the results as presented.

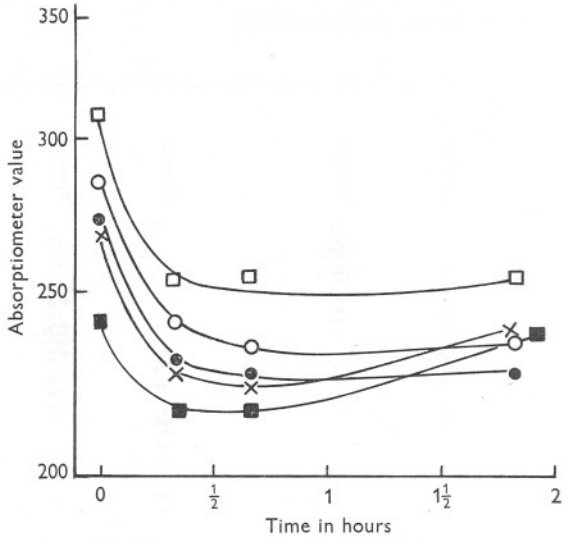

Fig. 8. The filtering activity of *Lasaea* in suspensions of marine bacteria. Filtering ceased after 40 min, and the slight increase in optical density in some of the experiments after 2 h is probably to be attributed to bacterial division and the resuspension of bacteria from released pseudofaeces.

TABLE VI. REMOVAL OF THE HAEMOGLOBIN OF THE BLOOD OF *ARENICOLA MARINA*, AFTER 12 H

Expt. no.		Readings at 415 mμ peak* 1st reading	2nd reading	% fall	Readings at 540 mμ peak 1st reading	2nd reading	% fall
1	20 *Lasaea* used in each expt.	1350	1150	15	—	—	—
2		1350	1150	15	—	—	—
3		1350	1275	5·5	210	195	9·3
4		1350	1275	5·5	210	180	8·6
5	Controls (No animals)	1350	1350	—	210	210	—
6		1350	1360	—	210	210	—

* A slight shift of peak between the first and second readings occurred both in experiments and controls, possibly due to oxidative changes.

Filtering, Feeding and Digestion

In experiments for the comparison of filtering, feeding and digestion rates (Tables VII and VIII), *Lasaea rubra* was found to ingest *Phaeodactylum* rapidly to the full capacity of the stomach. With the use of a thick suspension, the amount required to fill the gut is only a small fraction of the total filtered.

The surplus of the filtered material is extruded from the pallial cavity as loosely compacted pseudofaeces. The results obtained using cultures of *Phaeodactylum* show that after 2 h 40% of the material within the stomach was digested, after continuous heavy feeding up to the time of examination. The results from Table XIV indicate that within a short time the total food ingested may be submitted to digestion.

Inspection of the curves for the four experiments shown (Fig. 9A) reveals that the rate of filtering continued to increase over the 2 h experimental period. Since, however, the curve for each of the four experiments closely agrees with the curve obtained by plotting the end-points of Expts. 1–4, it is considered that this artificial curve gives an accurate picture of the conditions obtaining in any of the individual experiments. The larger value of the filtering rate towards the end of the experiments (Table VII), as compared with the values cited in Table I, may be ascribed to the greater number of large-sized *Lasaea* used in order to facilitate dissection of the gut. The shape of the curves,

TABLE VII. SYNOPSIS OF RESULTS IN EXPERIMENT ON FILTRATION AND FEEDING IN *LASAEA RUBRA*

Time from start	Filtering rate (calculated from fall in density of supernatant) (ml./h/ 20 animals)	Total no. of *Phaeodactylum* filtered	Total no. of *Phaeodactylum* in pseudofaeces (from counts and calibration curve)	No. of *Phaeodactylum* in stomachs of 20 animals
½ h	0·815	$0{\cdot}77 \times 10^6$	$0{\cdot}7 \times 10^6$	$0{\cdot}0725 \times 10^6$
1 h	1·100	$2{\cdot}99 \times 10^6$	$2{\cdot}06 \times 10^6$	$0{\cdot}93 \times 10^6$
1½ h	1·205	$6{\cdot}63 \times 10^6$	$3{\cdot}67 \times 10^6$	$2{\cdot}90 \times 10^6$
2 h	1·825	$13{\cdot}39 \times 10^6$	$8{\cdot}79 \times 10^6$	$4{\cdot}60 \times 10^6$

and the progressive increase in filtering rate, is probably due to the increased delay shown by these larger animals in acclimatizing themselves and resuming natural behaviour in the cuvettes. The total of digested cells can be seen to have risen steadily throughout the experiment (Fig. 9B), and the proportion of digested cells is probably rather greater than is apparent here, as some cells must have been completely digested, and have disappeared altogether. This number is not likely, however, to be large, as with experience even the faintest 'ghost' cells can be recognized, and the total time of the experiment was not sufficient for very extensive digestion. Fig. 10 sums up the evidence from these experiments in a single diagram.

Most recent opinion agrees that any sorting that occurs on the gills or labial palps of lamellibranchs takes place by size and weight of particles, and that 'selection on other grounds, as according to organic or inorganic content, or according to digestibility or unpalatability is out of the question' (Verwey, 1952). But since Fox (1936) earlier maintained that particles could be sorted according to their acceptability, and Loosanoff (1949) states that feeding *Ostrea* are capable of choosing between different flagellates, it was decided to

TABLE VIII. FEEDING AND DIGESTION IN A CULTURE OF *PHAEODACTYLUM*

(Starting time of experiment 10.00 h, 18 August 1954.)

		Total *Phaeodactylum* in stomach in thousands								
		Animal 1			Animal 2			Average		
Expt. no.	Time	Intact cells	Digested 'ghosts'	Total	Intact cells	Digested 'ghosts'	Total	Intact cells	Digested 'ghosts'	Total
1	10.30	4	—	4	3	0·25	3·25	3·5	0·12	3·62
2	11·00	37	8	45	28	10	38	32·5	9	41·5
3	11.30	82	40	122	134	40	174	108	40	148
4	12.00	102	80	182	172	106	278	137	93	230
5*	12.30	16	7·5	23·5	24	8	32	20	7·75	27·75

* The rate of Expt. 5 was noticeably slower than that of the other 4; the values at 12.30 h approximate most closely to those of Expt. 2 at 11.00 h. These figures (Expt. 5) have not been expressed in the graphs in Fig. 9.

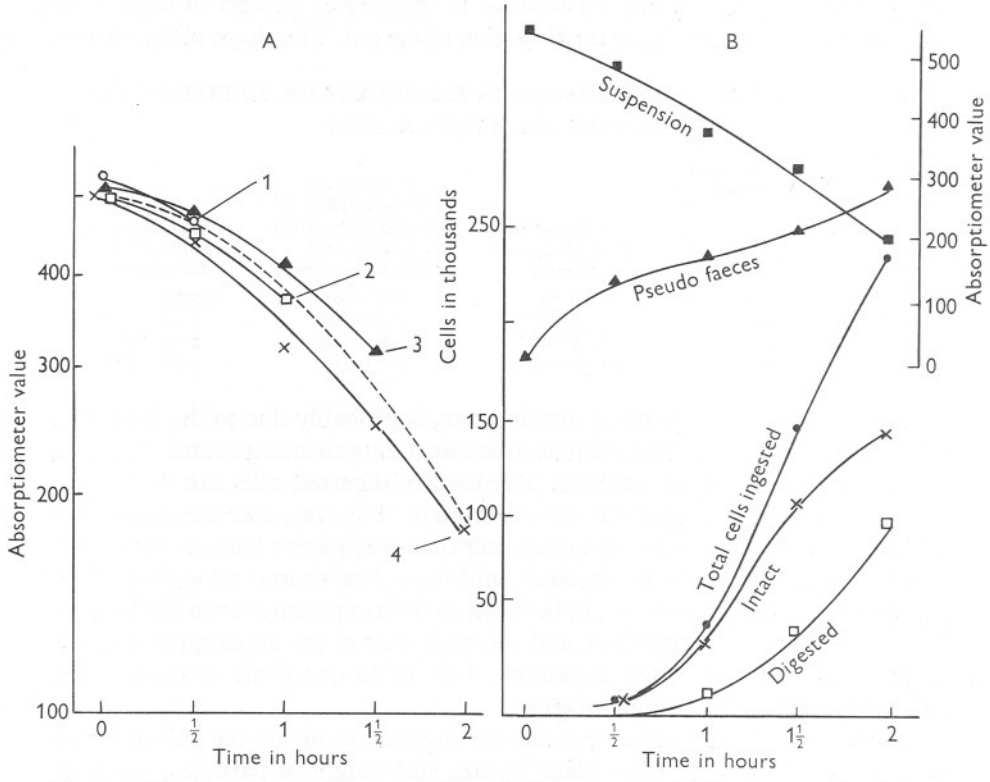

Fig. 9. A: the filtering rate of *Lasaea* in *Phaeodactylum* over 2 h in Expts. 1–4 referred to on p. 252. The broken line represents the hypothetical curve obtained by joining the points at which Expts. 1–4 were discontinued. B: above—the optical density of the suspension of *Phaeodactylum* and of the resuspended pseudofaeces, over 2 h, in the experiments referred to on p. 252; below—the numbers of cells (total, intact and digested) in the stomach of a single *Lasaea* at the same intervals (numbers estimated by haemacytometer counts).

carry out some experiments on discrimination in *Lasaea*. It seemed likely that, in a small lamellibranch able to digest naked or thin-walled organisms better than diatoms and or armoured dinoflagellates, any sorting mechanisms available for rejecting large or unsuitable organisms would operate very effectively. Experiments were set up with the following mixtures, each cuvette containing 20 *Lasaea*: (*a*) *Phaeodactylum tricornutum* / *Dicrateria inornata*; (*b*) *Peridinium trochoideum* / *Dicrateria*; (*c*) *Phaeodactylum* / *Chromulina pusilla*; (*d*) *Phaeodactylum* / *Thalassiosira fluviatilis*; (*e*) *Phaeodactylum* / 'Kieselguhr' (diatomaceous earth).

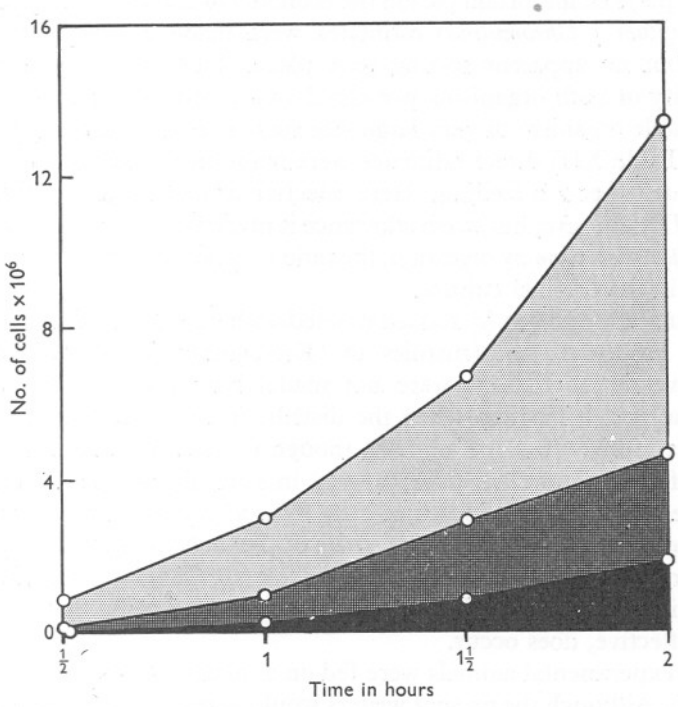

Fig. 10. Cumulative numbers of *Phaeodactylum* cells filtered, ingested and digested over a period of 2 h in the experiments referred to on p. 252. Black area represents digested cells, dark stipple the intact cells within the stomach, light stipple the cells rejected as pseudofaeces, and the unshaded area cells remaining unfiltered.

In (*a*) (Table IX) proportions of the two organisms in the supernatant were estimated before and after the animal had filtered, and, as might have been expected, no significant differences were found, i.e. no selection takes place between organisms as they enter the inhalant siphon. If any noxious or toxic substance is present the animal evidently ceases filtering and closes the shell (see p. 247, *Gymnodinium veneficum*). In (*b*) (Table X) estimates of the proportions of the two organisms were made in the original mixture and in the

resuspended pseudofaeces after 6 h feeding. This would give a measure of the sorting by the gills and labial palps, after filtration, but before entry into the mouth. There appears to be a significantly greater proportion of *Peridinium* in the pseudofaeces, and therefore some selection of *Dicrateria* in the food taken in. From experimental results below (Tables XII and XV) we know that *Peridinium* is indigestible, and, further, is much larger (25 μ) than *Dicrateria* (5 μ). There is thus the interesting possibility that sorting by size and weight may take place; it would be desirable, however, to do further experiments before concluding that this type of sorting between different organisms plays an important part in the economy of *Lasaea*.[1] In (*c*) (Table XI) (*Phaeodactylum* / *Chromulina*) estimates were made as in (*b*), after 10 h filtering, but no apparent sorting took place. This would agree with the acceptability of both organisms presented singly and with the absence of an easily sortable organism of very large size such as *Peridinium* in (*b*).

