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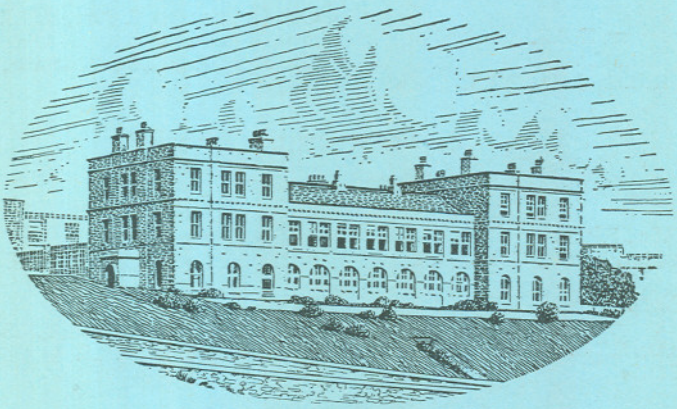

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THE DISTRIBUTION OF IRON IN THE WATERS OF THE WESTERN ENGLISH CHANNEL

By L. H. N. Cooper, D.Sc., F.R.I.C.
Chemist at the Plymouth Laboratory

(Text-figs. 1–2)

CONTENTS

Introduction

Iron is essential for all plants and animals, but only a small supply is available in sea water. Since most compounds of iron are very sparingly soluble in neutral or alkaline solution, their distribution in the sea may follow a very different pattern from that of phosphate and nitrate. Earlier it had been shown (Cooper, 1935; Thompson & Bremner, 1935*b*; Rakestraw, Mahncke & Beach, 1936) that the distribution of iron in the sea was very erratic, even under conditions when vertical mixing would be expected to be thorough, so that much of it must be particulate and subject to random distribution (cf. Harvey, 1945, pp. 34, 136). The fluctuations in the iron content of small replicate samples of sea water may be large, so that either very large volumes of water must be examined, as in net methods for capturing plankton, or chemical analyses on small volumes of water must be submitted to statistical scrutiny. The precision of the analytical method has consequently been first assessed before proceeding to examine samples of water collected in the western

English Channel at the time of the midwinter maximum of dissolved inorganic phosphate. This approach has yielded considerable information as to the nature and distribution of iron in the sea and of the size and numbers of particles containing iron. Evidence has also accrued for the concentration of iron in the surface film and in the layers of water next the bottom. In certain restricted areas the bottom concentration of iron appears to be removed by suspension feeding animals. On occasion particulate ferric phosphate was present.

Statistical Design

The known random distribution of iron demanded statistical methods which were unnecessary for the parallel examination of phosphate and silicate. For total iron (and similarly for reducible iron) the plan had been to analyse three sub-samples at each depth worked at a limited number of stations, but two events interfered with this. First, the occurrence of several 'untrustworthy' phosphate results (see p. 298) caused samples meant for iron analyses to be used for replicate phosphates. Secondly, other samples brought in for duplicate phosphates were actually analysed singly for iron: more information, especially about ferric phosphate, was thus obtained at the expense of the symmetry of the experiment.

Throughout this paper, to have convenient units for the analyses of variance, results are reported as mg.-atom/m.^{3} × 100, that is the unit of measurement is 1 in 10^{11}. This, for brevity, will henceforth be called a 'unit'. Table I will enable the reader mentally to convert results to his own favoured units. For computation the atomic weight of iron should however be taken as 55·84.

Table I

Each vertical column represents a given quantity of iron in different units

mg.-atom/m.^{3} × 100 Fe	100	1	1·8	1·25
mg.-atom/m.^{3} Fe μg.-atom/l. Fe	1	0·01	0·018	0·0125
mg./m.^{3} Fe μg./l. Fe	56	0·56	1	0·7
mg./m.^{3} Fe_2O_3 μg./l. Fe_2O_3	80	0·80	1·4	1

Although, to comply with conventional chemical usage, statistical means and standard deviations are reported to no more significant figures than would be given for single analyses, all calculations have been made to four or five places by machine.

Analytical Methods

In 1936 the reagent, 2:2′-dipyridyl, was used exactly as before (Cooper, 1935, pp. 420–24). In 1946–47 a number of small but important alterations were made.

Reducible iron. Into a 250 ml. flask 150 ml. of sea water from a very well-shaken sample bottle were introduced and treated with 0·30 ml. 4 N-HCl and

1·5 ml. of freshly prepared 10% sodium sulphite to give a solution having a pH around 2·8. After at least 4 and preferably 18 hr. 0·4 ml. 4 N-ammonium acetate and 1·5 ml. 0·4% dipyridyl in 0·2 N-HCl were added to give a pH around 5·0. After a further 4 hr. the depth of colour of the sample in a 25 cm. absorption tube was determined with the Pulfrich photometer using the blue-green S 50 spectral filter.

As a result of Buch's (1942) careful examination of the method further modifications may be needed but at the time of the cruise a translation of his Swedish text was not available. This paper will be considered at a later date in connexion with the chemistry of ferrous-*tris*-dipyridyl.

Total iron sensu strictu. Strictly total iron can be determined only if the water and everything in it is digested with sulphuric acid to fuming as by Thompson & Bremner (1935*a*). This procedure is very time consuming and would have been impossible in a temporary laboratory such as that used at Newlyn for the present investigation.

'*Total*' *iron as determined.* The original method was modified precisely as that for reducible iron. Compounds of iron were broken down by boiling with acidified bromine water. This method sets free only 41% of the iron in a solution of Waksman's ferrolignoprotein in sea water, whilst the method for reducible iron will liberate 20–25%. In sea water, therefore, there may well be organic compounds which are resistant to this treatment and terrigeneous clay and mineral particles would certainly be so. None the less, the method measures a definable quantity, is quick, and with occasional recourse to a service gas-mask may be used in a laboratory not equipped with a fume chamber.

The evaluation of the correction terms and of random analytical errors is given in Appendix I.

Midwinter Results, 1946–47

A temporary analytical laboratory was set up at Newlyn, the most westerly fishing port in Cornwall, and occupied between 1 and 28 January 1947. During that period the Marine Biological Association's research vessel *Sabella* made her base there with Mr P. G. Corbin as scientific officer in charge of work at sea. The data on the distribution of salinity, temperature, phosphate, silicate and zooplankton will be published separately. Fig. 1 shows the position of the stations worked for iron samples; fuller details will be presented in the paper dealing with phosphate. A preliminary cruise was also made on 4 and 5 December 1946.

The results are set out in detail in Table II. Triplicate analyses did not agree. Moreover, the distribution of iron at the bottom was markedly different from that in the water between the surface and 50 m. The dispersion of all results for reducible and for total iron may be seen from Fig. 2 in which all separate analyses have been grouped in arguments of 5 mg.-atom/m.^{3} Fe × 100 or 'units'. The distribution of analyses of inorganic phosphate constructed

with the same argument was utterly different. The relatively few analyses for bottom water showed a very wide spread.

Much of the iron must be distributed very differently from inorganic phosphate and nitrate. The latter, except for a little ferric phosphate (see below), are truly dissolved in the water, are vertically uniformly distributed under uniform isothermal, isohaline conditions and usually show complete uniformity within a small sample.

At station A3 (= International Hydrographic Station E2) on 7 January, the weather was very heavy, no nets could be worked, the sounding of 110 m. was

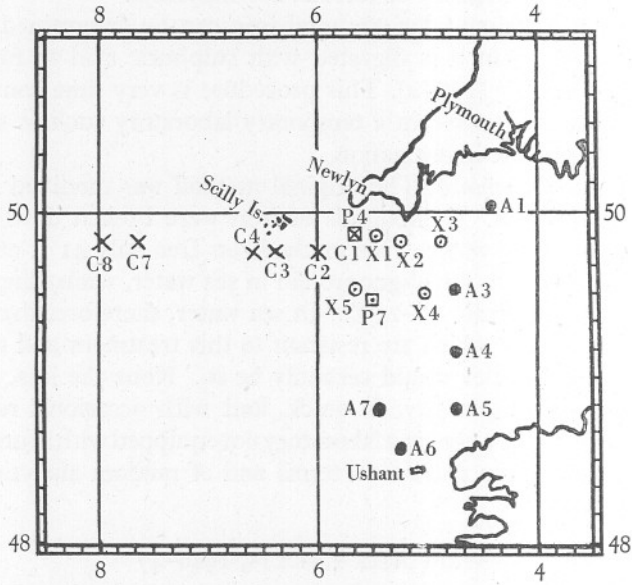

Fig. 1. Station positions. Cruise P, 4–5 December 1946. Cruise A, 6–7 January 1947. Cruise C, 17–18 January 1947. Cruise X, 21–22 January 1947. Equivalent international (E) stations: E1 = A1, E2 = A3, E3 = A6, E7 = P4 = C1, E25 = C4.

at least 15 m. too great and the angle on the warp when sounding and when sampling at 50 and 80 m. nominal depth was estimated at 35–40°. Whether this weather was the cause or not, the results were highly anomalous, and have had to be omitted from the statistical examination of all samples taken at standard depths, presented in detail in Appendices II and III and summarized in Table III.

Within the upper layers, analyses of both total and reducible iron at different depths (5, 25 and 50 m.) show no significant variation either in mean values or in the variation as shown between analyses. The whole of the variability of the total iron appears to be due to the reducible iron component. The difference of 6 units may be attributed to a dispersed unreducible fraction with a standard deviation not differing significantly from zero. There is present therefore a

small dispersed fraction detectable only when the water has first been oxidized with bromine water. It is most probably in organic combination and under the conditions prevailing in January 1947 must have been present in the bottom water in like amount. The analytical data require only that this unreducible fraction shall be well dispersed in the water. It does not necessarily require it to be truly dissolved.

TABLE II. TOTAL AND REDUCIBLE IRON IN THE ENGLISH CHANNEL, DECEMBER 1946 TO FEBRUARY 1947 AS MG.-ATOM/100 M.^{3} FE, REFERRED TO IN THE TEXT AS 'UNITS'. VOLUME ANALYSED THROUGHOUT WAS 150 ML.*

Station and sounding (m.)	Depth (m.)	Reducible iron	Total iron
	Cruise P, 4–5 December 1946		
P4 (=E7)	0	—	17
(76 m.)	5	9	—
	25	7	20
	50	22	26
	70	12	64
P7	0	27	19
(104 m.)	5	16	7
	25	9	10
	50	9	24
	70	14	53
	100	27	28
	Cruise A, 6–8 January 1947		
A1 (=E1)	5	—	37, 25, 18
(78 m.)	25	—	28, 32, 14
	50	—	39
	70	—	130, 73, 10
A3 (=E2)	5	—	6
(110 m. observed,	25	—	93
91 m. by chart)	50	—	6, 76
	80	—	69
A4	5	—	10
(94 m.)	25	—	7
	80	—	53
A5	5	—	34
(104 m.)	25	—	36
	50	—	26
	95	—	—
A6 (=E3)	25	—	0
(111 m.)	50	—	6
	75	—	28
	100	—	8
A7	0	—	23
(112 m.)	5	—	30, 21, 36
	25	—	31, 39, 32
	50	—	33
	75	—	36
	100	—	189, 26

* Though the results are given in terms of mg.-atom/100 m.^{3}, they are proportional to, and may be regarded equally as representing, weights of iron in single 150 ml. samples. For statistical purposes they are treated as such. For much of the development to follow, the distinction though subtle is fundamental. Mg.-atom/100 m.^{3} are equivalent to 0·8376 μg. per 150 ml. The very slight difference between the litre and the cubic decimetre is ignored.

TABLE II (*cont.*)

Station and sounding (m.)	Depth (m.)	Reducible iron	Total iron
	Cruise C, 17–18 January 1947		
C1 (=E7=P4)	5	—	8
(73 m.)	25	—	38
C2	60	—	26
(95 m.)	85	—	78
C3 (=N2)	0	—	24
(98 m.)	5	—	24
	50	—	42
	75	—	54
	90	—	68
C4 (=E25)	0	—	41
(100 m.)	25	56, 16, 12	32
	50	24, 8, 19	28, 10, 29
	75	6, 23, 12	38, 52, 78
	90	18, 8	22, 6, 24, 37
C7	0	—	15
(130 m.)	5	—	14
	25	—	20
	50	—	20
	120	—	64
C8	25	—	13
(127 m.)	50	—	16
	90	—	17
	Cruise X, 21–22 January 1947		
X1	0	—	25
(86 m.)	5	17	19
	25	20	34
	50	—	24
	75	79	44
X2	25	—	35
(90 m.)	50	—	30
	75	—	67
X3	0	20	—
(80 m.)	5	26	30
	25	29	52
	50	19	41
	75	37	36
X4	0	—	21
(100 m.)	5	19, 16, 12	24, 29, 32, 25
	25	10, 7, 10	22, 34, 16
	50	32, 43, 0	27, 16, 33
	90	0, 0, 0	8, 11
X5	0	53	—
(103 m.)	5	28	49
	25	4	19
	50	—	20
	95	—	52
	27 February 1947 (compare Appendix I, Table XIII)		
E1	5	7·4±3·0	—
(71 m.)	25	14·1±9·3	—
	67	11·6±3·5	—

In the bottom water the mean content of reducible iron is rather less than in the upper layers but the difference is not statistically significant. Within the error of observation, with limited numbers of analyses, the reducible iron is

randomly distributed throughout the whole water column. There is no significant variation with depth.

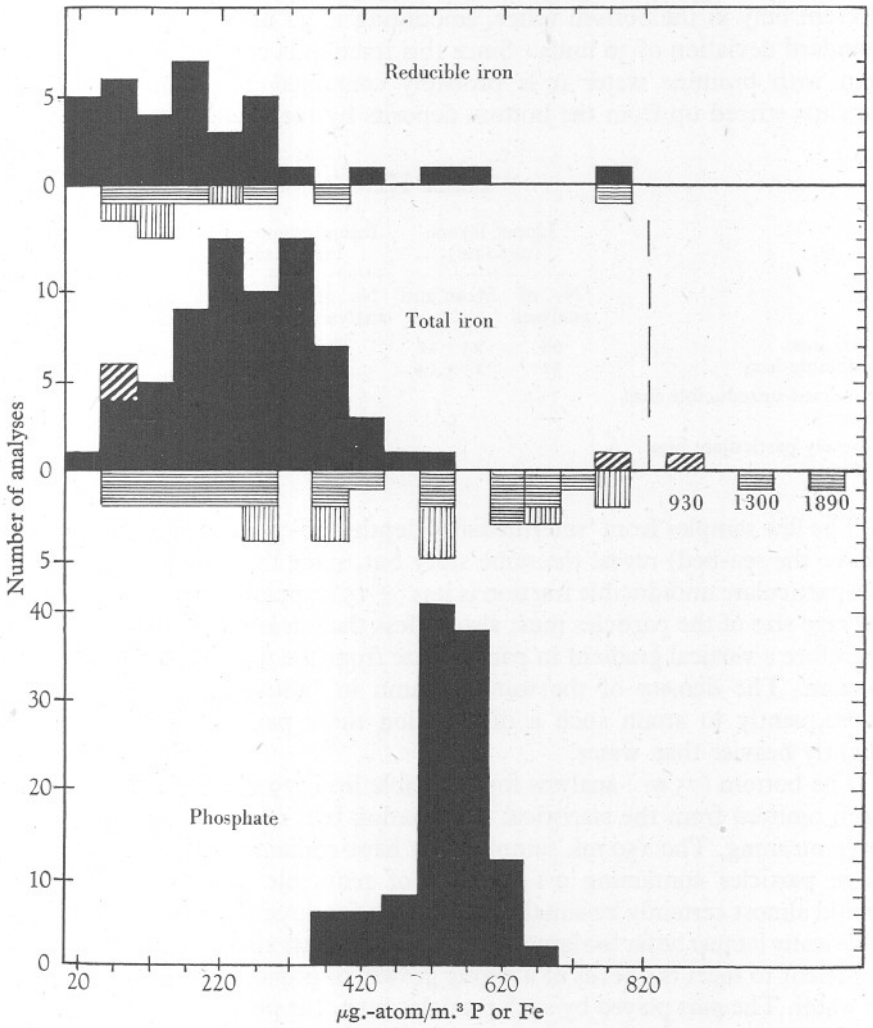

Fig. 2. Midwinter 1946–47. Numbers of iron and phosphate analyses grouped in classes with an argument of 50 μg.-atom/m.³. (The first class comprises analyses of 0, 10, 20, 30 and 40 μg.-atom/m.³, and is graphed as the median, 20 μg.-atom/m.³; and so on.) The iron analyses are separated as follows: *black*, samples from the upper water layers (between 0 and 50 m.); *horizontal hatching*, samples from bottom water; *vertical hatching*, samples from intermediate layer (23–37 m. above bottom). The upper water samples from station A 3 are *cross-hatched*.