In (*d*) (Table XII) direct estimates were made from the stomach contents of the *Lasaea* after 2 h feeding. Here selection at first sight appears to be in favour of *Thalassiosira*, but when allowance is made for the total disappearance of some *Phaeodactylum* by digestion, the ratio may not be significantly different from that in the original culture.

In the final experiment (*e*) *Lasaea* was fed with a mixture of *Phaeodactylum* and large empty diatom frustules of 'Kieselguhr' (diatomaceous earth). Quantitative determinations were not made, but animals were fixed and sectioned after 2 h feeding, when the distribution of 'Kieselguhr' could be followed carefully. In some animals, though not all, the stomach contained particles of 'Kieselguhr' reaching 30–35 μ in size. By far the greater part of the ingested material was a mass of pure *Phaeodactylum*, while the rejecting areas of the gill margin, labial palps, sides of the foot and ventral edges of the mantle were heavily loaded with diatom fragments. It is clear that, in a heavy concentration of *Phaeodactylum* and large diatom fragments, sorting, though not fully effective, does occur.

Further experimental animals were fed on a mixture of *Phaeodactylum* and 'Aquadag'. Although the present writers would agree with MacGinitie (1941) that this substance produces unnatural feeding behaviour, there is evidently no barrier to its ingestion. Large masses of 'Aquadag' were found embedded in mucus in the stomachs of sectioned animals. The 'Aquadag' did not appear to disperse within the stomach, but to pass in a compact bolus directly to the intestine.

[1] The *Peridinium*/*Dicrateria* mixture was presented in 'thin' culture so that the fraction taken into the gut and that rejected in the pseudofaeces might be of comparable order of size. Thus any difference in the proportions of the two organisms ingested are unlikely to be obscured by a markedly greater bulk of pseudofaeces.

TABLE IX. PROPORTIONS OF *PHAEODACTYLUM TRICORNUTUM* AND *DICRATERIA INORNATA* REMAINING IN THE SUPERNATANT AFTER 6 H FILTERING

		Ratio	
		Phaeodactylum	: *Dicrateria*
Control at 10.15 h		1·59	1
Control at 16.15 h		1·67	1
After filtering till 16.15 h	(1)	1·65	1
	(2)	1·53	1
	(3)	1·76	1
	(4)	1·53	1
	(5)	1·46	1
	(6)	1·55	1
Average value for six experiments		1·58	1

TABLE X. PROPORTIONS OF *PERIDINIUM TROCHOIDEUM* AND *DICRATERIA INORNATA* IN THE RESUSPENDED PSEUDOFAECES AFTER 6 H FILTERING

		Ratios	
		Dicrateria	: *Peridinium*
Control before experiment		2·55	1
In pseudofaeces after 6 h	(1)	2·36	1
	(2)	1·88	1
	(3)	1·53	1
	(4)	1·43	1
	(5)	1·21	1
	(6)	1·51	1
Average value for six experiments		1·65	1

TABLE XI. PROPORTIONS OF *PHAEODACTYLUM TRICORNUTUM* AND *CHROMULINA PUSILLA*, IN THE RESUSPENDED PSEUDOFAECES AFTER 10 H FILTERING

		Ratios	
		Phaeodactylum	: *Chromulina*
Control before experiment		1·7	1
In pseudofaeces after 10 h feeding	(1)	2·1	1
	(2)	1·98	1
	(3)	1·49	1
	(4)	2·3	1
	(5)	1·92	1
	(6)	1·47	1
Average value for six experiments		1·88	1

TABLE XII. PROPORTIONS OF *PHAEODACTYLUM TRICORNUTUM* AND *THALASSIOSIRA FLUVIATILIS* IN THE STOMACH CONTENTS OF *LASAEA RUBRA* AFTER 2 H FEEDING

		Ratios	
		Phaeodactylum	: *Thalassiosira*
Control count of mixture before feeding		7·6	1
Ratio in stomach contents after 2 h feeding	(1)	5·5	1
	(2)	7·5	1
	(3)	6·5	1
	(4)	4·6	1
	(5)	7·5	1
Average value for five experiments		6·3	1

Digestive Activity

Estimation of the digestive powers of *Lasaea rubra* was approached in two ways. First, the enzymic activity of a centrifuged homogenate of the whole animals was tested, using methods cited in Stephenson (1939). From much previous work on the digestive powers of lamellibranchs (see Yonge, 1926; Fox & Marks, in Fox, 1936) it was thought safe to assume the presence of a protease, amylase and lipase among the enzymes of the digestive gland. This was verified by confirmatory spotting tests, and in addition a slowly acting pectinase was found (a wedge of carrot flesh was reduced to pulp after 7 days, control wedges remaining intact). Protein, in the form of dogfish blood corpuscles, was found after 2 h to have undergone extracellular digestion within the lumen of the stomach, as seen in stained sections of *Lasaea*. After 12 h no result was obtained with the absorptiometric determination of cellulase activity (see Stephenson, p. 316) as used by Newell (1953) with *Ostrea*.

Secondly, the stomach contents of *Lasaea* were examined at intervals after feeding with possible natural foods. Experiments of the classical kind, using artificial 'type' substrates, such as cotton-wool, filter-paper and fibrin, in incompletely controlled conditions *in vitro*, seemed less likely to give an exact picture of the animal's natural digestion. The condition of the gut contents at stated times after feeding with the flagellates *Isochrysis galbana* and *Peridinium trochoideum*, and with *Phaeodactylum tricornutum* and *Thalassiosira fluviatilis* is shown in Tables XIII–XV. These results show a rapid and complete digestion of *Isochrysis* and *Phaeodactylum*. Whatever the true structure of the wall of *Phaeodactylum* (see Wilson, 1946, and Hendey, 1954) it is evidently very easily attacked by digestion to release the protoplasmic contents of the cell. The majority of ingested *Phaeodactylum* had become 'ghosts' in 45 min (Table XIV). Easy digestion of these two organisms is accompanied by rapid filtration, and they seem likely to be a nearly ideal food.

By contrast with these easily digested organisms we have the results using a heavily walled diatom, *Thalassiosira*, and an armoured dinoflagellate,

Peridinium. *Peridinium* is not digested, nor is this surprising, as its digestion would require a cellulase, which *Lasaea* lacks. Further, there is a correlation between low filtering rate and indigestibility. With *Thalassiosira* it was found difficult to make reliable absorptiometric measurements of uptake by *Lasaea*, as its normal habit in thick culture is to lie in a sludge close to the bottom of

TABLE XIII. CONDITION OF THE GUT CONTENTS OF *LASAEA* FED WITH VARIOUS ORGANISMS

(Examined in sectioned and stained material.)

Specimen no.	Feeding	Gut contents
1	1 h in *Peridinium trochoideum*	*Stomach*—cell walls of *Peridinium* all intact, many organisms still containing organized stainable protoplasm, somewhat shrunk due to fixation. No digestion. *Intestine*—well recognizable cell walls with contents. No digestion
2	1 h in *P. trochoideum*	*Intestine*—little evidence of digestion. Cell-wall plates still clearly recognizable
3	2 h in *Phaeodactylum tricornutum**	*Stomach*—filled entirely with 'ghost' cells. In a few cells a bluish staining material (azan), probably leucosin rather than protoplasm, remains (see Hendey, 1954). Digestion complete. *Digestive gland lumen*—entirely filled with 'ghost' cells in same condition as those in stomach. *Intestine*—'ghost' cells even more eroded than in stomach. Digestion complete
4	2 h in *Isochrysis galbana*	*Stomach*—no trace of organized protoplasm or intact cells. Digestion complete
5	2 h in *I. galbana*	*Stomach*—digestion complete as in (4)
6	2 h in *Thalassiosira fluviatilis*	*Stomach and intestine*—only a few cells digested, majority intact
7	2 h in *T. fluviatilis*	*Stomach and intestine*—little digestion. Some cells appear empty, but there is little breakdown of cell contents, even in faeces. Cell walls are always clearly defined. Digestion is apparently very slow, and the majority of cells are passed in faeces before digestion
8	2 h in *T. fluviatilis*	Less digestion than in (7). Almost all the cells show clearly defined protoplasmic contents
9	2 h in *T. fluviatilis*	*Stomach*—contained relatively few diatoms, showing only slight traces of digestion
10	2 h in *T. fluviatilis*	*Stomach*—diatoms almost all undigested. *Intestine*—faeces formed of compacted masses of cells, mostly quite undigested

* See also Table VIII, p. 254, and Table XIV, p. 260.

the vessel, though it is easily stirred into suspension and can thus become available for short periods for filtering. It is found that *Lasaea* can slowly digest *Thalassiosira*, presumably with the aid of the pectinase in the digestive gland, which would aid in separation of the siliceous valves. The process is, however, very slow, and it seems likely that diatoms are not in nature a good or sufficient food.

TABLE XIV. CONDITION OF THE STOMACH AND INTESTINE CONTENTS OF *LASAEA RUBRA* AFTER FEEDING WITH *PHAEODACTYLUM TRICORNUTUM*

(Animals fed on *Phaeodactylum* for 3 h, then placed in filtered sea water. Stomachs were dissected from living animals, contents diluted to 0·2 ml. with sea water and counts made.)

Animal no.	Time after feeding			Counts of cells in 0·1 mm³, after dilution				Total in stomach in thousands	Remarks
1	45 min	Stomach	Whole cells	48	56	28	32	82	Most of the 'ghost' cells heavily eroded, visible only with difficulty
			'ghosts'	64	76	80	64	142	
		Faeces	Whole cells	24	44	56	76	—	Also much detritus remaining from before feeding with *Phaeodactylum*
			'ghosts'	28	52	56	76		
2	1 h 20 min	Stomach	Whole cells	16	8			24	—
			'ghosts'	30	20			50	
		Faeces	Whole cells	0				—	Faeces with large no. of flagellates and detritus, in addition to *Phaeodactylum*
			'ghosts'	4					
3	1 h 45 min	Stomach	All digested to an advanced stage			—		32*	No cells remaining with contents and 'ghosts' much further digested than in 1 and 2—only visible with difficulty
4	2 h	Stomach	Whole cells	2	4			41*	—
			'ghosts'	18	17				
5	2 h 15 min	Stomach	Whole cells	6	7			75*	—
			'ghosts'	28	34				
6	2 h 30 min	Stomach	Whole cells	5	6			51*	'Ghost' cells as fully digested as possible while still remaining visible
			'ghosts'	22	18				

* 'Ghost' and whole cells not estimated separately in total in stomach.

DISCUSSION

Filtering Rates

Although all of the animals, so far as could be detected, kept their valves open and siphons out for most of the experimental period, an important cause of the variations in filtering rate recorded must have been the temporary cessation of activity of some of the animals. An attempt has been made to reduce these variations as far as possible by using 20 animals in each experiment and performing as many experiments as was practicable. It is also thought probable that the highest filtering rates have the greatest significance, and approximate most closely to a natural filtering rate in the field. On this basis, the filtering rate of *Lasaea* in *Phaeodactylum* compares closely with that of an adult (74 mm) *Mytilus californianus*, as estimated by the indirect method of Fox *et al.* (1937) (see Table II). A similar order of values was reported by Willemsen (1952) in adult *Cardium edule* and *Mytilus edulis* by a method very closely comparable to the one used here. Smaller specimens of *Mytilus edulis* have shown a faster

TABLE XV. CONDITION OF THE STOMACH CONTENTS OF *LASAEA RUBRA*, AFTER FEEDING WITH *PERIDINIUM TROCHOIDEUM* AND *THALASSIOSIRA FLUVIATILIS*

(Stomachs were dissected from living animals, contents diluted to 0·2 ml. with seawater, and counts made.)

1. *Peridinium trochoideum*

Animal no.	Fed (h)	Cells	Counts of cells in 1 mm³ after dilution	Estimated total cells in stomach
1	1	Cells with walls and contents intact	9 6* 2 4	1050
		Cells empty	– – – –	0
2	1	Intact	5 1 4 1	550
		Empty	– 1 1 –	100
3	1	Intact	1 1 – –	*c.* 100?
		Empty	– – – –	0
4	1	Intact	4 1 1 –	300
		Empty	– – – –	0
5	2	Intact	7* 4* 4 2	850
		Empty	– – – –	0
6	3½	Intact	4 1 1 –	300
		Empty	– – – –	0
7	3½	Intact	5 6 3 4	900
		Empty	– – – –	0

* Some cells showing signs of rupture of the wall.