By contrast the mean content of total iron in the bottom water is twice as great as in the upper layers. The disparity in the standard deviation is even

greater (3·5/1). This increase in the total iron content of bottom water has been shown to be due neither to the reducible nor to the dispersed unreducible fraction. It can be due only to a coarsely particulate unreducible fraction present only in the bottom water, amounting to 32 units with the very large standard deviation of 36 units. Since this fraction is only set free after oxidation with bromine water it is probably contained in particles of organic detritus stirred up from the bottom deposits by turbulent water movements.

TABLE III

	Upper layers (0–50 m.)		Intermediate layer (60–75 m.)		Bottom layer	
	No. of analyses	Mean and S.D.	No. of analyses	Mean and S.D.	No. of analyses	Mean and S.D.
Total iron	68	25 ± 11	8	46 ± 17	23	51 ± 38
Reducible iron	31	19 ± 14	4	14 ± 9	8	13 ± 14
Dispersed unreducible iron (max.)	—	6	—	6	—	6
Coarsely particulate iron (min.)	—	0	—	26 (± 15)	—	32 (± 36)

The few samples from 'intermediate' depths (60–75 m. depth and 23–37 m. above the sea-bed) reveal the same story but, since the standard deviation of the particulate unreducible fraction is less (± 15 compared with ± 36 units), the average size of the particles must also be less than nearer the bottom. There is therefore a vertical gradient in particle size from the upper layers towards the bottom. The density of the water column in January 1947 was uniform; consequently to attain such a distribution these particles must have been slightly heavier than water.

The bottom (75 m.) analysis for reducible iron (79 units) at station X1 has been omitted from the statistical examination but, none the less, it seems to have meaning. The 150 ml. sample must have contained one or possibly two large particles containing 0·1 μg.-atom of reducible iron. Such a particle would almost certainly be suitable chemically for direct assimilation by plants; physically it must be far too large to be handled by any single cell for it would be sufficient to meet the needs of a strong growth of phytoplankton in many litres of water. The part played by such particles cannot at present be fairly assessed.

To sum up, the bottom water contained:

(*a*) A well dispersed unreducible organic iron fraction amounting to about 6 units.

(*b*) A particulate reducible fraction amounting to about 13 units. This was probably flocculent ferric hydroxide or phosphate.

(*c*) A coarsely particulate or detrital unreducible fraction amounting to about 30 units.

(*d*) Occasional relatively large particles containing much reducible iron.

Ferric hydroxide or phosphate, precipitated, flocculated or excreted at mid-depths, will be caught by filter-feeding zooplankton, adsorbed upon phytoplankton or will settle slowly to the bottom. There, turbulence due to tides, currents and other water movements tend to keep these particles in intermittent movement. Sessile and free-swimming animals feeding on and near the bottom will also add their quota of iron-containing faeces. During passage through the intestines of filter feeders, some of the iron may be dissolved by acid digestive juices and excreted in solution whilst some will become incorporated in an organic matrix of mucilage and waste products which, after excretion, will tend to be broken down by bacteria so dispersing the iron in finer particles. The distribution of iron amongst these several fractions in the bottom water therefore must provide the key to the return of iron into circulation in shelf waters.

The earlier diatom outbursts in coastal areas believed by Gran (1931, 1933) to be due to iron derived from the land could equally well be ascribed to iron derived by vertical mixing from such an iron-rich layer of bottom water on a shelf. A heavy storm should increase the iron content of the photosynthetic layers at the expense of the bottom water and deposits. This may be the meaning of the very high and erratic results at station A3 (cf. Fig. 2).

Possible Removal of Particulate Iron from Bottom Water by Molluscs

Station X4 (49° 30′ N., 5° 00′ W.) is unique, for there in the bottom water triplicate analyses failed to detect any reducible iron, total iron was very low (the duplicate analyses of 8 and 11 units being 15 units less than in the overlying water) whilst the phosphate distribution indicates active removal of particulate ferric phosphate from the water (p. 299). Nothing remotely like this was found at any other station; that this water was of 'south-western' origin is no explanation. The absence of particulate matter near the bottom called to mind the conditions in the mussel-purification tanks at Conway, North Wales, when the molluscs placed in turbid estuarine water have been actively at work for a few hours. No physical cause for the state of affairs at station X4 is to be seen so that the conclusion that the water had been clarified by a large population of bottom suspension feeders seems the only reasonable alternative.

Mr H. P. Sherwood (private communication) at Conway placed a single mussel at the bottom of a litre-measuring cylinder about 0·4 m. high and covered it with a thick suspension of red oxide (Fe_2O_3) in estuarine water. In 1 hr. the mussel had cleared much of the suspension from the water whilst in 2 hr. no trace of the opacity could be seen. Given a certain amount of tidal mixing a bed of mussels should have no difficulty in the course of a few days in clarifying a bottom layer of water 5–10 m. thick.

Mussels do not live at a depth of 90 m. but, according to Mr G. A. Steven, an organism with similar feeding habits, the large mollusc, *Pinna*, does.

Capt. Creese of the Laboratory's research vessel *Sabella*, when asked where he would look for *Pinna*, gave a ground including the position of station X4 and no other position investigated during the winter cruises. Furthermore, on 20 July 1936 at station 1, well within this ground, zero reducible and total iron was found at the bottom (Table V). If a bed of molluscs, such as *Pinna*, does have such a marked effect on the iron content of the bottom water, further lines of investigation would open up. Acting over a period of time the molluscs would strip the iron from the water column, converting it first into faeces and much pseudo-faeces which would tend to accumulate in bottom deposits and return only slowly to the water. Failing replenishment by water from areas outside the beds, the surface flora would in time be adversely affected both qualitatively and quantitatively by shortage of iron. Thus, in certain hydrological circumstances, an inverse relationship may develop between a dense bottom molluscan fauna and the phytoplankton production many metres above. Such an area may prove to be adverse to early spring outbursts of phytoplankton (cf. p. 287).

Distribution and Size of Large Particles of Ferric Hydroxide

The whole of the variability of total iron in the uppermost 50 m. appears to be due to the reducible fraction composed, presumably, of flocks of ferric hydroxide and phosphate. In January 1947 it amounted to 19·3 units (mg.-atom/m.^{3} × 100) (p. 310). The dispersed unreducible fraction (6 units) contributed little to the variance[1] of total iron for which most analyses are available. However, the variance for neither total nor reducible iron as calculated in Appendices II and III is appropriate for calculating the average size and numbers of the particles, since variation between stations would be included. Eight samples drawn from between 0 and 50 m. had been analysed in triplicate or quadruplicate for total iron, viz. A1, 5 m.; A1, 25 m.; A7, 5 m.; A7, 25 m.; C4, 50 m.; X4, 5 m.; X4, 25 m. and X4, 50 m. For these there are twenty-five analyses on sub-samples, eight means and seventeen degrees of freedom, the variance between subsamples being 64·8 square units (p. 310).

A fair estimate of the variance introduced by the chemical technique is to be had from Appendix I, Table XIII. The standard deviation of 3·08 units for station E1, 25 m. Series 1 corresponds to a variance of 9·5 square units. Therefore 64·8 less 9·5 (=55·3) is the final estimate of the variance between different readings of total iron from one position in the water column when the size of each water sample is 150 ml. That is, 55·3 represents the sampling variance for iron in a volume of 150 ml. of sea water in the western English Channel in January 1947. Furthermore, the amount of iron subjected to this

[1] The limitation that the variance applies only to the sample volume of 150 ml. needs constantly to be borne in mind, e.g. it is not a function of the cubic metre.

sampling variance is 19·3 units. A variance of such magnitude (55·3), it would seem, can arise only because the iron is aggregated in particles. Two questions may be posed: (1) how many particles are there? and (2) what is their size expressed in terms of their iron content?

According to Mr G. M. Spooner (private communication) random variation in numbers of particles dispersed in a volume of fluid (of which they form an insignificant fraction) may be expected to conform to the Poisson distribution.

The Poisson frequency distribution is a special limiting case of the binomial distribution, derived from the binomial expansion $(p+q)^n$, when p is a very small fraction but n correspondingly large so that np remains an ordinary number. In our example the volume of water is regarded as an aggregate of a large number (n) of particle-sized units, of which a small proportion (p) belong to the special iron-containing particles under investigation, the rest, a proportion q, to the medium. The mean number of iron-containing particles in the given volume is np. The variance amongst different samples is also np, since the general binomial variance of npq becomes np when q is not significantly different from unity. It is a characteristic property of the Poisson distribution that the Mean and Variance are equal. Identical values should therefore be obtained for the mean and variance when these are expressed as numbers of particles instead of as weights of iron per unit volume.

In the present instance the mean is 19·3 units of iron and the variance 55·3 sq.units. If it is supposed that the iron is distributed in a number of equal aggregates or particles, it at once follows that 55·3/19·3 (=2·865 units) represents the amount of iron per particle. (Converting by the factor given in the footnote to Table II this equals 0·240 μg. per particle.) There will thus be a mean number of 19·3/2·865 (=6·74) particles per 150 ml. This is equivalent to 45 particles per litre. This estimated mean number will scarcely be affected even if there is an appreciable range of variation in either particle-size or in iron-content of particles. An assessment of the accuracy of this result, not rigorously statistical, suggests that the number of particles could just possibly have been as low as 30 or as high as 80 particles per litre and almost certainly was not outside these limits.

To arrive at a rough estimate of the size of such a particle let us assume it to have a specific gravity of 1·1 and an iron content of 10%. Such a particle would occupy

$$\frac{0{\cdot}24 \times 10^{-6} \times 10}{1{\cdot}1} = 2{\cdot}18 \times 10^{-6} \text{ c.c.}$$

If the particle is spherical and of radius r, then $r = 0{\cdot}08$ mm.

That is, there were about 45 large particles per litre of water with a diameter averaging about 160 μ. If the iron content had been assumed to be $1\frac{1}{4}$% instead of 10%, this diameter would have worked out at 320 μ.

The result means that in the sea water of the English Channel between the surface and 50 m. at midwinter in 1946–47 four-fifths of the iron was

associated with a very few large particles. We have to ask ourselves whether such a surprising result is possible and what such particles might be.

No collections for phytoplankton were made but, during the first two cruises, Mr P. S. B. Digby was on board and made some quantitative collections between 50 m. and the surface for his studies upon copepods. He used a Harvey quantitative net with 120 meshes per linear inch. Such a net would catch most, though not all, of the particles of the size we are considering. Two of these catches were generously placed at my disposal and Dr T. J. Hart has kindly examined them for me. In the catch from station C4 he found 45 'large particles' per litre which had diameters of the order of 100 μ. This number is minimal and half made up by partially disintegrated colonies of *Phaeocystis*. In the catch from station A7 he was able to find only nine such particles per litre; nearly half were faecal pellets whilst a quarter were cells of the alga *Halosphaera viridis* (average 120 μ).

That diatoms may heavily adsorb ferric hydroxide and phosphate is known (Harvey, 1937*a*) but whether these particles did in fact contain sufficient iron to account for our analytical findings is not known. The material was collected for very different ends and no exact agreement could be expected. All that can be said is that particles in sufficient numbers and of the right size were present to make the analytical results not unreasonable.

As the spring advanced the numbers of large diatoms, of large herbivores and of their faecal pellets must have increased rapidly. The number of smaller organisms also would have increased. The iron associated with living matter must have passed through many cycles in the course of the spring, though only a small part may have been truly metabolized by plants or animals. The iron would have become distributed amongst a larger and larger number of organisms and faecal pellets whilst the percentage content of each one, on an average, would have become less and less. So far as iron is concerned, this is equivalent to more and smaller particles. With about 370 particles or organisms per litre the variance would become about 1 sq.unit and any possibility of establishing the presence of particulate iron by statistical study of replicate chemical analyses would vanish. There is therefore less chance of establishing the thesis in these waters at seasons other than midwinter.

Between 9 March and 14 November 1934, analyses of iron were carried out upon catches made at station L4 with the Harvey quantitative net (200 meshes per linear inch). The maximum iron content was found on 20 March coincident with the phytoplankton maximum, 41 μg.-atom per cu.m. of water filtered ($\equiv$4·1 units) (Harvey *et al.* 1935; Cooper 1935). During the next fortnight the plankton-iron fell to one-third whereas the plankton-phosphate remained almost constant. As the spring advanced the ratio of iron to phosphate expressed in gram-atoms fell from 2·55 to 0·45, whilst during the autumn it rose from 0·48 to 1·93 on 14 November. It is likely therefore that at midwinter the ratio would have been still higher. None the less, the gap

between the observed maximum of 4·1 units caught with the Harvey net on 20 March 1934 and 19·3 units indicated by the present study at midwinter 1946–47 is a large one to bridge.

The analytical method has been tested under conditions where the iron has intentionally been kept as highly dispersed as possible. There is evidence, not consistent enough to publish, that if an iron solution, ferric or ferrous, is added to sea water at its natural pH around 8·1, some of the added iron becomes undeterminable. Results agree poorly. Possibly some iron flocculates, though not visibly to the naked eye, and the method does not quickly attack these flocks (cf. Buch, 1942). If flocks or ferruginous particles are present analytical errors may be exaggerated, whereas with well-dispersed iron they will not arise. In such case, the general picture outlined here is correct but the variances may have been somewhat exaggerated so that the iron in the sea may have been distributed amongst more and somewhat smaller particles than the present study has suggested.

The Horizontal Distribution of Iron

The horizontal distribution of phosphate or other truly dissolved nutrient is best studied in terms of the integral mean content of the vertical water column which takes account of the unequal spacing of standard depths (Cooper, 1933, p. 722, as 'average nutrient salt content of the water column'). For a randomly distributed constituent such as total iron, this integral mean would have value only if arithmetic means of a large number of analyses were first available at each depth and station. The most convenient measure of the total stock of iron in the English Channel in winter is therefore the simplest, the arithmetic mean of all analyses at a station irrespective of depth. No clear picture emerges from charting single stations, but one can be obtained by arranging the data for stations at which iron and phosphate analyses are both available in three groups of increasing phosphate content (Table IV, fourth column).

Table IV. Ratio of Total Iron to Phosphate

(Mg.-atom/m.³ Fe and P × 100)

No. of stations n_1 (1)	No. of analyses P (2)	No. of analyses Fe n_2 (3)	Phosphate integral mean for whole water column: Range (4)	Phosphate integral mean for whole water column: Average for n_1 stations (5)	Total iron: Arith. mean of n_2 detns. (6)	Total iron: S.D. (7)	Total iron: S.E. of mean (8)	Ratio: Fe/P (9)
5	23	29	**37–50**	43·4	24·9	14·0	2·6	0·57
6	39	33	**51–55**	53·2	28·7	18·3	3·2	0·54
5	27	35	**56–63**	59·4	35·7	15·0	2·5	0·60

The standard error of the means (col. 8) shows that the increase in total iron with phosphate given in the sixth column is just statistically significant. The inclusion of bottom analyses somewhat increases the standard deviations

(col. 7) compared with those for 0–50 m. in Table XIV. The ratios of total iron to phosphate (arithmetic mean average of integral means) (col. 9) are not far from constant. The analyses of the midwinter maximum of phosphate therefore gave a measure of the statistical probability of occurrence of total iron (0·57 × concentration of phosphate). At any one station the total iron calculated from this ratio is as likely to represent the total iron content as the limited number of analyses actually performed.

For computing Table IV, stations C1 and C2 were treated as one and, in addition to station A3 (p. 282), the analyses at A1, 70 m. (130 units) and A7, 100 m. (189 units) were omitted. These analyses were grossly at variance with replicates and their inclusion, whilst increasing the spread of the mean of total iron in the fifth column, nearly doubles the standard errors of the mean in the two higher groups. Both analyses were on bottom water making it likely that single particles of bottom detritus of very high iron content were caught by the bottle; both are better left out.

The Distribution of Iron in 1933–34

When the 1933–34 work was reported (Cooper, 1935), the reason for the erratic results obtained was not clearly understood and to economize space figures for the average content of the water column only were presented. Though the data are not sufficiently homogeneous for a satisfactory statistical study, further information of value for the present account may be had from them.

(1) On 15 December 1933, at all the stations between L1 and L5 (Eddystone) and at E1, the total iron content of surface water sampled *with a wooden bucket* was high (32–43 units), whilst that at 5, 50 and 69 m. was a mere 7–9 units suggesting that about 30 units was in some way associated with the surface layer.

(2) On 12 February 1934 at stations L4, L6, E1 and at a position midway between E1 and E2 erratic results were obtained, the surface results for total and reducible iron being especially erratic and high.

(3) On 20 March 1934 at the peak of the spring outburst at L4 surface total iron was higher by 54 units than in deeper water whilst at E1 the water at 5 m. contained none. Thence to the bottom there was a graded increase to the very high bottom content of 54 units. At all depths reducible and total iron were identical. Clearly the diatoms had stripped the water in their zone of growth of iron which had found its way to the bottom in a readily attackable form.

The Surface Film of Ferric Hydroxide

In 1933–34 concentration of iron in the surface layers was frequent. Yet at midwinter 1946–47 statistical examination showed no such surface concentration. The answer to this contradiction arises from the different methods of

surface sampling used in the two years. In 1946–47 all surface samples were taken with the Nansen-Pettersson water-bottle submerged just beneath the surface and not breaking water. It sampled the uppermost metre of water but not the surface film. The wooden bucket used throughout 1933–34 definitely included an area of the surface film to which it is therefore reasonable to attribute the high surface results in that year.

Harvey (1937*b*) has shown that an unprotected sol of ferric hydroxide in sea water is electropositive whilst a similar sol protected by gum arabic is electronegative. A sol afforded definite protection by albumen carried either no charge or one too small to be measured with the apparatus employed. Mokrushin (1947) has found that on ageing colloidal solutions of positively charged ferric hydroxide a very thin invisible surface film is formed spontaneously by coagulation, whereas no such film is formed on sols of negatively charged ferric hydroxide. From these and many other observations he has concluded that this surface coagulation of electropositive sols results in the formation of a uni-micellar film, at first only a network—later becoming a compact solid film. The thickness of this layer of ferric hydroxide was 3–4 mμ and it was further deduced that micelles in the hydrosol were lamellar or plate-like with a diameter of 30–40 mμ. One such micelle would weigh about 10^{-17} g. and cannot be identified with the particles already discussed. A solid film of ferric hydroxide, 1 sq.cm. in area, 3·5 mμ thick and having a specific gravity of 3·5 would weigh 1·0 μg.

The wooden bucket used for surface sampling was about 30 cm. in diameter and was usually filled to a depth of about 20 cm. or a volume of 14 l. If we suppose that (*a*) the sea surface was uniformly covered with such a unimicellar layer, (*b*) that a disk of the surface 30 cm. in diameter (containing $\pi \times 15^2 \times 1{\cdot}0\,\mu\text{g.} = 700\,\mu$g. ferric hydroxide) was sampled by the bucket and (*c*) that pouring from bucket to storage bottle led to fair sampling of the superficial and bulk phases, then 700 μg. ferric hydroxide would have become distributed in 14 l. of water. That is, each litre would appear to contain 50 μg. ferric hydroxide or 0·47 μg.-atom of Fe. This figure gives a rough maximum for the amount of iron present in a sample taken from the surface by dipping up with a bucket and attributable to a surface unimicellar film of electropositive unprotected ferric hydroxide. The results of 15 December 1933 would agree with two-thirds of the surface of the sea being covered by such a film. Naturally only the order of magnitude of this calculation has significance, but its essential soundness is supported by Dr W. R. G. Atkins's observation (private communication) that the iridescent 'oily' film frequently observed on bog ponds consists largely of ferric hydroxide. This is apparently well known to limnologists.

TABLE V. HYDROLOGICAL DATA, CRUISE OF 20–22 JULY 1936

Station no.	Position	Date and time (G.M.T.) July	Depth (m.)	Temp. (° C.)	Sal. (‰)	PO_4-P (mg.-atom/m.³ × 100)	Iron (mg.-atom/m.³ × 100)			
							Total	Reducible		
								Unfiltered water	Membrane filtered at pH 8	Membrane filtered at pH 4
1	50° 08′ 30″ N., 4° 39′ 30″ W.	20, 1415	10	13·4	34·50	4	4	4	—	—
			65	10·9	34·59	19	0	0	—	—
2	49° 56′ N., 5° 13′ W.	20, 1750	10	11·65	34·67	16	13	9	—	—
			60	11·6	34·70	39	2	2	—	—
3	49° 47′ N., 5° 22′ 10″ W.	20, 2000	10	13·4	34·80	9	4	4	—	—
			85	11·52	35·18	31	23	23	—	—
4	49° 29′ 40″ N., 5° 38′ 20″ W.	20, 2330	10	15·57	34·99	6	21	0	—	—
			80	11·28	35·28	31	0	0	—	—
5	49° 13′ 20″ N., 5° 54′ 30″ W.	21, 0310	10	15·57	34·97	5	38	9	—	—
			90	11·0	34·39	56	36	11	—	—
6	49° 08′ 20″ N., 6° 24′ W.	21, 0655	10	15·0	34·98	4	23	9	—	—
			100	10·71	35·44	45	43	11	—	—
7	49° 25′ 40″ N., 6° 9′ 30″ W.	21, 1040	10	15·25	34·92	5	16	16	—	—
			100	10·87	35·34	38	18	9	—	—
8	49° 43′ N., 5° 55′ W.	21, 1410	10	14·3	34·99	3	18	9	—	—
			80	11·1	35·32	32	21	30	—	—
11	50° 2′ 30″ N., 4° 26′ 40″ W.	22, 1220	0	15·0	34·45	6	16	2	0	16
			5	14·1	34·45	6	16	2	0	9
			10	13·15	34·47	7	18	4	0	4
			25	11·85	34·61	24	2	2	0	2
			50	—	34·58	24	9	0	0	0
			65	11·29	34·56	24	0	0	0	2

The Distribution of Iron in July 1936

In July 1936 a cruise was made by S.S. *Salpa* to the southward of the Scilly Isles with Mr F. S. Russell and Mr P. H. T. Hartley on board. Ten stations were worked with the young fish trawl and eight with the Nansen-Pettersson water-bottle at 10 m. and near the bottom. The temperature, salinity (analyses by the Government Chemist), inorganic phosphate and reducible and total iron, determined on the water samples, are given in Table V. Station 11, about 3 miles to the westward from the position of station E 1, was worked for a full hydrographical depth series, but not for the young fish trawl.

Since there was a strongly marked thermocline throughout the area except at station 2, no physical or chemical resemblance need be expected between the water at 10 m. and that at the bottom. The young fish trawl may sample both layers and, in any event, the two species of *Sagitta*, of such value in characterizing water masses in the area, migrate daily across the thermocline. One species though perhaps initially associated with only one layer may become apparently associated with both. It is necessary, therefore, to compare the *Sagitta* distribution with the hydrological data for both layers separately.

(*a*) *Comparison in Bottom Waters*

The bottom waters may be divided into three clearly contrasted groups (Table VI).

Table VI. Hydrological Observations at the Bottom on 20–22 July 1936 compared with Catches of *Sagitta*

Sal. (‰)	Station	Depth (m.)	Phosphate (mg.-atom/ $m.^3 P \times 100$)	Iron (mg.-atom/$m.^3 \times 100$) Reducible	Total	*Sagitta* *elegans*	*Sagitta* *setosa*
Low salinity group:							
34·56	11	65	24	0	0	Not determined	
34·59	1	65	19	0	0	92	11
34·70	2	60	39	2	2	342	12
Medium salinity group:							
35·18	3	85	31	23	23	5	1
35·28	4	80	31	0	0	284*	0
35·32	8	80	32	30	21	0	0
35·34	7	100	38	9	18	54	0
High salinity group:							
35·39	5	90	56	11	36	970*	0
35·44	6	100	45	11	43	4110	0

* Night stations.

Low salinity group. Three stations of very low salinity extending right to the bottom, relatively low phosphate, very low or zero reducible and total iron and a few *Sagitta setosa*. *S. elegans* was present in numbers exceeding *S. setosa*.

Medium salinity group. Four stations with bottom salinity about 35·3 ‰, intermediate content of phosphate and of reducible and total iron and only a single *S. setosa*. *S. elegans* was present at three of the stations.

High salinity group. The two most westerly stations with salinities 35·39 and 35·44 ‰ had high phosphate and total iron, a large difference between reducible and total iron (28 units), no *S. setosa* and a great many *S. elegans*. These last stations were evidently occupied by truly 'elegans' water. Their high unreducible iron content supports the 1946–47 midwinter results in showing 'elegans' water to be relatively rich in this form of iron at the bottom.

(*b*) *Comparison of* 10 *m. Observations*

The 10 m. results are most conveniently arranged in ascending order of total iron content (Table VII). It will be seen than low total iron is associated

TABLE VII. HYDROLOGICAL OBSERVATIONS AT 10 M. DEPTH ON 20–22 JULY 1936 COMPARED WITH CATCHES OF *SAGITTA*

Iron (mg.-atom/m.³ × 100)				*Sagitta*		Phosphate (mg.-atom/ m.³ × 100)
Total	Reducible	Station	Salinity (‰)	*elegans*	*setosa*	
Low total iron and salinity group:						
4	4	3	34·80	5	1	9
4	4	1	34·50	92	11	4
13	9	2	34·67	342	12	16
18	4	11	34·45	Not determined		6
High total iron and salinity group:						
16	16	7	34·92	54	0	5
18	9	8	34·99	0	0	3
21	0	4	34·99	284*	0	6
23	9	6	34·98	4110	0	4
38	9	5	34·97	970*	0	5

* Night stations.

with the lower salinity water and presence of a few *S. setosa*. Relatively high total iron is associated with higher salinity water, complete absence of *S. setosa* and includes the stations with large catches of *S. elegans*. Reducible iron and phosphate tell little, but as far as a correlation can be seen low total iron marches with low reducible iron and relatively high and erratic phosphate, high total iron with relatively high reducible iron and uniformly low phosphate (3–6 mg.-atom/m.³).

The fresh water which had reduced the salinity of the stations nearer Plymouth had in no way enriched the store of iron; there the slender stock of iron was all reducible. The typical 'elegans' water at 6° W. was much richer in both total iron and phosphate but the additional iron was mostly unreducible.

The complete vertical series at station 11 near E 1 is of interest. In one set of analyses the sea water was brought to pH 4 (required acid determined by titration with bromophenol blue as indicator to an end-point matched against

the same indicator in M/20 potassium hydrogen phthalate) and left for 30 min. before filtration through a collodion membrane of filtration time 20 sec. Whereas membrane filtration at pH 8·1 removed all the iron, at pH 4 part had become dissolved and passed into the filtrate. Again nearly all the iron, in marked contrast to phosphate, had become concentrated above the thermocline. This partition of iron upwards is difficult to understand on physical grounds.

Comparable Results from North American Waters

Rakestraw, Mahncke & Beach (1936) filtered a number of samples through Whatman No. 42 filter paper and then analysed the content of iron by co-precipitation of ferrous sulphide with magnesium hydroxide. Water from the Gulf of Maine, all collected in summer, contained very little iron; usually it was zero. Positive results (8–20 units) were, however, found on a number of surface samples, but whether these included the surface film or were more truly 0·5 m. samples is not stated. Filtered samples from four deep stations in the Atlantic Ocean off New England were also analysed. Here also there was a tendency for surface results to be high and at other depths ranging from 50 to 3830 m. iron was always detectable (2–25 units). Thompson & Bremner (1935*b*), 25–100 miles off the continental shelf in the north-east Pacific, obtained very similar results for 'soluble' iron, i.e. iron which had passed a Whatman no. 42 filter paper. Seiwell (1935), by a method which included iron in detritus and organisms trapped in the water-bottle and is similar to the method for 'total' iron of the present paper, examined waters collected in December and August, also from the Gulf of Maine. The distribution there in December 1933 was similar to that in the English Channel in December–January 1946–47. For two stations at which phosphate analyses were also made (Conseil International, 1933) the total-iron/phosphate ratio may be worked out. At station 1880 (42° 45′ N., 68° 48′ W.) on 6 December 1933, the uppermost 50 m. showed uniform distribution of salinity (33·06 ‰), temperature (7·14° C.) and phosphate (99 units) and high and *variable* iron content yielding a Fe/P ratio ranging from 0·67 and 0·91. In the underlying water of markedly different structure, this ratio was only 0·44. A position 70 miles to the eastward at station 1897 (42° 25′ N., 67° 06′ W.) was characterized by a very much lower ratio (0·13–0·25), not notably affected by a marked discontinuity at 100 m. Comparable English Channel ratios are summarized in Table IV. It is evident that the relative abundance of iron and phosphate varies in different parts of the world much more than do nitrate and phosphate.

Particulate Ferric Phosphate

Occasionally in phosphate analyses, results are obtained which appear high compared with those from adjacent positions, vertically and horizontally. Contamination, or the widely recognized difficulties inherent in methods

involving complex molybdates, provide explanations ready to hand. Routine calibration runs to determine salt error in phosphate analyses are now made in triplicate to enable 'untrustworthy' results to be rejected. It would now seem that these causes have been invoked too frequently, at any rate by the writer, to explain what is a truly variable property of the sea water itself.

Harvey (1937*a*) has pointed out that ferric phosphate as well as hydroxide is likely to be present in sea water in particulate form. If the distribution of ferric phosphate is as erratic as that of total iron, we should have an explanation of these 'untrustworthy' phosphate analyses. Cruise X provided several such, with vertical variations greater than would be anticipated in isothermal, isohaline water in unsettled weather. Let us assume that dissolved inorganic phosphate was indeed vertically uniformly distributed under the isothermal conditions obtaining and was represented by the smallest analysis found in each vertical series. Phosphate in other samples or sub-samples in excess of this smallest value would then represent particulate ferric phosphate randomly distributed. Although there is no clear correlation between this and the total iron content, one may be found with the total iron in excess of the smallest analysis for total iron found for each vertical series. Table VIII shows the first step in the calculation for station X4.

TABLE VIII. APPROXIMATE CALCULATION OF PARTICULATE FERRIC PHOSPHATE AT STATION X4

(mg.-mol./m.^{3} $FePO_4 \times 100$) or (mg.-atom/m.^{3} Fe or P $\times$ 100)

				Total iron			
Depth (m.)	Phosphate found	Assumed dissolved phosphate	Particulate $FePO_4$	Found	Mean	Smallest analysis	Excess over smallest analysis
0	40·0	37·1	2·9	21	21·0	8	13·0
5	39·1	37·1	2·0	24, 29, 32	28·3	8	20·3
25	43·5	37·1	6·4	22, 34, 16	24·0	8	16·0
50	44·3, 42·2	37·1	7·2, 5·1	27, 16, 33	25·3	8	17·3
90	37·6, 37·1, 37·3	37·1	0·5, 0·0, 0·2	8, 11	9·5	8	1·5

The 'particulate $FePO_4$' (col. 4) found in this way from all separate phosphate analyses for the five Cruise X stations respectively were then arranged in groups of increasing $FePO_4$ content and compared with the corresponding mean 'excess total iron' figures (as in the last column of Table VIII). The result of the calculation is set out in Table IX. Figures such as the duplicate phosphate results at station X4, 50 m. were entered twice as 7·2 and 5·1, the same excess total iron figure, 17·3, being entered for both.

The results for station X5, 5 m. ('particulate $FePO_4$', 0, and excess total iron, 30 units) are anomalous and affect the average for the first group in the way shown. The inference is strong that the irregularities in the determinations of inorganic phosphate are in fact due to random occurrence of particulate ferric

phosphate and that this particulate ferric phosphate is associated with the largest particles or least well-dispersed fraction of the particulate iron. The ferric phosphate was most probably included in faecal pellets (Cooper, 1948*b*).