2. *Thalassiosira fluviatilis*

Animal no.	Fed (h)	Cells	Count of cells in 1 mm³ after dilution	Estimated total cells in stomach
1	1	Frustules with contents intact	2 2 – 1	250
		Frustules empty*	1 – – –	50
2	1	Intact	1 1 1 –	150
		Empty	– – 1 –	50
3	2	Intact	1 – – –	Very few taken into stomach
		Empty	– – – –	
4	2	Intact	2 1 – 1	200
		Empty	– 1 – 1	100
5	2½	Intact	4 2 – –	300
		Empty	– 2 – –	100
6	2½	Intact	2 2 – 3	350
		Empty	2 1 – 3	300
7	4½	Intact	4 3 2 1	500
		Empty	– 2 1 1	200
8	4½	Intact	4 3 3 2	600
		Empty	5 4 1 1	550
9	4½	Intact	7 3 6 6	1100
		Empty	2 3 1 2	400
10	16	Intact	1 – 1 1	150
		Empty	1 – – –	50
11	16	Intact	– 1 1 1	150
		Empty	1 – – –	50
12	16	Intact	6 3 1 –	500
		Empty	1 1 1 –	150
13	16	Intact	1 1 – 1	150
		Empty	– 2 1 –	150

* Sample counts of original thick culture for proportion of empty frustules: intact frustules 1:5.

rate, Jørgensen's figures giving ratios of *c.* 200 and 150 with animals 15 and 30 mm long respectively. Jørgensen appears to have calculated rates over relatively short periods of up to 1 h. All the *Lasaea* calculations are, however, for rates uniformly sustained for at least 3 h. The high initial rate almost always obtained by Jørgensen with single *Mytilus* was never observed in *Lasaea*. Much higher values were also twice reported with oysters, when the direct method of measuring water pumped out of the exhalant aperture of the pallial cavity was used. Here, if the volume of the animals has been estimated correctly in Table II, the ratio is considerably higher than with the indirect method of measuring filtration.

Control experiments were carried out to determine whether variations in the density of the culture had any effect on the filtering rate. Loosanoff & Engle (1947) suggest that in conditions of high turbidity, feeding, as distinct from filtering, might cease in oysters. Variation in concentration of *Phaeodactylum* produced no significant difference in filtering rates, though in dense cultures the output of pseudofaeces is markedly increased. In the experiments with various flagellates recorded above, no correlation could be found between initial density of the experimental culture and the rate of filtering. It seems likely that a high filtering rate can be maintained at the highest densities used in these experiments, as will be seen from the values with *Chromulina* and *Isochrysis*, which were both used in thick cultures. On the other hand, a thin *Prorocentrum* culture was filtered almost as rapidly as the dense *Isochrysis*. Thus, with pure suspensions of food organisms, the density of the suspension appears to be without effect on the filtering rate.

Of the variations in filtering rate recorded with different organisms, perhaps the most striking result was obtained with the naked dinoflagellate *Gymnodinium veneficum*, in which no sustained filtering was observed at all. The remaining organisms may be arranged—by comparison with *Phaeodactylum*—in two groups. First, *Nannochloris atomus*, *Chlorella stigmatophora*, *Peridinium trochoideum* and *Dicrateria inornata* were filtered at rates significantly lower than that for *Phaeodactylum*. With each of the remaining flagellates, namely *Isochrysis galbana*, *Exuviaella baltica*, *Chromulina pusilla* and *Prorocentrum micans*, the average rate was faster than that for *Phaeodactylum*. The most rapidly filtered organism in any of the experiments was *Isochrysis galbana*, the fastest rate with this flagellate being 2·181 ml./h for 20 animals. The fastest rates with *Chromulina pusilla*, *Exuviaella baltica* and *Prorocentrum micans* were respectively 2·12, 1·184 and 1·365.

It will have been noted that micro-organisms were always presented to the animals at concentrations greatly exceeding those ever present in the sea; and the criticism might be made that the experimental results establish nothing more than the reaction of *Lasaea* to a series of unialgal cultures at artificially high concentrations. There are, however, several indications that the preferences disclosed may have some reality under natural conditions. Thus, with

the toxic *Gymnodinium veneficum* no filtering was obtained, and with *G. vitiligo* and *Chlorella stigmatophora*, both suspected by Bainbridge (1953) of mild toxicity, low filtering rates were recorded. However, *Dicrateria* and the minute *Nannochloris*, which also gave low rates, have never been suspected of toxicity. A recent report by Pinto (1953) states that *Exuviaella baltica* and *Prorocentrum micans* have both been found toxic in 'red water', but in our experiments using the Plymouth strains of these organisms we have found no evidence of toxicity. The filtering rates with these organisms were among the highest obtained.

Of the other flagellates filtered rapidly by *Lasaea rubra*, *Isochrysis galbana* has repeatedly been found highly acceptable as food to cultures of filter-feeding animals. *Chromulina pusilla*, a species also filtered rapidly, is probably identical with the 1–2 μ component of the phytoplankton that evidently contributes heavily to the natural food of *Lasaea* (Table XVI). With *Peridinium trochoideum*, on the other hand, there appears to be a correlation between low filtering rate and lack of digestibility (see Table XV).

Any differences in pumping-rate resulting from the different sizes of the organisms, and consequent higher efficiency of filtering large organisms, appear to be unlikely. Small *Chromulina* (1–2 μ) were in fact filtered rapidly, large *Peridinium* (25 μ) slowly. Nor could any correlation be found by plotting the sizes of each of the various species of flagellates against the respective filtering rates obtained.

No figures appear to exist for exact comparison of the filtering rates of other lamellibranchs with different types of flagellates. Jørgensen (1949) carried out experiments with *Mytilus*, using, in addition to *Phaeodactylum*, the flagellates *Dicrateria inornata* and *Isochrysis galbana*. He used single specimens of *Mytilus* of lengths 1·5, 2·9 and 3·2 mm, but his experiments, being not primarily directed towards this point, appear to be too few in number, and the results to fluctuate over too wide a range, to allow significant correlation of feeding rate with type of organism.

Further experiments might well be carried out on filtration rates with various organisms, and on possible mechanisms of discrimination. Bainbridge (1953) studied the effects of various phytoplanktonic organisms on planktonic animals, but little seems to have been done on the relation of sedentary benthic animals to particular constituents of the phytoplankton. With *Lasaea* itself nothing is yet known of the way in which the presence of a particular kind of flagellate exerts its effect on filtering rate. There may be different effects on the rate of beat of the gill cilia, different degrees of contraction of the gill mesh, or variations in the proportion of time engaged in filtering. It is hoped to carry out further experiments on some of these points with the use of a larger bivalve such as *Mytilus*. Of particular interest as a first study would be the direct observation of the action of various phytoplankton cultures on ciliary beat.

The Filtering Efficiency of the Gill

Jørgensen (1949) and Jørgensen & Goldberg (1953) discuss the mechanism of collection and transport of food particles by the lamellibranch gill, and whether this takes place by a continuous mucous sheet moving over the gill surface, as suggested by MacGinitie (1941), or with the aid of only a small amount of mucus. Using *Mytilus edulis*, Jørgensen (1949) found that, under certain conditions, graphite particles were filtered down to 1–2 μ in size, and at other times the majority of the $< 5\,\mu$ particles were rejected. He explains these differences by the presence or absence of a 'mucous net' constituting a fine filter upon the surface of the gill. When feeding takes place by this net, particles become intercepted by the mucus and little or no selection according to particle size is possible. Under certain conditions, however, the smaller sizes of graphite particles are allowed to pass through the gill ostia in the absence of a mucous net, at which times filtration will take place merely by the gill ostia or the efficiency of the straining cilia. Later Jørgensen & Goldberg (1953) found retention in *Ciona* and in *Mytilus* of graphite particles of 1–2 μ. In *Crassostrea virginica*, 2–3 μ particles were retained, while 1–2 μ particles appeared to pass through the gill. It was assumed that in *Ciona* and *Mytilus* a mucous net contributed to food collecting, after the manner described by MacGinitie, and that in *Crassostrea* the filtering efficiency of the gill was decreased in the absence of mucus.

Our experiments with *Chromulina pusilla* (1–2 μ diameter) and with bacteria showed no evidence of a falling off in efficiency of filtering in *Lasaea* with smaller particle size. With one exception, the 5 μ flagellate *Isochrysis galbana*, the rates with *Chromulina* were the highest recorded. The great potential value of these small and unarmoured forms seem to necessitate a high retentive efficiency of the gill, since it is likely that *Lasaea* feeding in nature must derive a high proportion of its food from abundant flagellates of this size (see Table XVI).

Jørgensen (1949) found evidence that *Mytilus* at times rejected 'Aquadag'. In general *Lasaea rubra* took up 'Aquadag' very vigorously over experimental periods of 5 h, sometimes at a rate as fast as any recorded with flagellates. Freshly made up 'Aquadag' gave contradictory results. In one set of experiments, the same effect was obtained as by Jørgensen & Goldberg (1953); 'new' 'Aquadag', made up for not more than 1 h was poorly filtered, while 'old' 'Aquadag' that had been left to stand for 7 h before use was filtered more than twice as fast. Jørgensen and Goldberg ascribed this effect to the tendency of particles to form larger aggregates on standing. In *Lasaea* the efficient filtering of the smallest particles of 'Aquadag' is probably not conclusive evidence of the presence of a mucous net; and it seems that bivalves may be very differently sensitive at different times to stimulation or irritation by graphite. This irregularity contrasts with the constant and easily

reproducible filtering rates obtained in *Lasaea* with suspension of natural foods.

The mesh size of the gill is by no means easy to determine in fixed and contracted sections. In *Lasaea rubra*, moreover, neither the secretion of a mucous net nor the contraction of the gill ostia may in fact be necessary to retain small flagellates. A far more efficient means of entrapping particles at the frontal surface seems to be the fine fringes of eu-latero-frontal and pro-latero-frontal cilia, which are very highly developed in *Lasaea* (see the papers of Atkins, 1936, 1938, for lamellibranchs in general; and Oldfield's recent work, 1955, on *Lasaea rubra*). In previous discussions about the mucous net, the presence of these filtering cilia has been largely ignored, only Verwey (1952) having called attention to their use. Further, there is the strong objection to the mucous-net theory, that such a continuous sheet covering the gill would impede or prevent the action of the elaborate systems of sorting cilia. Jørgensen (1949) has recognized this difficulty; no one has satisfactorily answered it. However valuable MacGinitie's work in this field may be, his interpretation fails to reconcile the freedom of action of the sorting cilia with the employment of a continuous mucous net. It seems at least that efficient filtering can be carried out in the absence of a mucous net, and that the greatest stimulus to mucus secretion when it occurs may be the presence of large amounts of indigestible debris. Certainly, the lack of obvious mucus in many lamellibranchs, which sort particles on the gills and palps, is very striking in comparison with the large amounts of mucus in ciliary-feeding gastropods (Yonge, 1938), *Amphioxus* and ascidians (Orton, 1914), none of which have highly developed sorting mechanisms on the gill surface.

Tammes & Dral, in a recent paper (1955), make a further criticism of McGinitie's theory of an 'ultra-sieve' and take the view that material may be retained by the gill, by a mechanism of adhesion to sticky cilia.

Comparison of Filtering, Feeding and Digestion

In a lamellibranch living in a high concentration of suspended material, it is important to distinguish three separate activities, filtering, feeding and digestion; and the rate of any of these functions will not necessarily be deducible from that of any other. It may be assumed that the formation of pseudofaeces in thick cultures is a consequence of the mechanical overloading of the gills and palps with a greater accumulation of particles than can be received into the gut (see Popham, 1940, for details of rejectory mechanisms of the mantle cavity). Probably because of the great quantities filtered, the pseudofaeces of *Lasaea* were not compacted in strings as in *Mytilus* and *Ostrea*, but lay in a thick sludge, surrounding each cluster of bivalves, removed a little way from the animals by the force of the exhalant current. True faeces were produced in very much smaller amount than pseudofaeces, and had the form

of compact oval pellets bound with mucus. Even in the thickest cultures that could be measured by the absorptiometer, it is unlikely that more than a very small fraction of organisms of *Phaeodactylum* size pass through the gill unfiltered; and in the present calculations this has been neglected and complete filtration assumed.

References in many papers to 'feeding' in lamellibranchs must clearly be interpreted as no more than 'filtering'. 'Food' is that which enters the mouth. Often, however, especially in experiments with 'thin' cultures, and probably as a general rule in natural sea water, feeding may approximate closely to filtering. It is also unsafe to speak of 'feeding' as equivalent to 'digestion'. Sometimes, such as in experiments using cultures, the amount of food digested may—with sufficient elapse of time—correspond to that ingested. But in other conditions, and probably in most situations in nature, much material can be shown to be ingested that will never be digested, either by reason of its own nature, or because of the overgorging of the animal's gut.

In several features *Lasaea rubra* appears to present an exception to the classical picture established by Yonge (1926) and later investigators of lamellibranch digestion. These aspects will be more fully discussed in a forthcoming paper. First, there is an entire lack of amoebocytic action within the stomach at any stage of digestion. Secondly, a large amount of extracellular digestion of phytoplanktonic organisms takes place within the stomach, before the products are absorbed, presumably for completion of digestion, by the digestive gland. In so far as this may involve proteolytic enzymes, it is thought that these must originate from the spheres passed into the stomach by holocrine fragmentation of the digestive cells. Probably in these features, as in much else, *Lasaea* is to be regarded as an exceptional lamellibranch, specialized by its small size and as a result of its 'difficult' habitat. It would certainly be unsafe to draw from it at this stage too many general conclusions about the nature of lamellibranch digestion.

The Animal in Nature: Filtering Rate and Feeding Efficiency

From several considerations it seems justifiable to regard the filtering rates observed with *Phaeodactylum* and some of the flagellates as not falling significantly short of the average natural rate. The animals used displayed normal visible activity and posture, and this was taken to imply normal filtering activity with little disturbance by experimental conditions. The relation of the volume of water filtered per hour to the volume of animal showed impressive agreement with figures recorded for other lamellibranchs (see Table II). Further, the filtering rate was found to vary predictably with temperature, with different kinds of flagellates, and in some cases with position on the shore (see below p. 271). It was at least true that variations due to such factors as these were not masked by depressing factors operating as the result of laboratory conditions.

It was also thought safe to assume that there is continuous filtering during the rather short period (*c.* 3 h) that upper tidal *Lasaea* are normally submerged on the shore. In experiments lasting 8–10 h, little falling off in the rate of activity was observed.

The quantity and composition of material suspended in natural sea water, varies enormously with the locality, the phases of the tide, the season of the year and many other factors. Verwey (1952) provides a good dis-

TABLE XVI. ESTIMATES OF AMOUNTS OF LIVING ORGANISMS AND SUSPENDED MATTER IN WATER SAMPLES FROM ROCKS BELOW PLYMOUTH HOE

(*a*) From surface sea water, a foot above the substratum (12 August 1954) and (*b*) from *c.* 1 cm above the surface of the substratum (20 September 1954).

	Counts per 1 mm^3 natural sea water							
	(*a*)				(*b*)			
Organisms under 2 μ	60	48			27	38	37	29
Organisms 2–5 μ	1	2			3	4	3	3
Organisms 5–10 μ	–	3			1	–	–	2
Totals	61	53			31	42	40	34
Over 10 μ	Much unidentifiable organic debris, sand grains and detritus				*Skeletonema*; several naviculoid diatoms; 3 chrysophycean flagellates; 1 euglenoid; fragments of *Enteromorpha* and '*Ectocarpus*'; and much unidentifiable debris			

cussion of the causes of such variation. The figures provided in Table XVI were obtained by haemacytometer counts of centrifuged sea water in which *Lasaea rubra* was naturally feeding, on the Hoe Front at Plymouth. Two such counts are given, (*a*) from sea water taken at the surface on an incoming tide, about a foot above the rocky bottom, and (*b*) from sea water taken by pipetting from approximately 1 cm above the surface of the rock. In composition and number of organisms these figures may be regarded as representative of water samples from near the substratum in the late summer. No information is at present available for other times of the year.

Any assessment of the annual food intake and the growth and metabolic requirements of *Lasaea* must await further work than is recorded here. We would now do no more than point out that, if we assume the late summer figures for density of phytoplankton given in Table XVI to approach a representative average for 12 months, and if we take the rate of filtering derived in this paper as coming close to the natural one in the field, then—working from living flagellates and diatoms alone—we find an annual food intake of no more than *c.* 0·07 mg for an animal weighing 1·0 mg. This figure, which—as was found by Fox & Coe (1943) with similar calculations for *Mytilus*—is obviously

inadequate, neglects other important sources of food. There is first the considerable amount of organic detritus, revealed in Table XVI and in analyses of stomach contents, that must be stirred into suspension during feeding, and which it is difficult to estimate even in samples from near the bottom. While it is unlikely that *Lasaea* augments its suspended food by sucking up detritus directly from the bottom (neither the gill nor the inhalant siphon show any of the adaptations characteristic of deposit feeders—see Yonge, 1949), particles of debris must be constantly stirred into temporary suspension, and will appear in large amounts in the food of a suspension feeder. There is further the rich spring outburst of phytoplankton, at which time the greatest growth of *Lasaea* must occur; there is also the enormous crop of gametes liberated by intertidal algae, which may densely cloud the water for short periods. Finally, the effective filtering time must often be extended by the lodgment of splash in crevices containing *Lasaea*. Even two or three drops of splash may be enough to stir up significant quantities of detritus from the substratum and make it available for filtering. The smaller the amount of splash—within reasonable limits—the greater will be the concentration of the suspension filtered.

An analysis of the stomach contents of *Lasaea rubra* feeding on the Hoe Front (see Table XVII) is quite comparable with previous reports of food ingested by lamellibranchs (see, for example, Fox & Coe, 1943; Coe, 1947). There is obvious difficulty in finding traces of minute or naked flagellates even a short time after their reception into the stomach, and the total of these ingested is best assumed to be the total amount present in, and filtered from, natural sea water. Their digestion by the animal is probably rapid and complete (see Table XIII). In addition, the stomach often contains diatoms sometimes of large size, up to 50 μ, either intact and little digested, or as open or broken frustules. Sponge spicules and siliceous spines of various kinds are a prominent item. Much organic detritus is also present, amorphous in nature, and almost always unidentifiable. In *Lasaea*, as in other lamellibranchs, it forms a normal part of the material taken into the stomach.

On the digestibility of organic debris it is difficult to assemble exact evidence. We have relied here on a careful examination of stomach contents and faeces in stained serial sections. Massive debris almost always appeared much less plentiful in the faeces than in the stomach contents. Faecal pellets were composed chiefly of fine broken spicules, occasional inorganic fragments such as small sand grains, and large diatom frustules, either empty or with stainable contents. It seems that some at least of the large fragments of organic detritus taken into the stomach are broken down by extracellular enzymes and perhaps assimilated. Some breakdown has probably taken place outside the animal by bacterial or autolytic action, and some of the products of this must be available to the animal. *Lasaea* is probably, however, without power to initiate the breakdown of cellulose walls (see results of the enzyme

experiments, and experiments with *Peridinium trochoideum*, p. 259). Of diatoms, the experiments with *Thalassiosira* indicate that digestion is slower and sometimes incomplete; certainly some of these, as well as armoured dinoflagellates, must pass through the gut intact. The question of the availability of detritus to *Lasaea* is an important one, on which it is hoped to make further and more critical experiments.

TABLE XVII. EXAMPLES OF THE GUT CONTENTS OF TYPICAL *LASAEA RUBRA*, COLLECTED FROM ROCKS BELOW PLYMOUTH HOE AFTER SEVERAL HOURS' FEEDING

(Examined in fixed and stained serial sections.)

Specimen	Region of gut	Remarks
1	Stomach	Large numbers of small, unidentifiable organisms, corresponding in size to the 1–2 μ flagellates of sea water. A number of broken spicules as of sponges. A diatom frustule, *c.* 20 μ, with no contents. A number of frustules of small naviculoids. Little trace of detritus, or unselected bottom deposits
	Intestine	A number of larger, inorganic particles, up to 30 μ in size, evidently rejected by the sorting area into the intestine
2	Stomach	A large amount of unorganized and unidentifiable detrital material. Maximum size of particles, *c.* 20–30 μ. A good deal of organic (or colloidal) material, staining pink with azan
	Digestive diverticulum	No large or identifiable particles
	Intestine	Contents similar to stomach, including some large, irregular particles and many small refractile specks
3	Stomach	Many particles of amorphous, azan-staining, (? organic) debris, particle size 30 μ and less. Several frustules of diatoms, empty, 10–15 μ
	Intestine	Diatom frustules, and several opaque, inorganic particles
4	Stomach	Large numbers of undetermined spicules, as of sponges, with traces of broken diatom frustules. Small, highly refractile, inorganic particles. A large *Coscinodiscus* of *c.* 50 μ diameter, with stainable contents, apparently not digested
	Digestive diverticulum	No identifiable particulate contents
	Intestine	Spicules, detrital particles, and fragments of inorganic material up to 15 μ across. A large *Coscinodiscus*, *c.* 50 μ across, with the contents not digested

Ecology: Intertidal Distribution

Morton (1954), in a paper on the animals inhabiting intertidal crevices, has briefly discussed the ecology of *Lasaea rubra*. The species extends essentially throughout the intertidal zone, except the uppermost *Littorina neritoides* fringe. Its upper limit at Plymouth is at about the top of the *Chthamalus* barnacle zone, and it ranges downwards to low-water mark, as was pointed

out by Glynne-Williams & Hobart (1952). Morton has recorded that at Wembury it may just reach crevices in the zone of black lichen, *Verrucaria maura*, commenting that 'its presence here is a remarkable achievement for a lamellibranch relying on suspension feeding'.

There is in general a good correlation of shell size with length of time of

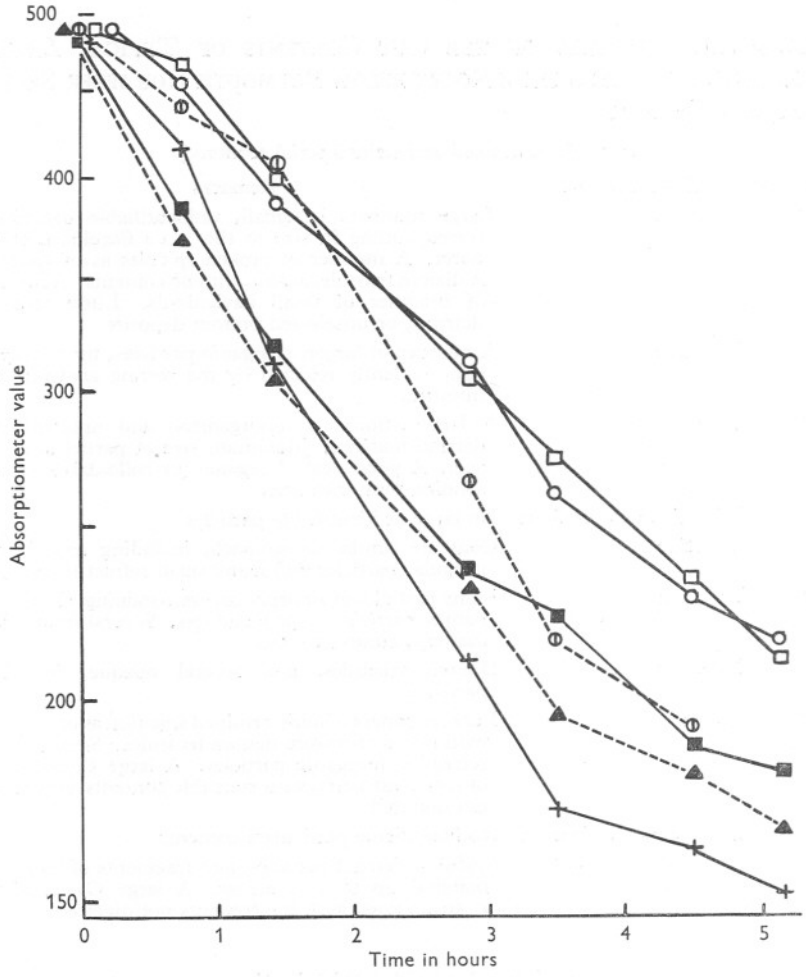

Fig. 11. Filtering rates in *Phaeodactylum* of three series, each of 10 large *Lasaea rubra*, from near low-water mark (location 1), and from the upper limit of the range (location 5–6), at Wembury. □——□, ○——○ and ◑– –◑ are from the lower level, and + ——+, ■——■ and ▲– –▲ from the upper level. The filtering rate in the high tidal series is initially greater, becoming reduced to that of the upper series after *c.* 3½ h. Of the low-tidal series, ◑– –◑ exhibited a higher filtering rate than the remaining two, reaching the same performance as the high tidal series after 1½ h.

submersion. The largest size (3·0 mm) is reached at the lower tidal positions (Morton's locations 1 and 2); and the smallest adult shells (never more than 2 mm in length) are found in tufts of *Pygmaea*, much higher on the shore (location 8). The latter habitat seems to be the most harsh which this species has to tolerate. The percentage exposure between tides may exceed 80, and at Wembury, the areas of *Pygmaea* on sun-exposed rock faces become desiccated and brittle between tides during summer. The shells of *Lasaea* become entirely dry. Yet in spite of the apparent difficulties of the habitat, the *Pygmaea* tufts are—as was pointed out by Colman (1940)—the most certain places to collect *Lasaea rubra* in large numbers alive.

In some places there appear to be exceptions to the rule of greater size with longer submersion. One example is shown by the *Lasaea* at Blackstone Rocks, Wembury, at the extreme upper limit of the *Chthamalus* zone, at Morton's locations 5 and 6 (percentage exposure *c.* 90). The shells here are large in modal and maximum size. Their size induced us to do some experiments on their filtering rates as compared with those of animals of comparable size selected from lower tidal levels (percentage exposure 45). The results appeared interesting, in showing a marked initial increase in the filtering rate of those higher on the shore as compared with those lower down (see Fig. 11).