TABLE IX. RELATION BETWEEN PARTICULATE $FePO_4$ DEDUCED FROM PHOSPHATE ANALYSES AND 'EXCESS TOTAL IRON'

(mg.-atom/m.^{3}) or (mg.-mol./m.^{3}) × 100

'Particulate $FePO_4$' range	No. of iron analyses	Excess total iron, average with standard deviation
0–1·1	8	3·9 (excluding X5, 5 m.) ± 3·8
	9	6·8 (including X5, 5 m.)
2·0–3·8	8	13·0 ± 8·9
4·4–7·8	10	17·1 ± 8·6
8·6–11·8	4	27·7 ± 17·9

The assumption that the sample from each station giving the lowest analysis for phosphate contained no particulate $FePO_4$ is, in general, unlikely to be true. The estimates of the distribution of ferric phosphate at the Cruise X stations set out in Table X are therefore minimal.

TABLE X. MINIMAL PARTICULATE FERRIC PHOSPHATE CONTENT AT STATIONS ON CRUISE X

(mg.-mol./m.^{3} × 100)

Station ...	X1	X2	X3	X4	X5	Mean
Depth (m.)						
0	0	—	0	2·9	4	1·7
5	4	9, 0, 5, 0	1	2·0	0	2·1
25	6	7	4	6·4	1	4·9
50	0, 9	4, 0	3	7·2, 5·1	3, 2	3·6
70	12	9	1	—	—	
90	—	—	—	0·5, 0, 0·2	—	5·7*
95	—	—	—	—	1·1, 0·7	

* Excluding X4, 90 m.; see below.

Particulate ferric phosphate, like total iron, may possibly be greatest in bottom water. Similar but less clear-cut deductions may be drawn from other stations worked in January 1947.

For station X4 evidence has already been produced (p. 287) that the very low reducible and total iron in the bottom-most layer of water (90 m.) might be attributed to removal of particulate iron by bottom-dwelling animals, such as the suspension feeding mollusc, *Pinna*. At the time of analysis the low bottom phosphate, compared with samples from 25 and 50 m., was disconcerting, resulting in triplicate analyses of bottom water made 43, 44 and 62 hr. after collection. The very close agreement, 37·6, 37·1 and 37·3 units now suggests that *Pinna* had done their work well, leaving no particulate phosphate at all in the bottom water. Under the isothermal, isohaline conditions prevailing, 37·3 units appears to be a precise measure of the truly dissolved inorganic phosphate of the water column. If this premise may be accepted,

the results for station X4 in Table X represent not the minimal but the actual distribution of particulate ferric phosphate, amounting at 50 m. to as much as 6 units (= 0·06 mg.-mol./m.^{3} $FePO_4$).

Classification of the Forms of Iron in Sea Water

The forms of iron which may be present in sea water may be classified as follows:

A. *Uniformly dispersed iron*

(1) *Ionic iron.* Cationic iron, Fe^{++}, $FeOH^{++}$ and Fe^{+++} in true solution in sea water at equilibrium will amount to not more than 10^{-8} mg.-atom/m.^{3} (Cooper, 1937). Since equilibrium is only slowly attained, somewhat more may exist in sea water in which iron is in process of regeneration and redispersion. Ferrite anion, H_2FeO_3', may also perhaps be present in similar amount (Cooper, 1948*b*). Ionic iron is never likely to be detectable by chemical analysis.

(2) *Dissolved inorganic complexes.* Ferrifluoride is readily hydrolysable by sea water (Cooper, 1948*b*) and no evidence for any other inorganic complexes has yet been produced.

(3) *Dissolved crystalloidal or dispersed colloidal organic compounds.* Ferricitrate, when added to sea water, proves to be one of the less readily hydrolysed complexes of iron and there is indirect evidence of the existence of citrate in the sea. Crystalline calcium citrate tetrahydrate was dredged by R.R.S. *Discovery II* at station 417, 71° 22′ S., 16° 34′ W. in 2580 m. and appears quite definitely to have been formed *in situ* (Bannister & Hey, 1936). The bottom water of the Weddell Sea therefore contains sufficient citrate-ion to exceed the solubility product of calcium citrate. Ferricitrate may therefore exist in natural sea water. Breakdown products of haemoglobin and cytochrome such as haematin are also likely. Haemoglobin and haematin have both been found to be unusable as sources of iron by diatoms (Harvey, 1945, p. 35). The iron found in filtered deep ocean water by Rakestraw *et al.* (1936) and Thompson & Bremner (1935*b*) may well be attributable to organic iron which may or may not be directly assimilable by plants.

(4) *Colloidal inorganic compounds* such as ferric hydroxide or phosphate held in a dispersed sol by mucilaginous or other organic 'protective' colloids. Harvey (1937*b*) has shown that hydroxylic substances impart considerable protection to ferric hydroxide sols which often then become electronegative. Given time enough they all hydrolyse and ferric hydroxide precipitates.

B. *Aggregated or particulate iron*

(1) *Discrete flocculent particles of ferric hydroxide or phosphate.* There is good evidence for the existence of both in sea water (Harvey, 1937*a*, *b*).

(2) *A unimicellar film of ferric hydroxide*, electropositive and unprotected by organic colloids, covering a considerable part of the sea surface.

(3) *Ferric hydroxide or phosphate adsorbed* upon the surface of organic or inorganic detritus, upon living diatoms (Harvey, 1937*a*) or upon other phytoplankton.

(4) *Iron in faecal pellets.* The particulate ferric phosphate indicated by analysis is likely to be associated with, or to arise from, faecal pellets (see Cooper, 1948*b*). Since the gut of many marine animals is considerably more acid than sea water, iron and phosphate there meet under conditions well suited to the formation and precipitation of ferric phosphate. There also ferric hydroxide and phosphate will receive 'protection' from slime and mucilage. It remains to be seen what happens to iron when bacteria break down excreta, returning the more soluble components to the water.

These four fractions must together be mostly responsible for the 'coarsely particulate reducible' fraction amounting at midwinter to 0·19 ± (S.D.) 0·12 mg.-atom/m.^{3} in the 0 to 50 m. layers. In summer water at least, much of this particulate reducible iron is soluble at pH 4. Many of the particles must be large, 100 μ or more in diameter.

(5) *Organically bound iron* in the cells and tissues of living and dead plants and animals. With this fraction and perhaps with faecal pellets also may be identified the 'very coarsely particulate unreducible fraction' found in the bottom water, there amounting to about 0·32 ± (S.D.) 0·36 mg.-atom/m.^{3}. There must be some such iron in the upper waters also.

(6) *Terrigeneous iron* in clay and suspended mineral matter derived from the land. Such, in general, will not be determinable by the methods employed here though they will figure in analyses by the methods of T. G. Thompson and his collaborators.

Possible Lines of Advance

Assessment of the distribution of iron and of a plankton population by examination of small samples of water suffer from similar drawbacks. Only very large numbers of samples can overcome the great variability due to swarming or concentration in some places and not in others of plants, animals and their excretory products. Further analyses of sea water for iron by existing methods do not therefore offer the most promising line of advance.

Single particles—diatoms, faecal pellets, organic and inorganic detritus—need to be examined by microscopic and microchemical techniques to assess the nature of the particulate distribution of iron. The particles in the bottom layers and their interchange with the upper layers especially need attention. The adsorption and assimilation of iron by diatoms, centric and pennate, flagellates and other planktonic algae demands further knowledge. Do the plants gather all the available inorganic iron to themselves leaving none for later generations except that arising from their own death? Must forms of iron other than hydroxide and phosphate first be hydrolysed? What is the function

of iron in auxospore formation and expansion? Are any organisms favoured by the concentration of iron and possibly ammonia (Cooper, 1948*a*) in the surface film? In what form is the iron in animal faeces and how quickly is this iron made available to plants? Again, do extensive beds of bottom-dwelling suspension feeders immobilize sufficient iron to affect the productivity of the overlying water?

Summary

With 2:2′-dipyridyl as reagent for reducible and total iron, determinations were made on samples from the western English Channel at midwinter 1946–47, at the time of the maximum of phosphate, and also in July 1936.

The midwinter results have been treated statistically and the errors of analysis assessed.

Unlike phosphate and nitrate, much of the inorganic iron occurs as particles of fair size, distributed at random in the water. In winter there is no statistically significant difference between the total iron content of samples drawn from 0·5, 5, 25 and 50 m. depth, the grand mean and standard deviation for the whole area being $0{\cdot}25 \pm 0{\cdot}11$ mg.-atom/m.^{3}. The reducible iron for the same depth range and area was $0{\cdot}19 \pm 0{\cdot}14$ mg.-atom/m.^{3}. There appears to be about 0·06 mg.-atom/m.^{3} of iron which is reasonably highly dispersed and not determinable until the water has been oxidized with bromine.

In the bottom layers of water, although the reducible fraction differs little from the overlying layers, the total iron shows a very much greater mean value and variance.

The very low content of total and reducible iron and of particulate ferric phosphate in bottom water at a position 49° 30′ N., 5° 00′ W. has been provisionally attributed to removal by a dense bed of suspension feeders, possibly the mollusc *Pinna*.

By a statistical examination of the analytical results it has been deduced that the particulate fraction in the upper 50 m. was distributed on an average amongst about 45 particles per litre, each containing about 0·24 μg. Fe and with a diameter of about 160 μ. This result has been compared with data on the distribution of iron in plankton organisms.

As a statistical average waters richer in phosphate were also richer in total iron, the atomic ratio Fe/P being about 0·57.

In July 1936 the 'elegans' water to the westward was much richer in both phosphate and total iron than the more eastern mixed water containing *Sagitta setosa*. The results agree well with the conclusions drawn from the cruises at midwinter 1946–47.

In 1933–34 concentration of iron was frequently observed in the surface layers; in 1946–47 it was not. In the earlier work the surface film was always cut and included in the sample, in the later it was not. The probable amount of

a surface unimicellar film of electropositive unprotected ferric hydroxide has been calculated and agrees well with observation.

Particulate ferric phosphate was found to the extent of about 0·06 mg.-mols/m.^{3} This appears to increase towards the bottom.

The success of the work at Newlyn in January 1947 owes much to the willing co-operation of Capt. Creese and the crew of R.V. *Sabella* under very adverse weather conditions. To Mr P. G. Corbin grateful thanks are due for undertaking single-handed the supervision of all the scientific work at sea on stations never more than two hours apart for two or three days on end. The statistical development would not have been possible without the unstinted assistance of Mr G. M. Spooner.

APPENDIX I

Statistical Methods Used and Correction Terms (see p. 281)

Two correction terms were needed, one for the extinction of light by the colour of ferrous-tris-dipyridyl formed from iron impurities in the reagents and determinable in freshly distilled water, the other for the turbidity or extinction due to scattering and absorption by substances dissolved or suspended in the sea-water samples. These were determined separately and the combined extinctions subtracted from the extinctions observed with the sea-water samples plus reagents. To measure each of these corrections at all accurately taxes the instrument and the observer, and in view of shortage of time four samples only from each cruise were examined for turbidity and the mean value applied as a correction to all analyses made on that cruise. The standard deviations of these mean values were actually less than the values reported on sub-samples of one sample. No error is therefore introduced into the statistical treatment. Later in the year with much more plankton variably distributed this procedure would not have been justified.

Finally in all work flasks, after all-night soaking with chromic cleaning mixture, were left for several hours with slightly acid tap water to leach away adsorbed contaminants from the glass. Flasks were covered with inverted crystallizing dishes except when an operation was in progress. Reagents were added from burettes which also were protected from dust by inverted test-tubes.

The large random variations in reducible and total iron discussed in the foregoing paper might reasonably be attributed to a defective method of analysis. That the method is sound for well-dispersed iron is shown by the following experiment. Samples from station E 1, 25 m. depth, were brought in on 27 February 1947 in Winchester bottles. Since no membrane filters were available, on 3 March part was filtered under suction through a Whatman No. 544 paper which is hardened, slow filtering and retentive, whilst part was left

unfiltered. Several series of analyses were carried out in sextuplicate to justify evaluation of the variance and standard deviation. Parallel determinations were made on water drawn from the same station at 5 and 67 m. depth.

Series 1. On 5 March, 1 l. of filtered 25 m. water was acidified with 2·5 ml. 4 N-HCl. From this were measured six 150 ml. subsamples for determination of reducible iron.

Series 2. As series 1 but on 5 March 51 units Fe as ferric alum were added to the litre of water after filtration and *acidification.*

Series 3. As series 1 except that the sea water was unfiltered, the storage Winchesters merely being well shaken.

*Series E*1, 5 *m., unfiltered* and *Series E*1, 67 *m., unfiltered.* Exactly as Series 3.

Series reagent blank. Distilled water was treated precisely as Series 1 but in triplicate only. On each subsample, however, eight drum readings were made instead of four to give the same total number.

Unfiltered turbidity series. Unfiltered water from 25 m. was transferred from the storage Winchester, without treatment other than shaking, to the absorption tube for measurement of colour due to absorption and scattering by constituents of the water.

Filtered turbidity series. Filtered 25 m. water was similarly examined.

Upon all these subsamples, measurements of colour had to be made with the S 50 filter of the Pulfrich Photometer with the minimum of instrumental and personal bias. All were made on one day, 7 March. There is a slight backlash in the mounting of our Pulfrich eyepiece, spectral filters and drums so that a jolt is always liable to cause a small shift of zero. This source of error is guarded against by a control reading with distilled water (against the distilled water always present in the twin tube) every eighth or tenth determination. No such error arose in this experiment. More serious is the tendency of most observers more or less unconsciously to force subsequent readings on a sample to fall into line with the first. This human weakness is particularly hard to overcome with the highly subjective matching of colours. On 6 March therefore, the arbitrarily numbered flasks of all the above series were placed in random order with the further restriction that no two flasks of the same series were allowed to come next to one another. When colour comparison was made the following day, readings in quadruplicate were entered on a separate sheet in the order in which the flasks presented themselves. The two correction terms which have to be applied are set out in Table XI, where d is the deviation of the observed extinction from the mean. This extinction (E) is the product of an extinction coefficient and the length of the absorption tube in appropriate units, here 25 cm.

The higher turbidity and variance of the filtered compared with the unfiltered water requires comment. On 7 March large numbers of phytoplankton cells were observed in the absorption tubes containing filtered water. Similar

cells were seen in the water in the last Winchester to be used but not in numbers in any of the other experimental flasks, whether the water had been filtered or not. On 3 March at the start of filtration the paper had not bedded down well in the Buchner funnel and consequently the faulty filtrate was filtered again. Evidently a few cells were left in the filtration flask and were transferred to the first Winchester to be filled and the last to be emptied. There in the next 4 days they must have found conditions favourable for rapid division which were absent in the remaining two Winchesters of filtered water and experimental flasks. Though these Winchesters were for part of their time in a cupboard, sufficient attention was not given to keeping them away from light. The Winchesters of unfiltered water were never removed from their cupboard except when required. The explanation of the very high variance is clear. Since the errant Winchester was supposed to contain homogeneous water it was not shaken very thoroughly before transferring the subsamples to flasks, which therefore, in fact, contained varying amounts of the cell culture causing varying extinction of light to be measured. Unfortunately at the time, the possible significance of the observation for work on cultures was not appreciated so that the species responsible was not identified. None of the other series of measurements with filtered water contained water from this Winchester.

TABLE XI. EXTINCTIONS REQUIRED AS CORRECTION TERMS

($E = \log_{10} I_0/I$)

Series	No. of sub-samples n	Arithmetic mean	Average deviation $\frac{\Sigma d}{n}$	Variance $\times 10^6$*	Standard deviation $\sqrt{\frac{\Sigma d^2}{n-1}}$
Reagent blank in dist. water	3	0·0106	0·0005	0·39	0·00062
Turbidity, unfiltered water	6	0·0288	0·0008	0·97	0·00098
Turbidity, filtered water	5	0·0318	0·0018	51·90	0·0071

* This and Table XII are the only ones where variance has to be multiplied by 10^6; in all others the units have been chosen to avoid this operation.