The ability to filter rapidly continued for a longer period than the normal time of submersion or contact with splash, which could never much exceed 2 h. Apparently *Lasaea rubra* living at this high level may be equipped to take rapid advantage by fast filtering of the presence of even the most temporary patches of water lodged by the splash.

The results of these preliminary experiments do no more than to point a field which it is hoped in the future to explore: the study of upper tidal *Lasaea rubra* as a filter-feeding animal living under hard conditions. It would be interesting to find whether *Lasaea* is regularly able to compensate for an unfavourable position on the shore by an increased filtering rate. In future experiments it is hoped to investigate possible differences in growth rate, metabolic rate and filtering rate, correlated with differences in shore level.

Summary

The rates of filtering, feeding and digestion have been experimentally investigated in the small intertidal lamellibranch *Lasaea rubra*. Absorptiometric determinations of filtering rates with cultures of *Phaeodactylum tricornutum* were carried out over 3 h periods. The filtering rate is strongly affected by temperature change, but not—it appears—by variations in the turbidity of the culture. For a group of 20 animals, the filtering rate reached an upper level of more than 1 ml. per hour. A very comparable order of value has been previously established for other lamellibranchs studied.

Variations in filtering rate were investigated with the use of unialgal cultures of other organisms. The toxic *Gymnodinium veneficum* was not filtered at all. *G. vitiligo*, *Chlorella stigmatophora*, *Nannochloris atomus*, *Peridinium trochoideum* and *Dicrateria inornata* were filtered at rates lower than for *Phaeodactylum*; *Exuviaella baltica*, *Prorocentrum micans*, *Isochrysis galbana* and *Chromulina pusilla* were filtered at higher rates. The cell size of the organism—like the density of the culture—had no detectable effect on filtering rate. Samples of *Lasaea* pre-treated with the toxic *Gymnodinium veneficum* showed a subsequent depression of the normal filtering rate with *Phaeodactylum*. Eventual recovery was obtained.

Haemacytometric counts of stomach contents were made after feeding with *Phaeodactylum* and the rate of digestion established over a 2 h period. Preliminary digestion, with the reduction of the cell to a 'ghost', takes place extracellularly.

The ability of *Lasaea* to carry out sorting of mixed suspensions was investigated, after feeding with varied particle sizes, by examination of the remaining supernatant culture, the pseudofaeces and the gut contents. Only with mixtures of *Peridinium* and *Dicrateria* and of *Phaeodactylum* and 'Kieselguhr' does evidence of sorting ability appear.

Lasaea was found to digest *Phaeodactylum* and *Isochrysis* rapidly, the diatom *Thalassiosira* slowly and *Peridinium* not at all. It is thought likely that organic detritus figures largely in its food budget.

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THE EFFECT OF WATER CONSERVATION ON THE STRUCTURE OF MARINE FISH EMBRYOS AND LARVAE

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(Plate I and Text-figs. 1–2)

Fish eggs can be divided into three main groups: marine pelagic eggs which float of their own accord, marine demersal, and freshwater demersal eggs. The embryological development of freshwater teleosts follows an accepted pattern, demonstrated by morphologists during the nineteenth century. Marine embryos, both pelagic and demersal, do not conform in all respects to this pattern, and misunderstandings have frequently arisen by lack of attention to known structural differences between the three groups. McIntosh & Prince (1890), in their comprehensive review of fish development, emphasized some outstanding anomalies in pelagic structure, but gave no adequate explanation of their occurrence.

The present purpose is to draw attention to a striking divergence in the morphology of these three groups; to relate it to the difference in habit; and to demonstrate how it affects the transport of yolk derivatives during the embryonic and larval phases. Here the term 'larva' refers to the yolk-sac stage after hatching. The post-larval phase begins with the final disappearance of the yolk.

The Subdermal Space of Pelagic Embryos and Larvae

Pl. I, fig. 1, is a transverse section through the trunk of a 4-day-old demersal trout (*Salmo trutta*) larva, showing the nerve cord, notochord, urinary vessels, gut and axial blood vessels, all surrounded by the heavy myotome musculature. The integument is closely apposed to the underlying mesoderm, and is extended dorsally and ventrally as the marginal or embryonal fin.

This orthodox picture should be compared with that shown in Pl. I, fig. 2, which is a transverse section through the trunk of a 2-day-old pelagic plaice (*Pleuronectes platessa*) larva. The myotomes are relatively weak, and the integument is well separated from the underlying mesoderm to give a voluminous subdermal space. This space has already been recorded by earlier workers. A day before his cod hatched, Meek (1924) observed: 'During the preceding day or two, a space has been gradually forming on each side of the body,

separating the ectoderm from the underlying parts. This space is now a prominent feature of the body and tail. It is the preparation for a subdermal space which forms an important larval feature; it is lined with mesenchyme and is apparently a lymph sinus.' McIntosh & Prince (1890) also described the same space as a late embryonic structure, filled with a jelly-like lymph and extraordinarily enlarged above the cephalic region.

In the cod (*Gadus callarias*) larva (Pl. I, fig. 3), the separation of integument from mesoderm is complete in the gut and pectoral region. There is no separation in front of the eyes and over the mandible, nor posteriorly in the caudal region, where the integument is closely knitted to the lateral mesoderm of the main axis, but separation dorsally and ventrally produces the lumen of the big marginal fin, which must be supported, in view of the lack of fin-rays, by turgor within the space.

When larval fin-rays are absent, or just developing, it is reasonable to suppose that the size of the marginal fin, in relation to the more compact axial structures, will vary according to the extent of the subdermal space. The transverse sections in Pl. I show a marked difference in relative fin size between a pelagic marine larva with a big subdermal space, and a demersal freshwater larva with little integumental separation.

I scoured the literature for drawings of newly hatched fish larvae from the three egg types, to see if there were consistent differences in marginal fin size. Ten representative drawings per group are displayed in Text-fig. 1. The drawings are simplified reproductions from various publications; the outlines are exact. My tentative conclusions from this visual comparison are that larvae from pelagic marine eggs, with consistently big fins, have extensive subdermal spaces, and that these spaces are not well defined in demersal

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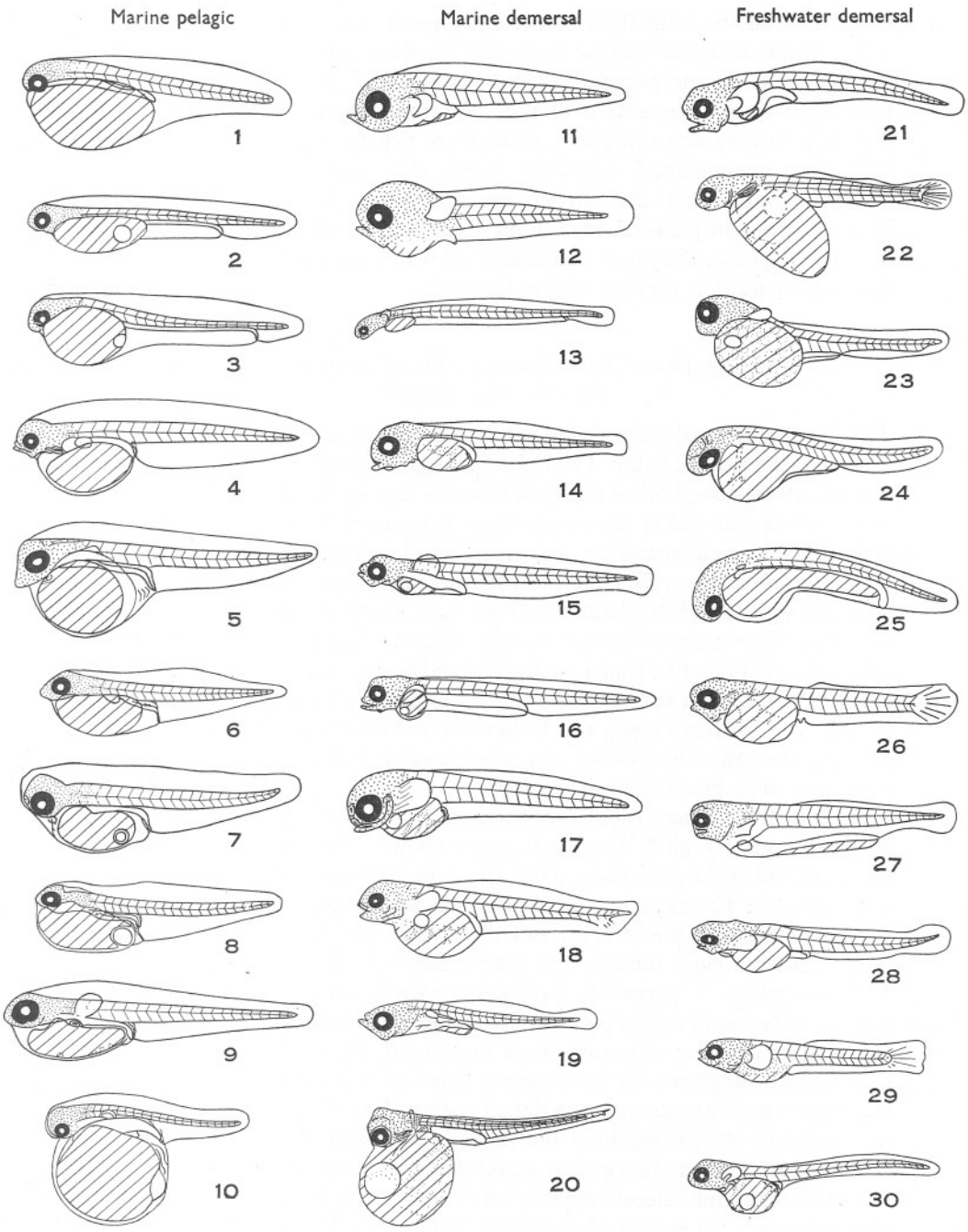
Legend to Text-fig. 1.

Text-fig. 1. A comparison of marginal fin size in early larvae from different types of teleost eggs, i.e. marine pelagic (left), marine demersal (centre), and freshwater demersal (right). 1, *Gadus luscus*, 3·0 mm; 2, *Argentina sphyraena*, 6·9 mm; 3, *Clupea pilchardus*, 3·5 mm; 4, *Pleuronectes platessa*, 8·0 mm; 5, *Solea vulgaris*, 3·2 mm; 6, *Callionymus lyra*, 2·2 mm; 7, *Rhombus laevis*, 4·0 mm; 8, *Scomber scombrus*, 3·5 mm; 9, *Trachinus vipera*, 3·3 mm; 10, *Lophius piscatorius*; 11, *Hypleurochilus geminatus*, 2·1 mm; 12, *Chasmodes bosquianus*, 3·6 mm; 13, *Clupea harengus*, 7·0 mm; 14, *Myoxocephalus scorpius*, 8·2 mm; 15, *Chirolophis galerita*, 12·0 mm; 16, *Pholis gunnellus*, 9·4 mm; 17, *Cyclogaster montagui*, 3·8 mm; 18, *Cottus gobio*, 8·0 mm; 19, *Gobius niger*, 3·0 mm; 20, *Anarrhichas lupus*, 12·0 mm.; 21, *Lota maculosa*, 3·5 mm; 22, *Salmo trutta*; 23, *Gasterosteus aculeatus*, 4·7 mm; 24, *Richardsonius balteatus*, 5·3 mm; 25, *Labeo gonius*, 3·6 mm; 26, *Crenichthys baileyi*, 4·3 mm; 27, *Notropis girardi*, 5·5 mm; 28, *Percopsis omiscomaycus*, 6·0 mm; 29, *Plancterus kansae*, 6·6 mm; 30, *Boleosoma n. nigrum*, 5·0 mm. Nos. 1, 4–10, 13 and 15–20 after Ehrenbaum, 1904, 1905–9; nos. 11 and 12 after Hildebrand & Cable, 1938; nos. 2 and 3 after D'Ancona & Sanzo, 1931; no. 14 after Bigelow & Welsh, 1925; nos. 21, 28 and 30 after Fish, 1931; no. 22 after Stuart, 1953; no. 23 after Ved Vrat, 1949; no. 24 after Weisel & Newman, 1951; no. 25 after Nazir Ahmad, 1944; no. 26 after Kopec, 1949; no. 27 after Moore, 1944; no. 29 after Koster, 1948.


Marine pelagic
Marine demersal
Freshwater demersal
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Text-fig. 1.

freshwater larvae, with their consistently small fins. The relative fin size of larvae from demersal marine eggs falls between these two extremes, with *Hypleurochilus* at the pelagic end and *Anarrhichas* approaching the demersal freshwater state. The extent of the subdermal space in this group can be expected to vary accordingly. Larvae from pelagic eggs are usually characterized by integumental separation above the medulla. This feature is the exception rather than the rule in demersal larvae from both environments. Whereas the conspicuous, rayless fin of pelagic larvae persists well into the post-larval phase, the fin of demersal freshwater larvae may begin to assume the adult condition very soon after hatching.

The Subdermal Space and the Problem of Buoyancy in Pelagic Fish Embryos

The development of voluminous subdermal spaces in fish embryos is clearly related to the pelagic habit. The immature egg of the plaice sinks in sea water. Fulton (1898) stated 'that the final changes in the maturation of the pelagic ovum, while still within the ovary, are accompanied by a comparatively rapid and relatively great accession of a watery fluid of low density from the ovary, which dissolves the yolk spherules, is associated with the dissolution of the germinal vesicle and the definite formation of the periblast, distends the ovum to three or four times its former volume, thinning the capsule correspondingly, renders it of crystalline transparency and reduces its specific gravity, so that it is enabled to float in sea water of ordinary density—in other words, to become pelagic. . . .' Milroy (1898) and Dakin (1912) found that the vitelline diluent entering the egg at maturation had a salt concentration with a greater resemblance to adult blood than to sea water.

McIntosh & Prince (1890) described the porous nature of certain fish egg-shells. Krogh, Krogh & Wernstedt (1938) showed that the 'amniotic' or perivitelline fluid of the pipe-fish egg had the same chloride concentration as the sea water outside. The experiments of Jacobsen & Johansen (1908) on the specific gravity of cod and plaice eggs, and the studies of Svetlow (1929) and Gray (1932) on trout eggs, indicate the permeability of the shell to salts and water in these species. If permeable capsules are the general rule among fish, then marine embryos, like their parents, are in constant danger of desiccation under the osmotic gradient. Osmotic work must be done, in the first place, by the peripheral protoplasm surrounding the yolk before gastrulation, and later, by the embryonal integument, in order to prevent loss of water and gain of salts. An unhealthy pelagic egg loses buoyancy in sea water; a dead egg sinks. This rule applies whether the egg has an oil globule or not.

An adult marine teleost replaces water lost under the osmotic gradient by swallowing sea water, absorbing water and some salts through the gut wall, and secreting the excess blood chloride to the outside, through special cells

in the gills (Keys, 1931; Keys & Willmer, 1932). This osmo-regulatory mechanism requires an open gut, a well-developed blood circulation and an advanced gill system, for efficient functioning.

The gut of a pelagic plaice embryo opens at a very late stage in incubation (Fullarton, 1891). The blood circulation is ill-developed, as will be shown in the next section, and the gill-bars lack filaments at hatching. The adult osmo-regulatory mechanism cannot apply, in its entirety, to the embryo. A method of direct water acceptance through the integument, to the exclusion of salts, is difficult to conceive in the prevailing osmotic conditions; we must assume, lacking concrete evidence to the contrary, that the permeability of the embryonal integument is normally maintained at a low level, by the expenditure of energy in the form of osmotic work. There is direct evidence to support this assumption in freshwater fish eggs. Krogh & Ussing (1937), using heavy water, confirmed the conclusions of Gray (1932) on the high degree of water impermeability of the trout egg membrane during early development. The evidence for marine eggs is not so substantial. Experiments by Loeb & Wasteneys (1915) led them to believe that the protoplasmic membrane of the fertilized *Fundulus heteroclitus* egg was impermeable to salts and almost impermeable to water. Conversely, Krogh *et al.* (1938) found that unfertilized eggs of *Pleuronectes flesus* and *Crenilabrus exoletus* exchanged water and ions with the surrounding solution. The act of fertilization, however, may have an important bearing on the subsequent permeability of the peripheral protoplasm.

The efficiency of the embryonal integument as a water barrier will depend largely on the health of the embryo and the favourability of its environment. Any osmotic water 'leak' must be followed by adjustment of the internal salt balance. The salt-regulatory mechanism of marine fish embryos and larvae is not yet known, although Krogh *et al.* (1938) showed that such a mechanism must exist in the egg of the pipe-fish *Nerophis ophidium*.

Apart from the question of salt balance, the embryo cannot afford to lose too much water if the egg is to remain buoyant. The limit of water loss, in this context, will be determined by the initial reserve buoyancy at liberation and the loss of weight due to the excretion of respiratory metabolites. A healthy plaice egg remains buoyant until hatching, even though its degree of reserve buoyancy at liberation is not great. If the embryonal weight decreases but little during incubation, then it follows from the simple relationship, mass/volume = density, that little change can take place in the volume enclosed by the embryonal integument. During development, there is a withdrawal of soluble nutrients from the dilute fluid yolk. They are used in the synthesis of the compact embryonic axis, and in respiration. But the low-density yolk diluent remains. It accumulates beneath the integument, forms and fills the voluminous subdermal spaces, and thus maintains an overall embryonal volume in keeping with the buoyancy requirements of the egg.

The problem of water conservation does not arise in a demersal freshwater embryo. One can, therefore, readily understand the lack of a subdermal space in this group, and hence, the ill-developed marginal fin.

A floating marine egg accepts sufficient yolk diluent at maturation to meet both osmotic and buoyancy requirements during early development. A non-buoyant marine egg, on the other hand, requires enough yolk diluent to provide against the osmotic hazard only. Accordingly, one might expect the subdermal spaces of this group to be less capacious than in pelagic eggs; to be more developed than in freshwater eggs; and to vary with specific differences in the reserve of yolk diluent below that necessary to float off the egg. The picture of relative fin size from Text-fig. 1 supports this view.

The Evolution of the Pelagic Egg

The palaeontological record suggests that modern marine teleosts are derived from freshwater ancestors (Romer & Grove, 1935; Moy-Thomas, 1939). The comparative anatomy of adult osmo-regulation is in accord with this view (Baldwin, 1937). There is no reason to suppose that the process of marine colonization by freshwater fish has now ended, nor that reversion back to freshwater is impossible (Hoar, Black & Black, 1951).

Complete physiological adaptation to one or other of these habitats implies the development of a mechanism capable of maintaining the internal environment against the external change, in the egg as well as the adult. The spawning migrations of the salmon, shad and eel can be interpreted as evidence that the embryo is unable to develop in the physico-chemical conditions of adult life.

Assuming teleost embryos to have only a limited tolerance to internal salt change, then what are the essential requirements for the adaptation of a freshwater type of egg to a truly marine existence? First of all, a vitelline membrane highly impermeable to salts and water. The evidence for early impermeability in modern teleost eggs had already been given. Secondly, a means of balancing the internal salt concentration should an osmotic water 'leak' occur in less than optimum conditions. A freshwater embryo, without a functional renal system, must then secrete salts from the outside in. The reverse applies in a sea embryo, and although mechanisms of embryonal salt control are not yet known, it is significant that the chloride secretory cells in the adult *Fundulus heteroclitus* can reverse their polarity when the fish is transferred into fresh water (Copeland, 1948). The ability of the cell membrane to work against a chloride gradient is the essential property of the chloride secretory cell (Hoar *et al.*, 1951). Do such cells occur in fish embryos?

When the external osmotic pressure exceeds that inside the embryo, sufficient water must be present, throughout early incubation at least, to supply the needs of the embryo in the event of osmotic loss. This third requirement is specific to a change from fresh water to marine conditions for egg

development. An egg is supplied with water, in the form of yolk diluent, at maturation within the ovary. The specific gravity of teleost body fluids is less than that of sea water. The degree of yolk dilution at liberation may be enough to supply embryonal water needs (in co-operation with a salt-regulatory mechanism) and yet not sufficiently advanced to float off the egg. This is the demersal marine condition. An extension of the process of yolk dilution must inevitably produce a buoyant form of egg, when the upthrust exerted on the distended egg by displaced sea water exceeds the gravitational pull.

Thus, by considering the embryonal adaptations necessary for colonization of the sea, it is possible to arrive at an intrinsically buoyant type of egg. The vast majority of pelagic eggs are, in fact, marine. Buoyancy in the freshwater environment involves a completely different mechanism. This attempt to trace the evolution of buoyancy in fish eggs is intended to be provocative rather than informative, particularly as the mechanism of embryonal salt-regulation is not yet understood and measurements of yolk dilution over a whole range of fish species are not yet available.

Some marine fish families are more advanced in the direction of pelagic egg production than others. The pleuronectids produce floating eggs only; the gadoids have a demersal representative in *Lota maculosa*. Among salmonids, the trout has a freshwater habit throughout its life-history; the salmon is not yet embryologically adapted to a marine existence; whilst the related *Argentina sphyraena* is completely marine, and has a pelagic egg. There are both demersal and pelagic egg producers among the clupeoids. Early larval representatives of all these families are displayed in Text-fig. 1. Those issuing from pelagic eggs have large, inflated embryonal fins, a feature clearly related to the pelagic habit rather than to family affinities.

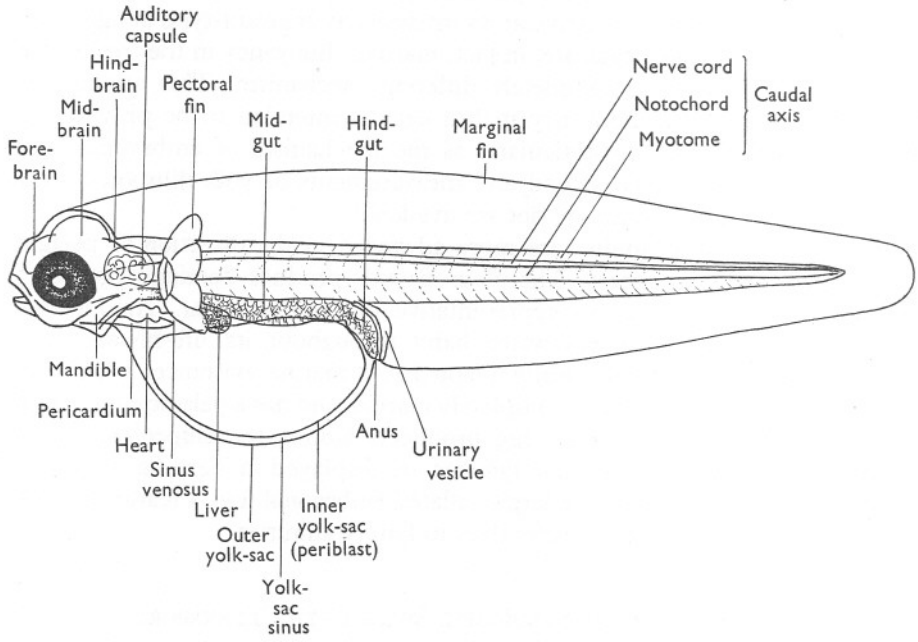
The Role of the Subdermal Space in the Transport of Yolk Derivatives

From the time when segmentation starts after the fertilization of a fish egg, to a stage preceding the formation of a pulsating heart and a primitive blood circulation, it is clear that the embryo appropriates yolk by a direct process of incorporation, without the aid of a blood-vascular system. With the subsequent thickening and lengthening of the main embryonic trunk, an increasing volume of tissue is moving out of contact with the yolk in its inner sac, thereby creating problems of food transport.

At this point, the yolk-sacs of most demersal freshwater eggs diverge structurally from those of pelagic marine forms. The former develop a complex system of circulatory vessels around the yolk; in pelagic eggs, this system is always absent, and its function is taken over by the yolk-sac sinus, into which the heart opens directly (Text-fig. 2). The yolk-sac sinus is, in reality, a subdermal space, formed when the extra-embryonal mesoderm

around the yolk breaks down into isolated mesenchyme cells during gastrulation (Meek, 1913).

At some stage in embryonal development the ventral yolk-sac sinus is put in lateral communication with the lumen of the rapidly growing marginal fin, on the dorsal side. Pl. I, fig. 3, is a slightly oblique transverse section of a cod larva through the pectoral fin region, showing the gross separation of the integument from the main larval axis, to give a lateral channel connecting the yolk-sac sinus ventrally, to the fin space dorsally.



Text-fig. 2 Diagram of a newly hatched plaice larva.

It would seem, therefore, that nutrient derivatives from the yolk of pelagic eggs have easy access to embryonic and larval tissues via the subdermal spaces. To test this supposition I immersed newly hatched plaice larvae in fast fixatives containing corrosive sublimate. A dense brown coagulation quickly appeared in the yolk, the yolk-sac sinus, beneath the integument around the base of the pectoral fins, and in the lumen of the marginal fin. In some normal early larvae, coagulation extended throughout the lumina of both dorsal and ventral portions of the fin (Pl. I, figs. 4, 5). The coagulated moiety of the plasma was frequently washed out of transverse sections during staining. If remaining, it appeared as a decolorized reticulation in the subdermal spaces of the marginal fin, in the cardial and pericardial cavities, and around the base of the pectoral fin.

In case yolk derivatives in the fin were a fixation artifact caused by a strongly contracting yolk-sac, I narcotized five normal larvae in menthol, and measured the length, depth and width of the inner sac. The larvae were then transferred to a mild fixative, Baker's calcium formaldehyde, for 5 min, and the measurements repeated. Later immersion in Heidenhain's Susa intensified the initial coagulation. Table I shows that the inner yolk-sacs of three of the five specimens contracted a little after fixation, but not enough to account for the volume of coagulated derivatives seen in the fin. There was no noticeable movement of plasma from the yolk-sac sinus to the fin during the experiment. The progress of coagulation in larval spaces was watched under a high-power dissecting microscope, and seen to be always from the outside inwards for the dorsal fin, compatible with the view that soluble proteins were already there, before the addition of preservative.