In consequence of this, the rather lower and more regular turbidity determination on the unfiltered water has been applied as the correction term for all samples. The accuracy but not the precision of these iron analyses may therefore have been slightly affected. However, this experiment was concerned more with precision than with absolute accuracy. The correction term to be subtracted from the iron determinations is therefore the sum of the first two extinctions in Table XI:

$$0{\cdot}0106 + 0{\cdot}0288 \pm 10^{-3} \times \sqrt{(0{\cdot}39 + 0{\cdot}97)} = 0{\cdot}0394 \pm 0{\cdot}00117.$$

The variance was $1{\cdot}357 \times 10^{-6}$.

The extinctions for the series made to determine reducible iron in filtered or unfiltered sea water are set out in Table XII.

TABLE XII. EXTINCTIONS FOR REDUCIBLE IRON IN THE SEA WATER SERIES

Series	No. of sub-samples	Arithmetic mean	Average deviation	Variance $\times 10^6$	Standard deviation
E1, 25 m.					
1	5	0·0012	0·0051	40·04	0·00633
2	5	0·1061	0·0027	14·398	0·00379
3	6	0·0289	0·0141	365·48	0·0191
E1, 5 m.	4	0·0152	0·0044	37·66	0·00614
E1, 67 m.	4	0·0238	0·0051	50·61	0·00711

The difference between series 1 and 2 showed that an addition of 51 units Fe to acidified sea water gave an increment of extinction amounting to $0{\cdot}1049 \pm 0{\cdot}0074$. The standard deviation is twelve times that in distilled water and is equivalent to 3·8 units Fe. This factor has been used for computing the contents of reducible iron set out in Table XIII.

TABLE XIII. REDUCIBLE IRON IN WATER COLLECTED 27. ii. 47

(mg.-atom/m.$^3 \times 100$)

Series	Abbreviated description	Reducible iron with standard deviation
E1, 25 m.		
1	Filtered, no added iron	$0{\cdot}6 \pm 3{\cdot}08$
2	Filtered and acidified before addition of iron (510 μg.-atom)	$51{\cdot}6 \pm 1{\cdot}84$
3	Unfiltered, no added iron	$14{\cdot}1 \pm 9{\cdot}29$
E1, 5 m.	Unfiltered, no added iron	$7{\cdot}4 \pm 2{\cdot}98$
E1, 67 m.	Unfiltered, no added iron	$11{\cdot}6 \pm 3{\cdot}46$
Integral mean for reducible iron at E1, 27. ii. 47		11·5

Within the limit of error of the method, filtration through Whatman No. 544 filter paper removed all the reducible iron in the water. Providing that the sea water is acidified before the addition of ferric salt the increment in colour is identical with that in distilled water (unpublished work). There is no 'salt error'. Unfiltered sea water from 25 m. therefore contained 14·1 units reducible iron, all of which was removable by filtration, whilst at 5 and 67 m. there was somewhat less. Again, the standard deviation on filtered water attributable to analytical error amounted to 3·1 units.

Except in considering particle size (pp. 288–91), no assumption has been made as to the mathematical nature of the statistical distribution other than that variance is an additive property. Clancey (1947) has concluded that many of the distributions met in chemical analysis depart radically from the 'normal' and are possibly to be regarded more correctly as forms of 'rare occurrence' distributions. These have to be combined with distributions, probably of the 'normal' or Poisson type, arising from the interplay of biological and chemical factors in the water.

APPENDIX II

STATISTICAL ANALYSIS OF THE 'TOTAL IRON' DATA, 1946–47, BY G. M. SPOONER, M.A.

The 'total iron' data presented in Table II have been arranged so that the values for each depth at different stations are grouped; except for the deepest sample at each station, taken less than 20 m. from the bottom. Such samples are separated out as 'bottom' samples.

When this is done it is at once apparent—as indeed is evident from inspection of Table II—that the bottom readings are more variable than those taken well away from the bottom ('upper water layers') and include most of the higher values. By contrast, depths from 0 to 50 m. give more uniform results, both separately and when compared with each other. It is also clear that one station, A3, which had been worked in rough weather, stands apart from the rest, and evidently presents some anomaly: it is excluded from the computations which follow.

The means and variances for the values obtained at different depths are given in Table XIV.

TABLE XIV. TOTAL IRON AT DIFFERENT DEPTHS

Depth (m.)	No. of analyses	Mean ±S.E.	Sum of squares	Degrees of freedom	Mean square (variance estimate)	S.D. ±S.E.
Upper water layers (0–50 m.):						
0	8	23·13 ± 2·83	448·9	7	64·13	8·01 ± 2·00
5	19	24·84 ± 2·55	2098·5	18	116·59	10·80 ± 1·75
25	22	25·64 ± 2·66	3275·1	21	155·96	12·49 ± 1·89
50	19	25·79 ± 2·24	1713·2	18	95·18	9·76 ± 1·58
Total	**68**	**25·16 ± 1·316**	**7535·65**	**64**	**117·74**	**10·851 ± 0·9305**
Intermediate water (60–75 m. below the surface and 23–37 m. above the sea bed):						
	8	45·6 ± 6·07	2059·9	7	294·27	17·15 ± 4·29
Bottom water:						
	23	50·87 ± 8·03	32,573·6	22	1480·62	38·48 ± 5·68

The following points deserve notice:

(1) The mean value for total iron at the bottom is larger than that in the upper layers. Though the estimate for the bottom is not very precise, it can be said to be roughly twice as great. (2) The variation between analyses at one depth is clearly greater on the bottom, the standard deviation being some 3 to 4·5 times that of analyses from the upper layers. (3) Within the 'upper water layers', analyses from different depths (0, 5, 25, 50 m.) do not show any marked differences, either in mean values, which indeed seem more uniform than might have been expected, or in variation between analyses. This phenomenon will be examined more closely below, but in the meantime it is

useful to call attention to the values for the total number (68) of analyses for the upper water layers, which are given in heavier type in Table XIV. (4) The few analyses from depths greater than 50 m., but not close to the bottom, here classed as 'intermediate' depths, clearly give results of an intermediate character—that is, intermediate between those from the shallower depths (considered separately or combined) and the bottom.

The figures suggest that close to the bottom there is rather more iron than in the main part of the water column, and that it is more erratically distributed—presumably because part of it there occurs in coarse particulate aggregations which are normally absent from the upper layers. The influence of the bottom is felt some little distance above, but for the area under survey, where depths ranged from 80 to 120 m., it can be said that the top 50 m. at least was free from this influence. Throughout this top 50 m. conditions appear to be comparable.

Attention will now be confined to the 68 analyses from the top 50 m. (Fig. 2, upper part of middle histogram less readings for station A 3), and the variation found amongst them examined in greater detail. These 68 analyses, with a mean of 25·16 units, have a 'Sum of Squares' of 7583·2, giving (for 67 degrees of freedom) a mean square of 113·18 and S.D. of 10·64 (see foot of page). The latter is relatively high, and indicates that 5% of the analyses may be expected to lie outside the (approximate) limits of 4 to 47 units. How much of this variation, it must be asked, is due to differences between the several stations at which samples were taken, or to differences between depths? And how far has it been reduced by triplicating the analyses of some of the samples? To attempt an answer, 'analysis of variance' technique must be used.

At the outset it is evident that difficulties may arise through the lack of symmetry of the original data. As has been pointed out (p. 280), circumstances prevented measurements at all depths at every station, while it proved possible to replicate some of the samples, but by no means all. It is thus necessary to proceed with caution in attempting a partition of the variance, and to guard against any bias which might lead to invalid conclusions. As will be seen, however, difficulties largely resolve themselves, owing to the high degree of homogeneity which the data possess. The main object is to arrive at an estimate of the sampling variance when the effects of any differences between depths or between stations have been excluded.

The figures in Table XIV have already indicated that depths down to 50 m. give comparable results. Analysis of the variance shows:

	Degrees of freedom	Sum of squares	Mean square
Between depths	3	47·57	15·9
Within depths*	64	7535·65	117·74
Total	67	7583·2	113·18

* Sum of the separate items given in Table XIV.

Depth difference has therefore introduced no extra variation, since the value 117·8 is not less than 113·18. Indeed the effect shown is all the other way, though for only 3 degrees of freedom the value 15·9 is not quite significantly low even in a strictly orthogonal comparison. By chance the readings from the four depths average out more closely than might have been expected. The result gives a wide margin for any small bias which the lack of symmetry of the data as a whole may have introduced.

We can therefore in full confidence disregard differences in depth, when considering the upper layers. This fact is most fortunate, as the original data can now be re-arranged ignoring depth, and much of the asymmetry disappears. The main irregularity now lies in the distribution of replicate readings. These, however, gave a consistent form of result whenever they were made, and they can be safely regarded as representative of the whole sample. (An analysis of the samples with which they are concerned is given below.) Analysis may now proceed to separate off the influences of station-diversity and replicate sampling, as follows:

	Degrees of freedom	Sum of squares	Mean square (variance)	S.D.
Between stations	16	3933·2	247·8	
Between subsamples	17	1101·7	64·8	8·05 (±1·34)
Between samples (within stations)	34	2548·4	74·95	
Total	67	7583·2	113·18	10·64 (±0·913)

The higher variance for the 'between stations' component is significantly different from the other two, and indicates, not surprisingly, that the variability of the analyses has been somewhat increased by taking samples from different places. (Diversity between stations is mainly due to the low-value stations A4 and A6 and the high-value station X3: there seems to be little difference between the majority of the stations.)

The variance between the subsamples of those samples for which there are replicate analyses (64·8 sq.units) is lower than the other components, though very little less than that of the residual variance (74·95 sq.units), thus emphasizing the relative uniformity of the distribution of iron at any given station.

Note. The eight samples for which there is more than one analysis, and which therefore are involved in the 'subsample' data, happen to be quite uniform among themselves, though four different stations are represented. Analysis gives:

	Degrees of freedom	Sum of squares	Mean square	S.D.
Between subsamples	17	1101·7	64·8	8·05
Between samples	7	275·3	39·3	6·27
Total	24	1377·0	57·4	7·57

(The data include twenty-five analyses, made up of three each from samples A1, 5 m.; A1, 25 m.; A7, 5 m.; A7, 25 m.; C4, 50 m.; X4, 25 m.; X4, 50 m.; and four from X4, 5 m.).

By eliminating the effects due to the multiplicity of stations and to the occasional subsampling, the variance of 'total iron' analyses is reduced from the first over-all estimate of 113·18 ($=10·64^2$) to 74·95 ($=8·66^2$). This represents a value for the variance which might be found between a number of analyses from the same water-column (down to 50 m.) at any one station. If the analyses are confined to *one position* in the water-column, i.e. become replicates of a single sample in the sense employed here, the value for the variance will almost necessarily be less than 74·95, though not necessarily much less. Our direct and independent estimate of this value is 64·8, the significance of which is thus enhanced.

The 'refined' variance value of 64·8 is important, as it is a measure of the variation which remains between samples which should duplicate each other, and should be virtually identical in chemical composition. It is much larger than any possible variance resulting from errors introduced during the procedure of chemical analysis (see Appendix I). The conclusion reached therefore is that *even in the upper water layers the iron is distributed in particulate form*. The size of the particles, though less than that of the coarse particles which seem to occur on the bottom (p. 286), must be such that there is quite a small average number in a volume of 150 c.c., and random variations from that number can produce considerable differences in total iron between adjacent bodies of that volume.

From the actual derived value for the variance (64·8), the possibility is open for estimating this degree of aggregation of the iron, i.e. of the size and density of the particles with which it is associated, once allowance has been made for the error inherent in the chemical technique used in handling the samples in the laboratory.

APPENDIX III

Statistical Analysis of the 'Reducible Iron' Data, 1946–47

The data for reducible iron have been examined (Table XV) by the method used for total iron by Mr Spooner in Appendix II. The number of analyses is considerably less and the conclusions are to that extent less certain. The result at X1, 75 m. depth (79 units) is omitted.

(1) The results at 5, 25 and 50 m. are uniform, indeed more so than might have been expected. There are only three surface (0·5 m.) results and these are all relatively high (27, 20 and 53 units). These, standing by themselves, have little significance and there is no good reason for leaving them out of the further statistical analysis. This leads to a mean reducible iron content of the upper layers (0·5, 5, 25 and 50 m.) of 19·3 units.

(2) The mean reducible iron content of the bottom waters was less than in the upper layers though probably not significantly so. The result is in strong contrast with that for total iron.

(3) The variance and standard deviation was much the same in bottom water as in the upper layers. The number of analyses scarcely justifies a further analysis on the lines of that for total iron.

(4) The difference between the mean values for total and for reducible iron gives a measure of the unreducible fraction which has been worked out for the several standard depths other than the surface (Table XVI). The variance estimate, also found by difference, is sometimes negative. For the upper layers (5, 25 and 50 m.), the unreducible iron amounts to about 6 units. The ratios of the variances of total and of reducible iron examined in terms of their degrees of freedom suggests that the variance estimate of the unreducible iron does not differ significantly from zero. It is safe to say that the variance

TABLE XV. REDUCIBLE IRON AT DIFFERENT DEPTHS

Depth (m.)	No. of analyses	Mean ± S.E.	Sum of squares	Degrees of freedom	Mean square (=variance estimate)	S.D. ± S.E.
Upper water layers (0–50 m.):						
0	3	33·33 ± 10·56	607	2	303·5	17·39 ± 7·48
5	8	17·88 ± 2·28	291	7	41·6	6·45 ± 1·61
25	11	16·36 ± 4·50	2228	10	222·8	14·92 ± 3·18
50	9	19·55 ± 4·37	1377	8	172·4	13·02 ± 3·09
Total	31	19·32 ± 2·49	5193	27	192·3	13·87 ± 1·76
Intermediate water:						
	4	13·75 ± 4·28	220	3	73·3	8·56 ± 3·02
Bottom water:						
	8	12·75 ± 4·81	1302	7	186·0	13·64 ± 3·40

TABLE XVI. UNREDUCIBLE IRON AT DIFFERENT DEPTHS AS DIFFERENCE BETWEEN MEANS FOR TOTAL AND REDUCIBLE IRON

Depth (m.)	Difference of means	Difference of mean squares (=variance estimate)
Upper water layers (0–50 m.):		
5	6·96	+75·0
25	9·28	−66·8
50	6·24	−77·2
Total	5·84	−74·6
Intermediate water:	31·8	+221·0
Bottom water:	38·1	+1295

estimate of the unreducible iron is negligible or at least quite small compared with that of reducible iron in the upper layers. This fraction (about 6 units) is therefore reasonably uniformly distributed throughout the upper layers. At midwinter with vertical mixing of the water proceeding freely this must be true of the bottom water also. Also the random distribution of total iron in the upper layers is entirely due to the reducible component, i.e. ferric hydroxide

and phosphate. (These deductions assume that there is no inverse correlation between reducible and unreducible iron. Only further investigation can rule out this unlikely event.)

TABLE XVII. STATISTICAL ANALYSIS OF THE IRON CONTENT OF BOTTOM WATER

Fraction	No. of analyses	Mean	Variance estimate	Standard deviation
Total iron	23	50·87	1480·62	38·48
Reducible iron	8	12·75	186·0	13·64
Dispersed unreducible iron	—	6	Approx. zero	Approx. zero
Particulate unreducible iron	—	32·1	1294·6	36·0

It will be seen from Table XVII that when the reducible and dispersed unreducible fractions are separated from the total iron in bottom water, that a very large particulate unreducible fraction is left—32 units with the very large standard deviation of 36.

A similar examination of the results for the intermediate layer (Table XVIII) gives positive but rather lower values of the mean and standard deviation.

TABLE XVIII. STATISTICAL ANALYSIS OF THE IRON CONTENT OF INTERMEDIATE WATER

Fraction	No. of analyses	Mean	Variance estimate	Standard deviation
Total iron	8	45·6	294·27	17·15
Reducible iron	4	13·75	73·3	8·36
Dispersed unreducible iron	—	6	Approx. zero	Approx. zero
Particulate unreducible iron	—	25·85	221·0	14·9

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SOME CHEMICAL CONSIDERATIONS ON THE DISTRIBUTION OF IRON IN THE SEA

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Three purely chemical topics which have arisen in the course of the investigation described in the preceding paper (Cooper, 1948) will be discussed; first, a theoretical treatment of the formation and stability of ferric phosphate in sea water; secondly, an explanation as to how 2:2′-dipyridyl may react with ferric iron at high dilutions in natural waters to give the red ferrous complex; and thirdly, a short account of an experiment which showed ferrifluoride to be unstable in sea water.

Formation and Stability of Ferric Phosphate in Sea Water

Two series of experiments in this laboratory by Harvey (1937) and by the writer (unpublished) on the precipitation of iron from sea water containing phosphate and hydroxyl ions (pH 8·1) do not agree. Sometimes much of the phosphate is precipitated, sometimes none. Much closer control is necessary but it has not been clear what form this should take. The following theoretical examination, imperfect though it is, may help in the design of such control.

Pugh & du Toit (1936) examined the ionic exchange or hydrolysis of ferric phosphate and silicate in relatively concentrated systems. Solutions of ferric chloride were titrated with solutions of phosphate or silicate containing varying amounts of sodium hydroxide. A ferric phosphate with a higher PO_4/Fe_2O_3 mol-ratio than 0·71 could not be prepared since compounds richer in phosphate hydrolysed until this ratio was reached. There is an optimum value of pH above which the ferric phosphate hydrolyses further with increasing hydroxyl-ion concentration. The same is true of ferric silicate. At pH 8·0 and above, in presence of hydroxyl, silicate and phosphate in solution, ferric hydroxide would be the stable solid phase. The isoelectric point of ferric hydroxide prepared from its chloride is said to be pH 7·1 so that at pH 8·1 ferric hydroxide should be regarded rather as the very sparingly soluble ferrous acid, H_3FeO_3, which may be expected to dissociate

$$H_3FeO_3 \rightleftharpoons H^+ + H_2FeO_3'.$$

Since $FeOH^{++}$ is the dominant ferric cation at the isoelectric point, for electroneutrality of the micelle, the concentration of its anion, H_2FeO_3', should be twice that of its cation, $FeOH^{++}$. This (Cooper, 1937*b*, equation 12) will be about $10^{-12.54}$ at 18° C. The solubility product of ferrous acid, $(H^+)\,(H_2FeO_3')$ will therefore be of the order

$$10^{-7.1} \times 12 \times 10^{-12.54} = 10^{-19.3}.$$

At pH 8·1 the molar concentration of the ferrite ion would be about $10^{-11.2}$ or 0·006 μg.-atom/m.^{3}. This figure cannot be very accurate since the isoelectric point of ferric hydroxide sols depends greatly upon the method of preparation.

Revut (1936) also states that on standing ferric phosphate sols slowly hydrolyse. The complexes may vary much in structure and calcium phosphate may be included (Kheifetz, 1936).

In the sea ferric hydroxide and phosphate will almost always be colloidal, conceivably but very rarely micro-crystalline and never macro-crystalline. In an azoic world, given sufficient time, thermodynamic equilibrium would no doubt in the end be reached, whereas in temperate surface waters of the sea, the speed of the life cycle is likely never to allow equilibrium conditions to be even remotely approached. Equilibrium values for ferric hydroxide have been discussed (Cooper, 1937*b*) and at *p*OH 6·5 (pH 8 at 10° C.) (Cooper 1937*a*) we should expect $10^{-10.9}$ μg.-atom/m.^{3} Fe^{+++}, $10^{-5.3}$ μg.-atom/m.^{3} $FeOH^{++}$ and possibly 10^{-3} μg.-atom/m.^{3} H_2FeO_3'.

The amount of undissociated phosphoric acid, H_3PO_4, present in sea water must be minute (Nims, 1934). Phosphate occurs mostly as the ions, H_2PO_4' and HPO_4''. The second dissociation constant in terms of activities at infinite dilution has been most carefully determined by Nims (1933):

$$K_2 = \frac{a_H\, a_{HPO_4}}{a_{H_2PO_4}} = 5{\cdot}98 \times 10^{-8} \text{ at } 18^\circ \text{ C.} \qquad (1)$$

At finite concentrations of solute, activity coefficients have to be introduced. Extrapolation from Nims' experimental upper limit of ionic strength, 0·15, to the ionic strength of sea water, 0·7, would involve laborious computations which the problem does not at present justify. Nevertheless, to give shape to the problem, the concentration of the tribasic ion, PO_4''' will be calculated at pH 8·0 and 8·3 in a solution 'at infinite dilution', assuming that activity coefficients are all unity and that activities may be equated to molarities, written within parentheses. Since the concentrations of H_3PO_4 and PO_4''' are negligible compared with H_2PO_4' and HPO_4'', the total concentration, of dissolved inorganic phosphate, ΣP may be written

$$\Sigma P = (H_2PO_4') + (HPO_4'') = \frac{(H^+)\,(HPO_4'')}{K_2} + (HPO_4'')$$

$$= \left[1 + \frac{(H^+)}{K_2}\right](HPO_4''). \qquad (2)$$

For the third dissociation constant,

$$K_3 = \frac{(H^+)\,(PO_4''')}{(HPO_4'')}, \qquad (3)$$

the literature values are divergent, Abbott & Bray (1909) giving $3{\cdot}6 \times 10^{-13}$ and Blanc (1920) 2×10^{-12}. A round figure of 10^{-12} will be assumed. Combining equations (2) and (3), we have:

$$(PO_4''') = \frac{K_2 K_3}{(H^+)\,[K_2 + (H^+)]}\,\Sigma P = Q\Sigma P. \qquad (4)$$

At pH 8·0, $Q = 0{\cdot}86 \times 10^{-4}$,

and at pH 8·3, $Q = 1{\cdot}84 \times 10^{-4}$.

That is, if the alkalinity of sea water be raised from pH 8·0 to pH 8·3, the amount of tribasic ion 'at infinite dilution' would be doubled. In sea water the apparent dissociation constants will certainly differ markedly from those quoted, but the relative values of Q will be much less in error.

Now let us consider the partition of iron between hydroxide and phosphate in sea water. Sea water is sufficiently well buffered for the hydroxyl-ion concentration to be considered constant. By contrast the total concentration of phosphate ions, ΣP, is very low and not much greater than the amount of particulate ferric phosphate inferred from analysis (Cooper, 1948).

Let us *assume* that the solubility product of ferric phosphate,

$$K_{FePO_4} = (Fe^{+++})\,(PO_4''') = 10^{-31{\cdot}5}. \qquad (5)$$

The equilibrium concentrations of ferric ion (Cooper, 1937*b*) would be $10^{-19{\cdot}9}$ at pH 8·0 and $10^{-21{\cdot}3}$ at pH 8·3.

By combining equations (4) and (5):

$$(Fe^{+++})\,Q\Sigma P = 10^{-31{\cdot}5},$$

so that at pH 8·0, $\Sigma P = 34$ and at pH 8·3, $\Sigma P = 110$ μg.-atom/m.^{3}.

This theoretical approach confirms the experimental finding of Pugh & du Toit (above) in relatively very concentrated solution that the hydrolysis of ferric phosphate becomes greater the more alkaline the water. The conditions least favourable to the stability of ferric phosphate must therefore arise in the upper layers of the sea, during active plant growth. The formation and stability of ferric phosphate is likely to be most favoured under the more acid conditions found in the gut of many animals. Ferric phosphate is likely therefore to be introduced into sea water in faeces. It should be stressed that only the relative and not the absolute magnitudes of these calculations have meaning since the solubility product of ferric phosphate, $10^{-31{\cdot}5}$, was arbitrarily selected to give an answer within the realm of experience.

Doubt as to the correctness of these deductions springs less from the rather arbitrary nature of the calculation than from our complete ignorance as to how rapidly equilibrium conditions are attained. In all experimental work involving ferric phosphate in sea water, the need is indicated for very accurate control of hydroxyl-ion concentration (strictly activity) and for attention to the time factor and to the physical condition of the precipitated

phase. The topic merits study not only from a biological but from a geochemical point of view. The equilibrium between ferric, hydroxyl and phosphate ions would seem to be precariously balanced and not always rapidly attained. It is likely to be strongly displaced by hydrostatic pressure which will affect the co-precipitation of iron and phosphate in deep-water deposits.

Ferrous Iron in the Sea and in Lakes

2:2′-Dipyridyl is now often used for characterizing ferrous as opposed to ferric iron at high dilutions in lake water (e.g. cf. Mortimer, 1941). Determinations made in the English Channel by the writer in 1933–34 (Cooper, 1935, p. 422) and attributed to ferrous iron now appear to be nothing of the sort. An explanation is due. In the earlier work 100 ml. samples of sea water were treated with two drops of 4 N-HCl and 1 ml. 1 % dipyridyl in 0·2 N-HCl. Harvey (1937) has shown, and the writer has confirmed, that at high dilutions ferric iron will give rise to the red ferrous-*tris*-dipyridyl complex.

In sea water particulate and colloidal ferric hydroxide, phosphate and the like will approach an equilibrium with Fe^{+++} and $FeOH^{++}$ whose concentration at the analytical pH of about 4·5 will not exceed about 50 μg.-atom/m.^{3}:

$$Fe(OH)_3 \rightleftharpoons FeOH^{++} + 2OH' \rightleftharpoons Fe^{+++} + 3OH', \qquad (1)$$

$$FePO_4 + 2H_2O \rightleftharpoons Fe^{+++} + H_2PO_4' + 2OH'. \qquad (2)$$

These ferric ions will seek an equilibrium with ferrous ions which will be governed by the poise and pH of the system

$$Fe^{+++} + e \rightleftharpoons Fe^{++}, \qquad (3)$$

$$FeOH^{++} + H^+ + e \rightleftharpoons Fe^{++} + H_2O. \qquad (4)$$

When undissociated dipyridyl (dipy) is present the ferrous ions will be rapidly removed.

$$Fe^{++} + 3 \text{ dipy} \rightleftharpoons Fe(\text{dipy})_3^{++}.$$

Reactions (1) to (4) will then proceed to restore the ferrous-ion concentration, providing that the necessary reducing electron can be found. If the oxygen electrode system, considered as a thermodynamically reversible system, be examined as the source of the electron:

$$O_2 + 2H_2O + 4e \rightleftharpoons 4OH',$$

we find that reduction of 200 μg.-atom/m.^{3} of ferric ion to ferrous ion would result in the oxidation of hydroxyl ion to 0·006 ml./l. dissolved oxygen. The change of poise of the system would be negligible as would the change in *p*OH or pH of even a weakly buffered water.

The reaction may also be formulated as proceeding through the weakly blue ferric-dipyridyl complex:

$$Fe^{+++} + 3 \text{ dipy} \rightleftharpoons Fe(\text{dipy})_3^{+++},$$

$$Fe(\text{dipy})_3^{+++} + e \rightleftharpoons Fe(\text{dipy})_3^{++}.$$

The redox potential of this last reaction exceeds unity.

The source of the reducing electron should not be ascribed solely to the hydroxyl ion-oxygen system which, in fact, is not thermodynamically reversible but rather to the poising system of the water considered as a whole. The point of the argument is to show that in presence of a powerful combining reagent for ferrous ion such as 2:2′-dipyridyl, ferric iron in a natural water at high dilution may seem to combine directly to give the ferrous-dipyridyl complex without needing the presence of a definite reducing agent in the usual macrochemical sense of the phrase. Thus the results in 1933 and 1934 reported as ferrous iron certainly included some ferric iron as well.

Ferrifluoride in Sea Water

It was earlier suggested (Thompson, Bremner & Jamieson, 1932; Cooper, 1935) that ferrifluoride may play a part in the iron cycle in the sea. An experiment made in May 1936 now shows this to be unlikely.

A well-washed, freshly prepared precipitate of ferric hydroxide was redissolved by treatment with excess hydrofluoric acid in a platinum dish. Addition of an equivalent solution of sodium carbonate gave a white precipitate of sodium ferrifluoride which was well washed by decantation to remove excess hydrofluoric acid. A few crystals of the moist precipitate on a loop of platinum wire were added to 250 ml. of sea water. The suspension slowly became yellow and finally ferric hydroxide separated. This experiment was repeated several times and indicates that, due to one of the reactions:

$$FeF_6''' + 3H_2O \rightarrow Fe(OH)_3 + 3HF + 3F'$$

or

$$FeF_6''' + Al^{+++} + 3H_2O \rightarrow AlF_6''' + Fe(OH)_3 + 3H^+$$

ferrifluoride in sea water is unstable and plays no part in the iron economy of the sea.

Summary

A theoretical study of the behaviour of ferric phosphate in sea water has been made with existing data. The conditions least favourable for the continued existence of ferric phosphate should occur in the upper layers of the sea during active plant growth. The formation and stability of ferric phosphate is likely to be most favoured under the more acid conditions found in the gut of many animals. Ferric phosphate is likely to be introduced into sea water in animal faeces. Experimental confirmation of this deduction is essential.

In all experimental work involving ferric phosphate in sea water, the need is indicated for very accurate control of hydroxyl-ion activity and probably pressure, and for attention to the time factor and to the physical condition of the precipitated phase. The topic is of geochemical as well as of biological interest.

Dipyridyl is a reagent for ferrous iron. Reasons are set out why in sea and lake water at high dilutions it may also determine ferric iron without presence of an added reducing agent.

Evidence is presented that ferrifluoride plays no part in the iron cycle in he sea.

Addendum

The Editor has shown me the proofs of a contribution to this Volume (pp. 360–79) by S. M. Marshall & A. P. Orr (1948) on the effect of different plant nutrients upon the phytoplankton in Loch Craiglin. The deepest water (4–5 m.) was frequently oxygen-free, contained sulphide, and much resembled the freshwater studied by Mortimer (1941) in Esthwaite Water (English Lake District). Such a stratification is likely to lead to the following distribution of iron and phosphate.

Oxygenated upper layers. Any iron present is likely to be largely colloidal or particulate ferric hydroxide which may or may not combine with phosphate to give particulate ferric phosphate. The balance of evidence has suggested that normal sea water may be too alkaline for this to happen. In Loch Craiglin the pH of surface water may even rise to over 9.

Sulphide containing bottom water. In distilled water precipitated ferrous sulphide has a solubility of 70 mg.-mol/m.^{3} (Weigel, *cit.* Seidell, 1920, p. 345). Its solubility in sea water is likely to have the same order of magnitude. Consequently, any amount of iron likely to be present in the foul bottom water of Loch Craiglin could be retained in solution as dissolved ferrous sulphide. The flagellate, *Euglena proxima*, was present mostly in or near the boundary between the two layers.

On 1 August 1944 the loch had been fertilized with large quantities of superphosphate and sodium nitrate, most of which had been removed from the water by 7 August (Orr, 1947). Dissolved oxygen at 2 m. had risen to 292% 'saturated' and the pH to 9·82. On samples taken on 9 August Mr H. Barnes made some iron analyses by the method used at Plymouth and in the oxygen-rich, very alkaline water at 0, 1 and 2 m. was able to detect no iron. By contrast in the sulphide-containing water at 3 m. about 0·36 mg.-atom/m.^{3} ferrous iron was found. The criticism, made above, as to the soundness of analyses for ferrous iron by this method are not valid for reducing waters such as that at 3 m.

Iron, when once it has been immobilized in the bottom in any insoluble form, may be returned to the water physically by turbulence, by the agency of certain classes of animals, or by both together. Bacteria alone cannot bring it about under aerobic conditions. The important part played by faecal pellets in the bottom muds of the Clyde Sea area has been well shown by Moore (1931). The writer is being led more and more towards the view that detritus and suspension feeding animals hold a key position in the return of iron into

circulation in a form usable by plants. It is surmised that these, either by triturating their food or by dissolving it in acid digestive juices, disperse iron surplus to their needs in their faeces and give it a measure of 'protection' by embedding it in organic colloids. After excretion into the overlying water, bacterial breakdown would further disperse this protected iron into a form usable by plants. This is a development of the argument already put forward by Harvey (1945, p. 137) as to the part played by zooplankton in the upper layers of the sea.