TABLE I. INNER YOLK-SAC MEASUREMENTS IN MICROMETER UNITS

Larva no.	Narcotized			Fixed			Coagulation in Susa
	Length	Depth	Width	Length	Depth	Width	
1	29	31	32	28	31	32+	Heavy
2	10	24	25	10	24	25	Heavy
3	12	25	26	12	25+	26	Heavy
4	46	27	29	43	27+	29	Heavy
5	11	26	32	10	26	31	Heavy

We think of the heart and developing vascular system as becoming vital to the transport of yolk nutrients at an early stage in fish egg development. This may be substantially true for the demersal freshwater egg, with its yolk capillaries and closed vascular system. But in pelagic marine embryos there is an unusual extension of the primary method of direct yolk appropriation, independent of a blood vascular system, well into the larval phase. This is undoubtedly a consequence of the formation of subdermal spaces, dictated by the requirements of water conservation in the marine environment, and of buoyancy.

In the third group of fish eggs, demersal marine, yolk-sac vascularization is as variable as marginal fin development, and probably for the same reason. McIntosh & Prince (1890) related the presence of a vitelline circulation to the demersal habit, irrespective of environment. This is an over-generalization. The herring egg has a yolk-sac sinus and lacks capillaries; *Anarrhichas*, on the other hand, has a conspicuous yolk circulation.

If there is an early separation of the embryonal integument from the inner yolk-sac endoderm, causing the breakdown of the extra-embryonal mesoderm noticed by Meek (1913), then capillary formation becomes impossible. The degree of separation in this region will depend on the volume of yolk diluent left between the two membranes, on the completion of gastrulation. This, in turn, will depend upon the extent of yolk dilution. I have already suggested

that variable fin size in demersal marine eggs may be related to variable conditions of water reserve, or yolk dilution. The similar inconsistency in yolk-sac vascularization is in direct line with this view.

In conclusion, it is necessary to reformulate the ideas put forward in this paper, and state that some major differences in the structure and habit of fish embryos and larvae appear to be closely related to a very simple factor, namely, the concentration of soluble nutrients per unit volume of yolk, at the time of egg liberation.

Summary

Fish eggs can be divided into three groups according to habit: pelagic marine, demersal marine, and demersal freshwater. Embryos emerging from these eggs show group differences in the extent of the subdermal space, as inferred from the relative size of their embryonal or marginal fins. Pelagic marine embryos have large, inflated marginal fins, in contrast with the ill-developed fin of the demersal freshwater embryo. The fins of demersal marine embryos lie between these two extremes.

There is a parallel variation in the degree of vascularization of the yolk-sac: pelagic marine embryos have no vitelline circulation, demersal freshwater forms have good vascularization, whilst that of demersal marine embryos is variable.

These differences in both structure and habit can be related to group differences in the degree of dilution of the yolk by ovarian fluid at maturation, and the subsequent need for marine embryos to conserve water under the osmotic gradient. It is possible to construct an argument to explain the evolution of the pelagic marine egg, by considering the embryonic osmoregulatory adaptations necessary for the complete colonization of the sea by ancestral freshwater fish.

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EXPLANATION OF PLATE I

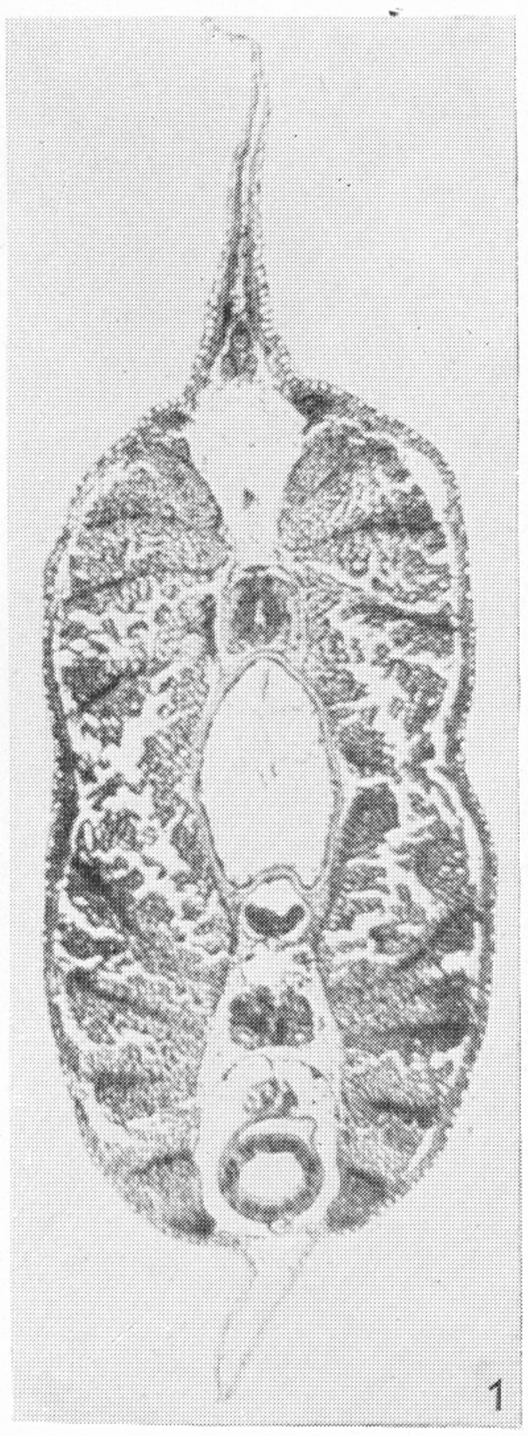
Fig. 1. Transverse section through the trunk of a 4-day-old demersal trout larva, showing the close connexion between the integument and underlying mesoderm. × 50.

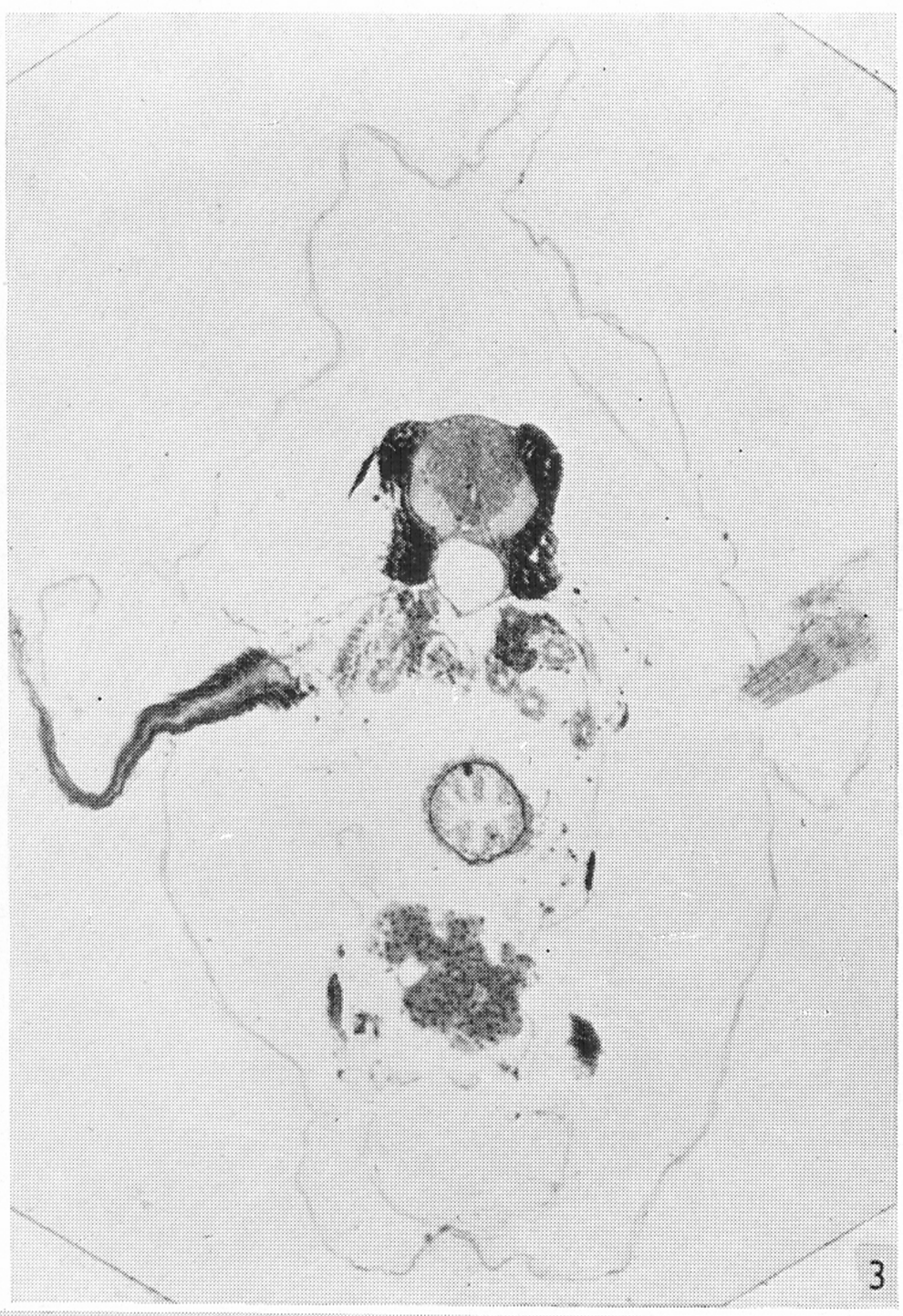
Fig. 2. Transverse section through the trunk of a 2-day-old pelagic plaice larva, showing the separation of the integument from the underlying mesoderm to give the superficial subdermal space. × 100.

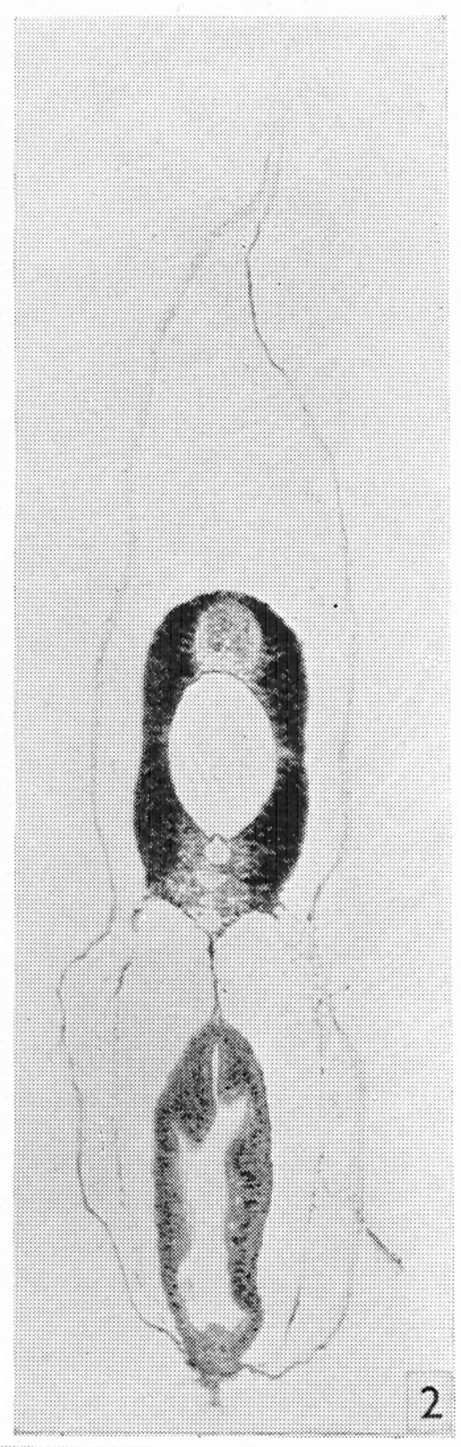
Fig. 3. A slightly oblique transverse section through the pectoral region of an early cod larva, showing gross integumental separation, and the continuity of the yolk-sac sinus ventrally, with the lumen of the dorsal marginal fin. × 100.