Though the minimal requirements of diatoms for iron are much less than those for phosphorus (Harvey, 1937), some iron in a usable form is essential. It would now seem likely that the planktonic plants over the continental shelf require a proper balance of animals, both planktonic and bottom dwelling, to maintain their supply of iron in such a usable form. If therefore after addition of phosphatic and nitrogenous fertilizers, the plant population should become so great as to exclude a balanced growth of animals then the circulation of iron in a usable form would cease completely. Unlike phosphorus and nitrogen, bacterial decay alone would be unable to redress the balance. Subsequent addition of further phosphate and combined nitrogen to an enclosed body of water would then cause little further production of phytoplankton. Something of this sort may have happened on occasion in Loch Craiglin though it is not possible to separate the effects due to possible lack of usable iron from those due to high pH and disturbance of the carbon dioxide-bicarbonate system.

I am much indebted to Dr A. P. Orr for allowing me to see the typescript of his paper which is to appear in the Proceedings of the Royal Society of Edinburgh, for a criticism of this addendum and for a statement of his views. It is only fair to add that the Millport work was carried out during the war years concurrently with other work of higher priority. Many factors, including those discussed here, were well appreciated but in the very limited time available could not be examined. It is surprising indeed how much valuable data he and Dr Marshall were able to collect.

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PARTICULATE AMMONIA IN SEA WATER

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In the English Channel during the winter months vertical mixing is thorough, so that in 50–100 m. of water temperature and salinity are usually uniform from surface to bottom. Other truly dissolved substances would also be expected to be uniformly distributed. The erratic distribution of iron has been shown to arise from its particulate nature, whilst small vertical variations of inorganic phosphate in winter as well as occasional poor agreement between replicate phosphate analyses are most probably due to particulate ferric phosphate (Cooper, 1948).

Two features of the ammonia analyses made in 1930–31 have always caused the writer concern. That ammonia, with its ready solubility and inability to form insoluble compounds, must be in true solution seems axiomatic. Yet, on the three cruises to International Hydrographic Station E1 (72 m. depth) on 4 December 1930, 13 January and 4 February 1931, there were marked maxima of ammonia at surface and bottom (Cooper, 1933, fig. 6). Rapid though regeneration of ammonia from decaying material may have been, it is odd that vertical mixing could have allowed dissolved ammonia so to accumulate. Surface samples were taken with a wooden bucket and so included a portion of the surface film.

Wattenberg's absorptiometric method (1928), which was then used, is not highly accurate within the range of concentrations studied. It was difficult completely to ensure that the prepared sea water used for making standards was ammonia-free. Though absolute accuracy may have been none too good, the relative precision of measurement between duplicates compared at the same time not only with standards but with one another was much better. Frequently the difference between duplicates or triplicates amounted to 0·7 mg.-atom/m.^{3} or more. Notebook comments such as 'results ignored; high figures attributable to contamination' were frequent. Such an analytical distribution is very reminiscent of that for iron.

Again, Redfield & Keys (1938) made a series of most careful analyses in the Gulf of Maine by the vacuum distillation technique of Krogh (1934). They remark that 'to be reliable, all determinations must be made in duplicate, since unforeseen contamination frequently occurred'. In other words, duplicates did not agree. Further (p. 87), they say: 'The distribution of ammonia appeared very irregular when the observations were first made.... It appeared, however, that great differences might be observed in similar situations at any time and in the same situation at different times.'

One is entitled to question whether these erratic results were really due to analytical error or contamination.

In April and May 1936 in Japanese coastal waters very many analyses were made by the research vessel *Syunpū Maru* (1938). The text is in Japanese, but the table of results has English headings. The distribution of temperature, salinity, density, oxygen, pH, silicate, phosphate, nitrite and nitrate agree with what might be expected of dissolved substances in shallow, partially stratified, temperate waters in spring. On any accepted view of the distribution of dissolved substances, the many results for iron and ammonia are chaotic even at stations which were isothermal and isohaline. The iron results now fall into line with the picture of particulate iron drawn for the English Channel. Is it reasonable to ascribe the Japanese results for ammonia also to a particulate distribution?

In spite of determined efforts by a large number of workers, no nitrifiers able to oxidize ammonia to nitrite have ever been found in sea water as such. These attempts are listed by Carey (1938) and ZoBell (1946, pp. 151–53). Nitrifying organisms have, however, been found associated with bottom deposits and with animal and plant plankton collected near shore in relatively shallow places (Waksman, Reuszer, Carey, Hotchkiss & Renn, 1933; Carey, 1938).

When Harvey (1940) presented diatom cultures with mixtures of ammonium- and nitrate-*N*, analysis showed that, in the light, ammonium-*N* was preferentially removed whereas in the dark the content of ammonium-*N* in the culture as found by analysis remained unchanged. Nevertheless, the dark experiments were compatible with some of the ammonium-*N* having been adsorbed by the cells whilst remaining accessible to bulk analysis of the water including everything in it.

A possible key to the riddle is provided by the work of Lees & Quastel (1946) on soil nitrification of ammonia. They concluded that soil ammonia is held on the surfaces of the soil crumbs by base-exchange combination and that the nitrifying bacteria proliferate only at the expense of such adsorbed ammonium cations. Some such adsorption of part of the ammonia in sea water upon particulate matter would provide an explanation of the experimental findings more reasonable than the exasperatingly frequent 'contamination'. Undissociated ammonia (NH_3) and ammonium-ion (NH_4^+) are present in sea water in not dissimilar amounts (Cooper, 1937) so that adsorption of either might occur.

All these observations may be brought within the following working hypothesis. In the sea ammonia is formed by the breakdown of amino- and amido-*N* in decaying matter, most of which is particulate. It may not be immediately given up to the water but may be retained on the surfaces of the particles as adsorbed ammonia (NH_3), ammonium-ion (NH_4^+) or conceivably as a weak, readily hydrolysable amido-grouping (—$CO.NH_2$). Such particles

with densities slightly different from that of the water will accumulate in the surface or bottom strata of the water mass or may be entrained by interfacial tension in the sea surface itself. None the less, their adsorbed ammonia would be determinable by any of the methods of analysis for dissolved ammonia. On such particles—perhaps nowhere else—nitrifying bacteria may find not only a structure on which physically to support themselves as seems necessary in soils but also the ammonium substrate they need. Success in culturing nitrifying bacteria would therefore demand a particulate rather than a plate technique. Ammonia added in solution to phytoplankton cultures would tend of itself to become adsorbed upon the plants in a way which would only be revealed if the plants were removed before analysis. Such capacity of ammonia-*N* for preferential adsorbtion would make it a more favourable plant food than nitrite or nitrate.

If the possibility of removal of ammonia from the aqueous phase of sea water by adsorption upon particles be admitted, 'contamination' as an explanation of the failure of replicate analyses to agree is no longer necessary. Occasionally it must occur, but more often results would be due to random distribution of 'particulate ammonia' of the type now known to apply to iron.

Though this hypothesis gives a coherent explanation of a number of observations, previously somewhat intractable, it is none the less highly speculative. It is put forward here since an opportunity for the writer to test it for himself is unlikely to arise in the near future.

Summary

The hypothesis is proposed that inorganic ammonia may be adsorbed on particulate matter in the sea. The random distribution of such particulate ammonia would account for the frequent 'contamination' reported in ammonia analyses and for the finding of nitrifying bacteria associated only with particulate matter, but never with sea water itself.

Addendum

The Editor has shown me the proofs of a contribution to this Volume (pp. 360–79) by S. M. Marshall & A. P. Orr (1948) on the effect of different plant nutrients upon the phytoplankton in Loch Craiglin. In certain experiments added nutrients disappeared from the water of this shallow loch very rapidly indeed. This suggested to the authors either that the nutrients were speedily absorbed by the phytoplankton and fixed algae, or that they were adsorbed by mud or suspended dead particulate matter. For salts of ammonia, notably the experiments of 2 April 1945 and 10 January 1946, the second view accords with the hypothesis here presented. Dr Orr (*in litt.*) states that Dr Steemann Nielsen in informal discussion has expressed the same idea to Dr Marshall. The uncertainty inherent in analytical data for nitrate allow of no conclusion

at present as to possible particulate distribution of adsorbed nitrate in the sea. Such a distribution must be considered unlikely until positive evidence can be produced in its favour. There is nothing in the known distribution of phosphate in the open sea to lend support to a particulate hypothesis for this nutrient on the scale required by the Loch Craiglin results. None the less it would be of interest to know whether the moorland stream or burn brings any considerable amount of iron into Loch Craiglin and, if so, whether ferric phosphate might be formed and precipitated. The pH of Loch Craiglin varied widely during the investigation and, as will be seen from a companion paper to this, ferric phosphate is more likely to be formed when pH is low.

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PHOSPHATE AND FISHERIES

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(Text-figs. 1–3)

A number of links in the food chain intervene between the presence of nutrient salts over fishing grounds and the marketing of commercial fish. Indeed not a chain but a network provides the better metaphor. The necessary study of each and every mesh must occupy many years. None the less as an empirical short cut it is well worth while to examine quantitatively whether a change in the nutrient resources of an area may be reflected in commercial landings.

The purpose of the paper is not to make positive assertions of fact but to suggest possibly valuable lines of investigation.

Phosphate and the Spurdog

There are four ports exploiting the Eddystone-Lizard grounds of which International Hydrographical Station E 1 is more or less the centre. Returns from the port of Plymouth and, to a lesser degree, Brixham are likely to be vitiated from the point of view of this study by landings from deep-sea trawlers operating on occasion hundreds of miles from their home port. The fishery of the port of Mevagissey is, however, carried on by small craft almost entirely on the Eddystone-Lizard grounds. Throughout this paper the serious limitations inherent in all fisheries statistics taken at small ports must constantly be borne in mind. Market conditions at Mevagissey sometimes lead to concentration of all effort on the capture of one species.

It was immediately apparent that dogfish landings at Mevagissey would repay study (Fig. 1, curve *A*). The species there completely dominant in the landings is the spurdog, *Squalus acanthias* (= *Acanthias vulgaris*), which, though classified with other dogfish in the fisheries statistics as demersal, is in fact truly pelagic. It is caught by an autumn line fishery on the Eddystone-Lizard grounds and is often landed by drifters working the winter drift-net fishery from Plymouth.

S. acanthias is noteworthy in that the eggs are developed into young fish within the mother (Ford, 1921) so that the young are never exposed to the vicissitudes of an independent planktonic existence.

The steady increase in landings between 1921 and 1926 might have been due to increased intensity of fishing, change in fishing gear or market conditions. That these cannot account for the whole of the increase is shown by the ratio of landings of dogfish at Mevagissey to (i) the total weight of demersal fish other than dogfish landed at Mevagissey (Fig. 1, curve *B*), and to (ii) the total landings of dogfish (all species) at English and Welsh ports (curve *C*). Both ratios show the same trends as curve *A* suggesting that the increased landings

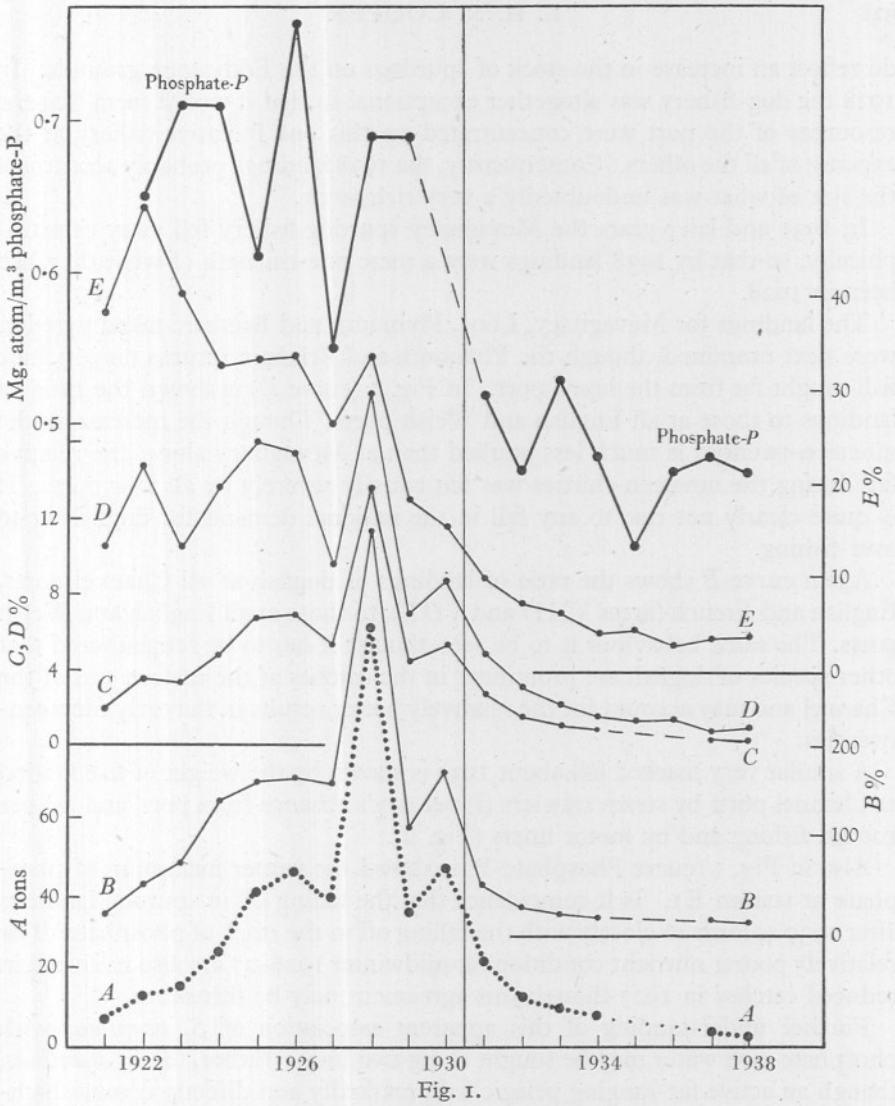

Fig. 1.

A, weight of dogfish landed at Mevagissey (tons).

B, percentage ratio: $\frac{\text{Dogfish landed at Mevagissey}}{\text{Total demersal fish less dogfish landed at Mevagissey}}$.

C, percentage ratio: $\frac{\text{Dogfish landed at Mevagissey}}{\text{Dogfish landed at English and Welsh Ports}}$.

D, percentage ratio: $\frac{\text{Dogfish landed at Brixham, Plymouth, Looe and Mevagissey}}{\text{Dogfish landed at English and Welsh Ports}}$.

E, percentage ratio: $\frac{\text{Dogfish landed from the English Channel (Areas VII D and E)}}{\text{Dogfish landed at English and Welsh Ports}}$.

Phosphate-*P*, mg.-atom/m.³, maximum content at E 1 in preceding winter.

The vertical line marks the change-over from predominantly phosphate-rich (*elegans*) to phosphate poor (*setosa*) conditions.

do reflect an increase in the stock of spurdogs on the Eddystone grounds. In 1928 the dog-fishery was altogether exceptional so that it would seem that the resources of the port were concentrated on this one lucrative fishery at the expense of all the others. Consequently, the 1928 landings probably accentuate the size of what was undoubtedly a very rich stock.

In 1931 and later years the Mevagissey spurdog fishery fell away catastrophically, so that by 1938 landings were a mere one-thirtieth of what they had been in 1928.

The landings for Mevagissey, Looe, Plymouth and Brixham taken together were next examined, though the Plymouth and Brixham returns may include fish caught far from the home port. In Fig. 1 (curve *D*) is shown the ratio of landings to those at all English and Welsh ports. Though the increase in the nineteen-twenties is much less marked than at Mevagissey alone, the relative fall during the nineteen-thirties was felt equally severely by all four ports. It is quite clearly not due to any fall in the national demand for dogfish or to over-fishing.

Again curve *E* shows the ratio of landings of dogfish at all Channel ports, English and French (areas VIID and VIIE), to those at all English and Welsh ports. The same behaviour is to be seen though it has to be remembered that other species of dogfish are prominent in the catches at the eastern end of the Channel and may account for the relatively better results in the early nineteen-twenties.

A similar very marked fall about 1930 is shown by the weight of fish landed at Channel ports by steam trawlers (i) per day's absence from port, and (ii) per 100 hr. fishing and by motor liners (Fig. 2).