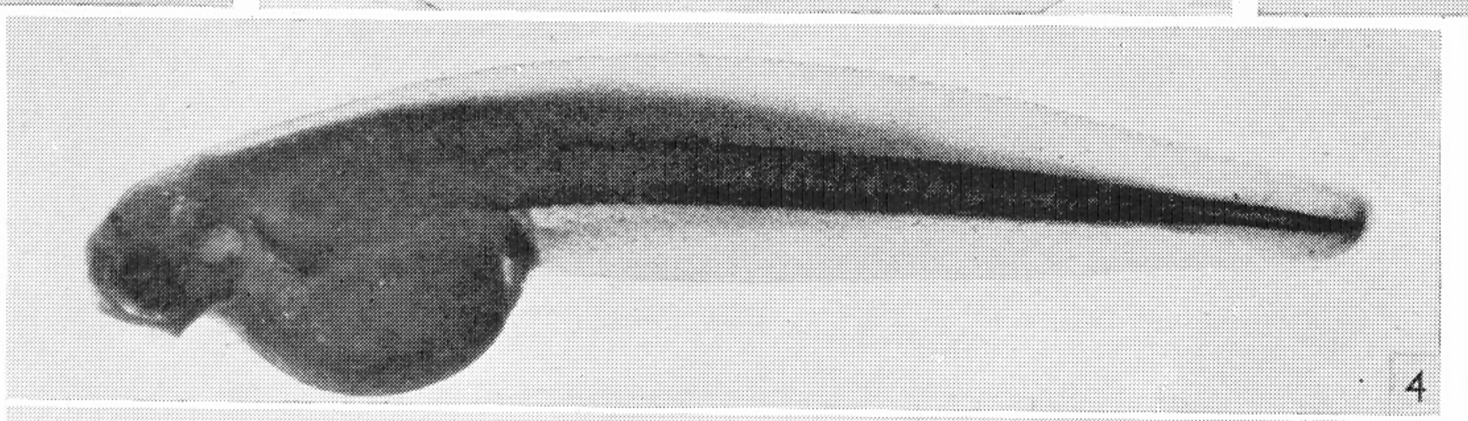
Figs. 4, 5. Newly hatched plaice larvae with extensive coagulation of plasma in dorsal and ventral portions of the marginal fin, after fixation in Heidenhain's Susa. × 12.

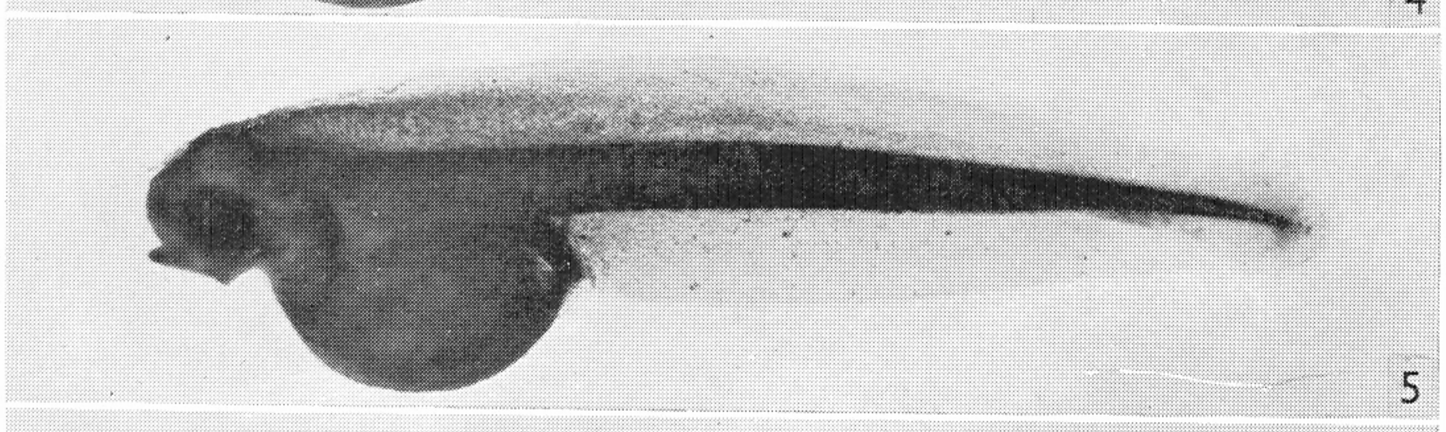
Figs. 6, 7. Day-old plaice larvae with heavy plasma coagulation in the anterior part of the dorsal marginal fin, above the head and gut. Fixed in Heidenhain's Susa. × 12.

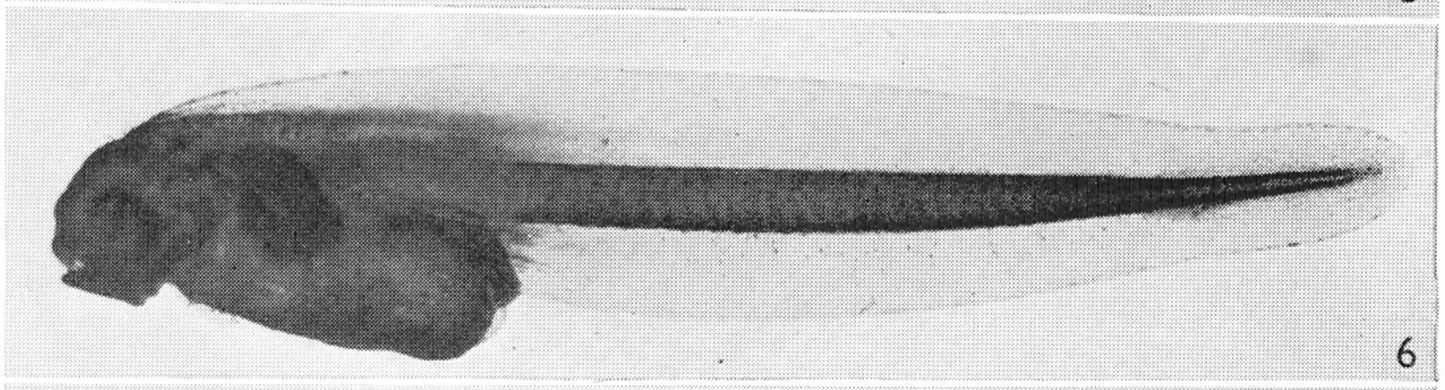


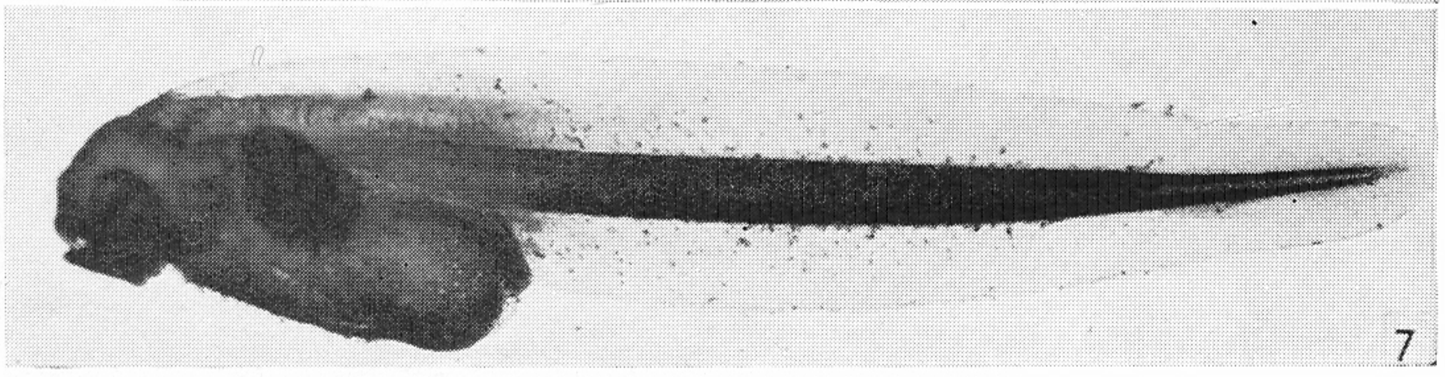












Figs. 1–7.

(*Facing p.* 286)

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

Note on the Use of Diphenylbenzidine for the Estimation of Nitrate in Sea Water

By W. R. G. Atkins

J. Cons. int. Explor. Mer, 1954, Vol. 20 (2), pp. 153–55

It was shown in 1932 that this reagent, once recrystallized from boiling toluene, can be used for years. The sensitivity is adequate and the colour stable even in bright daylight. All nitrate reagents used in strong sulphuric acid may give low results in presence of organic matter. This error is reduced when 20 mg. of the solid is dissolved in strong sulphuric acid and 7·5 ml. is run into a tube. The sample, 2·5 ml., is then added and is well stirred. The blue colour produced is an oxidation, not a nitration. The reaction is useful also in testing river and well waters and is quicker than the production of picric acid—a nitration product—in the usual test. Methods for the removal of traces of nitric acid from sulphuric acid were cited. Ramachandran heats with a very little formaldehyde, which goes to formic acid, or Pfeilsticker's ammonium chloride method may be used. With known amounts of potassium nitrate a relation may be found between concentration and intensity and this allows for impurity in the sulphuric acid also.

W.R.G.A.

Use of the Globe Photometer

By W. R. G. Atkins and H. H. Poole

Nature, Lond., 1955, Vol. 175, p. 1003

The illumination in a shaded site may be expressed as a percentage of the diffuse light received, from the whole sky, upon a horizontal plate or one may measure the effect at a point, by the action of light on uranium oxalate solution in a flask. The latter is preferable when studying the effect upon plant growth. We had used a selenium rectifier cell under the usual opal diffusing plate, surmounted by an opal hemisphere and an opal globe, so as to render the disc equally sensitive to light at any angle. More recently we assembled a globe photometer with a thin-film caesium-on-silver-oxide vacuum photo cell, a type proved constant in sensitivity for over three years on a roof. This is free from temperature error and always gives a rectilinear relation between current and illumination.

W.R.G.A.

Identification of Water-Masses by their Suspended Matter

By W. R. G. Atkins and Pamela G. Jenkins

Nature, Lond., 1955, Vol. 175, p. 951

For such identifications temperature, salinity and oxygen concentration have been used, also in special cases silica or hydrogen ion concentrations. Jerlov has shown the value of the visual determination of scattering in the Tyndall beam, which Poole and Atkins have measured using a photomultiplier tube. From September 1951 to July 1954 we filtered water from station E 1 through collodion discs. The albedo of the discs gave a good measure of the suspended matter. Apart from the living cells, the basis of the suspension was an amorphous mud, grey, yellowish or chocolate. The albedos were determined with a photo-electric disc, or visual comparisons were made by matching against Klincksieck's or Ridgway's plates. A. G. Lowndes examined the suspended particles using a petrological microscope, and especially important was his finding of microcline on discs from E 1 on 29 March, 1954. It was most abundant on the 5 m disc, which suggests that it had come out in fresh water and was slowly settling. The colour tests suggested the Permian Red Sandstone between Torquay and Exmouth as the source of suspensions found in October (1952 and 1953). W.R.G.A.

Observations on Luminescence in *Renilla* (Pennatulacea)

By J. A. C. Nicol

J. exp. Biol., 1955, Vol. 32, pp. 299–300

A study has been made of the luminescent responses of the sea pansy *Renilla köllikeri*. This is a pennatulid found in shallow water along the Californian coast. The responses take the form of light-waves which run over the surface of the rachis. They are controlled by an unpolarized nerve net, and are subject to facilitation.

By photo-electric recording it has been possible to analyse certain details of the luminescent response. At moderate rates of stimulation, several electrical shocks are usually necessary to evoke a luminescent wave, and subsequent flashes increase in intensity (facilitation). By lowering the frequency of stimulation, it is found that more stimuli become necessary to produce the first flash, owing to decay of facilitator.

Maximal estimates of latent period and flash-duration are of the order of 0·5 and 0·9 sec. respectively.

With repeated stimulation the luminescent response of *Renilla* becomes fatigued. Luminescence, furthermore, is inhibited by illumination. After exposure to light the ability to luminesce is lost and slowly recovers over the course of an hour.

Facilitation normally occurs terminally, at the level of the photocytes. By cutting the animal in various ways it has been possible to produce preparations in which neuro-neural facilitation becomes evident. This is occasioned by internuncial fatigue at residual interneural junctions. Normally, however, transmission is 1:1 throughout the nerve net.

J.A.C.N.

BOOK REVIEW

The Chemistry and Fertility of Sea Waters

By H. W. Harvey Sc.D., F.R.S.

Cambridge University Press 1955

This forms a useful companion to two earlier books by the same author and deals with the physics and chemistry of sea water, especially as these affect or are affected by plant and animal life. The aim throughout is to deal with factors affecting the productivity of the sea and it contains much hitherto unpublished information.

The first part has a short introduction on the composition of sea water and the currents which cause transport and mixing. The changes caused by plants and animals are described, especially the carbon, phosphorus and nitrogen cycles, as well as the role of bacteria in effecting these changes. There is an account of the influence on plant growth of various physical factors. Of special interest is the section dealing with nutrients including those less known such as vitamin B_{12}. The dependence of animal communities on plant production is outlined.

The second part deals with the chemistry of sea water, including both major and minor constituents and the dissolved gases; there is a full account of the carbon dioxide system. A very useful section (in conjunction with F. A. J. Armstrong) gives details of the methods for estimating silicate and the different forms of phosphorus and nitrogen.

There is a good bibliography covering the most important recent literature.

A.P.O.

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

		£	s.	d.
Annual Members	per annum	1	1	0
Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

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

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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