Also in Fig. 1 (curve Phosphate-P) is shown the winter maximum of phosphate at station E1. Is it coincidence that the falling off in spurdog landings after 1930 accords so closely with the falling off in the stock of phosphate? The relatively poorer nutrient conditions at midwinter 1926–27 are also mirrored in reduced catches in 1927 though this agreement may be fortuitous.

Further understanding of this apparent association of *S. acanthias* with phosphate-rich water may be sought along two lines. Either, (*a*) *S. acanthias*, though an active far-ranging pelagic fish, markedly and directly favours high-phosphate western 'elegans' water of which it would then be an indicator; or, more probably, (*b*) the association traces directly through shoals of clupeoids and mackerel which it attacks and voraciously devours. A correlation exists between herring and phosphate. Presentation of this demands first a study of the 'fine structure' of phosphate distribution which will be attempted in a later paper. Pilchard eggs frequently occur in water of a very poor type (Russell, 1937–40). A quantitative study of the abundance of *S. acanthias* associated with shoals of (*a*) the (eastern) Channel race of herring, (*b*) the 'western' race of herring (Ford, 1928), (*c*) pilchard and (*d*) mackerel might therefore lead to an advance on the central problem of productivity.

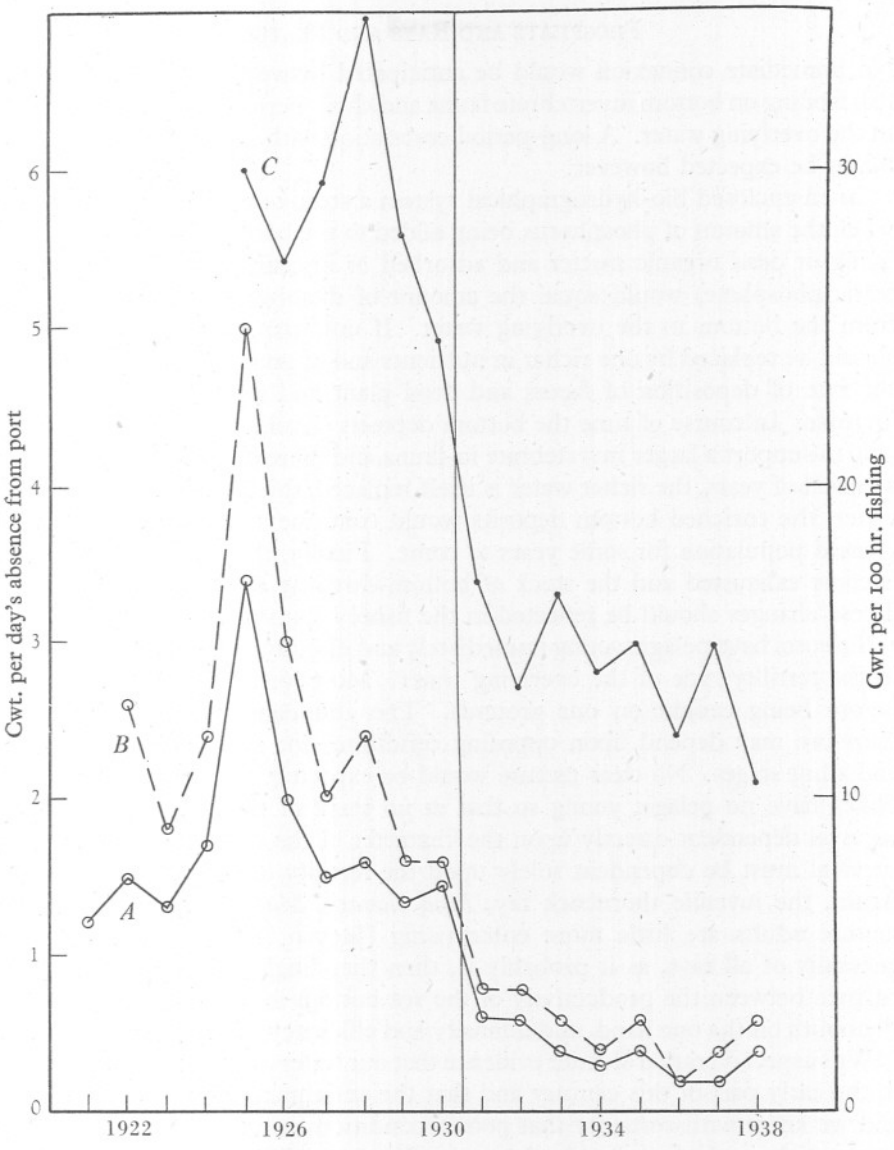

Fig. 2. Landings of dogfish (cwt.), at English ports in the English Channel from:
A, steam trawlers per day's absence from port.
B, steam trawlers per 100 hr. fishing.
C, motor liners per day's absence from port.
The vertical line marks the change-over from phosphate-rich (*elegans*) water to phosphate poor (*setosa*) water.

Phosphate and Rays and Skates

No immediate connexion would be anticipated between quantity of bottom fish feeding on bottom invertebrate fauna and short-period changes in nutrients in the overlying water. A long-period correlation with a considerable time lag might be expected however.

In an enclosed bio-hydrographical system a steady state should be reached when the amount of phosphorus being added to the bottom deposits as faeces, living or dead organic matter and adsorbed or insoluble phosphate (such as ferric phosphate) would equal the amount of dissolved phosphate returned from the bottom to the overlying water. If now this overlying water mass should be replaced by one richer in nutrients and of greater biological fertility, the rate of deposition of faeces and dead plant and animal remains should increase. In course of time the bottom deposits should become enriched and able to support a larger invertebrate in-fauna and more demersal fish. If, after a period of years, the richer water is itself replaced, this time by nutrient-poor water, the enriched bottom deposits would continue able to support the increased population for some years to come. Finally, the reserve store would become exhausted and the stock of bottom-dwelling animals would decline. These changes should be reflected in the fishery statistics.

Teleosts have pelagic young immediately and directly influenced by a change in the fertility type of the overlying water. Moreover, they may voyage far before being caught on our grounds. The abundance of mature teleosts therefore may depend upon opposing conditions for survival in their larval and adult stages. No clear picture would be expected. By contrast, rays and skates have no pelagic young so that at no stage of their existence is their survival dependent directly upon the character of the overlying water. Their survival must be dependent solely upon the fertility of the bottom deposits. Again, the juvenile thornback ray, *Raia clavata*, does not migrate and the mature adults are little more enterprising (Steven, 1936). If this is true generally of all rays, as it probably is, then they ought to reflect closely the balance between the productivity of the sea bottom in the neighbourhood of Plymouth on the one hand, and intensity and efficiency of fishing on the other.

We suspect on rather slender evidence that our waters were poor in phosphate in the early part of this century and that the enrichment occured about 1921 and we know with certainty that poorer conditions returned to the overlying water at the end of 1930 (Matthews, cf. Cooper, 1938) (cf. Fig. 1, curve *P*). If the argument is sound, some years would have had to pass before the enrichment of the water was reflected in increased catches of rays. Similarly, once the stock of fish and bottom food was built up, a lucrative fishery should have persisted for some time after the rich water had departed.

With this hypothesis in mind the landings of rays and skates at Mevagissey between 1921 and 1938 were examined (Fig. 3, curve *F*). The drop in landings

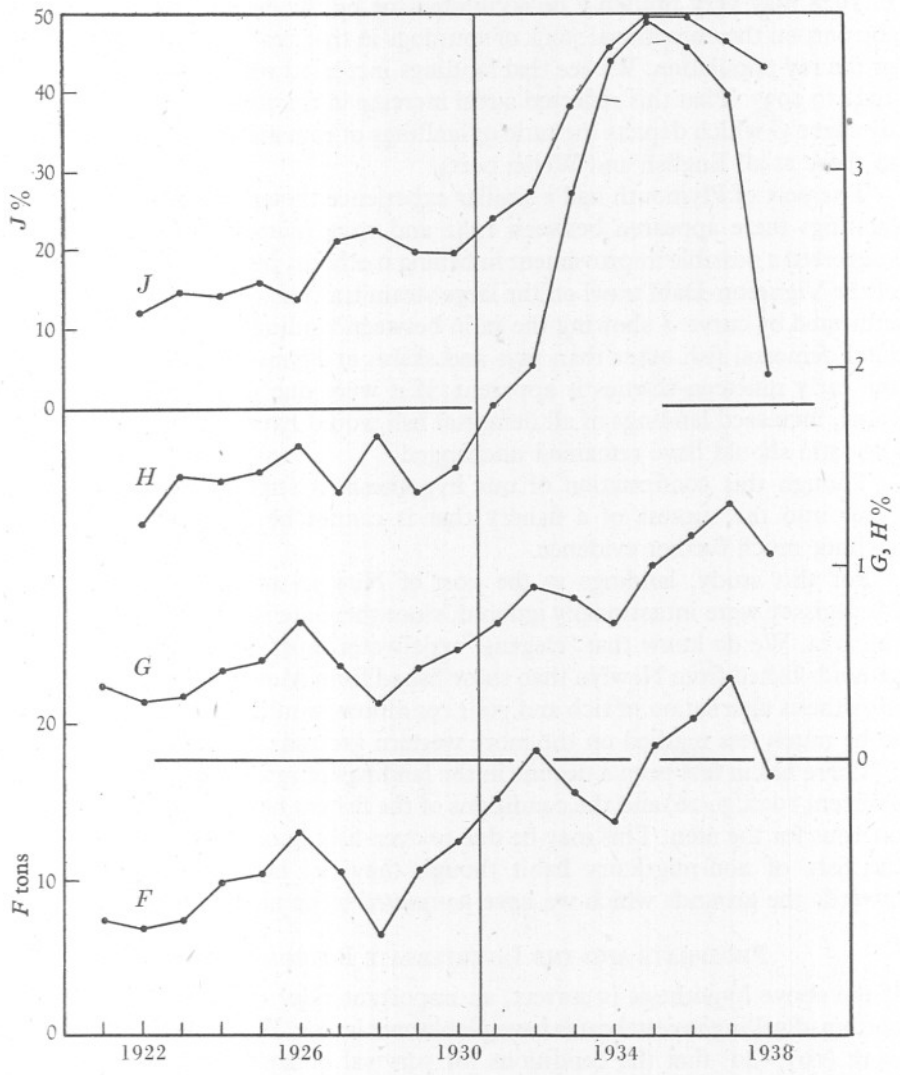

Fig. 3.

F, landings of rays and skates at Mevagissey (tons).

G, percentage ratio: $\frac{\text{Rays and skates landed at Mevagissey}}{\text{Rays and skates landed at all English and Welsh ports}}$.

H, percentage ratio: $\frac{\text{Rays and skates landed at Plymouth}}{\text{Rays and skates landed at all English and Welsh ports}}$.

J, percentage ratio: $\frac{\text{Rays and skates landed at Plymouth}}{\text{Demersal fish other than rays and skates landed at Plymouth}}$.

in 1928 may very probably be accounted for by the evident concentration of the port on the exceptional stock of spurdogs in that year and not to any decline in the ray population. We see that landings increased reasonably steadily from 1921 to 1937. That this reflected a real increase in the stock of fish is suggested by curve *G* which depicts the ratio of landings of rays and skates at Mevagissey to those at all English and Welsh ports.

The port of Plymouth had a similar experience though the main increase in landings there appeared between 1932 and 1934 (curve *H*). This sharp rise suggested a possible improvement in fishing methods, perhaps the introduction of the Vigneron-Dahl trawl on the large steam trawlers. That this was not so is indicated by curve *J* showing the ratio between landings of rays and skates to total demersal fish other than rays and skates at Plymouth. The same rise in the early nineteen-thirties is apparent; if it were due to use of an improved trawl, increased landings of all demersal fish would have been anticipated and the ratio should have remained unchanged.

Though this confirmation of our hypothesis is striking, so many factors enter into the success of a fishery that it cannot be considered as proved without much further evidence.

For this study, landings at the port of Newlyn 40 miles to the west of Mevagissey were intentionally ignored, since there are no nutrient salt data for the area. We do know that 'elegans'-type water must more often overlie the grounds fished from Newlyn than those fished from Mevagissey. On the above hypothesis alternation of rich and poor conditions would therefore be expected to be much less marked on the more western grounds.

There has in fact been a decline in the landings of rays and skates at Newlyn (Steven, 1932, p. 26) and the conditions of the fishery have become much more onerous for the men. This may be due to over-fishing or it is just possible that the fish, of non-migratory habit though they be, had moved up-Channel towards the grounds which we have *postulated* to be newly enriched.

Phosphate and the Invertebrate Bottom In-fauna

If the above hypothesis is correct, an important rider follows. Practically all bottom-dwelling invertebrates have planktonic larvae. We know from Russell's work (1937–40) that the conditions for survival of zooplankton deteriorated sharply in 1931 and in subsequent years became steadily worse. This seems to be true for all species without exception. The number of larvae of bottom invertebrates surviving to settle and metamorphose on the bottom must therefore have been considerably less in the nineteen-thirties than in the nineteen-twenties. Once successfully metamorphosed and established in their adult home they should have found richer food for which there would have been reduced competition. Each single organism had therefore the opportunity to grow to a larger size. In the first place this argument applies to detritus feeders. Probably it may be extended to the whole of the bottom in-fauna, but not necessarily to

the members of the epi-fauna taking their food by filter-feeding mechanisms and the like from the overlying water. The rider is therefore that during the nineteen-thirties the in-fauna probably decreased in numbers but that each organism became relatively larger.

No estimate can be made as to whether the *mass* of invertebrate food available for demersal fish changed for better or worse. An increase in the average size of the prey might however adversely affect the ability of small fish to catch and swallow their food, leading to relatively greater mortality during the first years of life.

The improvement in the ray fishery discussed above would suggest that the mass of food must have increased. As against this the adverse conditions for the pelagic young of teleost fish and of carnivorous invertebrates may have resulted in the rays being subjected to less severe competition for the available food.

That arguments such as these, by themselves, prove nothing needs to be stressed. As Mr G. A. Steven has remarked to the writer, we have no positive evidence that food supply is dominant amongst the many factors which may govern the survival and numbers of elasmobranch fish. A single coincidence between the stock of a nutrient in the water and the landings of a commercial fish may be due to chance or to some associated more fundamental but unrealized variable. Phosphate itself, primary though it is as a *plant* nutrient, is better regarded as an indicator of water type comparable with salinity. Though water with a high content of phosphate and probably of other nutrients and growth-promoting substances has the potentiality for producing a greater biomass than one with a lower content, it need not be better fitted for a particular species of animal and the food on which it feeds.

Fertilizing the Ocean

There is nothing new in the idea that the productivity of a limited body of water may be improved by adding plant nutrients, and in some circumstances it may be economic as in carp ponds and Norwegian oyster pools. That enclosed arms of the sea may be treated in the same way is now the subject of study by Gross and his colleagues (1942–6) and shows distinct promise. To fertilize considerable areas of the open sea provides a fascinating theme for dialectic with little factual data for a real assessment. Ritchie (1944), with a natural distaste for the compulsory restriction or moderation of fishing activity which is the remedy for over-fishing offered by fisheries naturalists, proposes that the International Council should arrange the allocation of sums of money contributed by the several nations for chemical nutrients with the assurance that these distributed in the sea will support a larger fish population and an increased rate of fishing. Qualitatively none would disagree with this proposal which has received approval, unsupported by figures, from experience of the fisheries of Mauritius (Wheeler, 1945). Quantitatively the idea seems hopelessly uneconomic and has been assailed by Graham (1944) and by

Atkins (1944) on account of the 'mountainous' amounts of phosphate and other nutrients which would be needed. Ritchie riposted with figures which do not bear examination. He pointed out that 201,000 tons of phosphatic nutrients (as P_2O_5 which equals 88,000 tons as P) were used in agriculture in the United Kingdom and Eire in 1937 and that this is considerably in excess of the phosphate turnover in the English Channel. Maybe, but the phosphate *added* to the land helped to produce about 42,000,000 tons of crops and supported 12,900,000 cattle as well as numerous horses, sheep and pigs (figures are for 1939 from Whitaker's *Almanac*, 1941, pp. 632 and 704). The same amount of phosphate *present* in the English Channel in 1937 resulted in the landing of 76,000 tons of fish. Though doubling the amount of phosphate and other nutrients in the Channel might more than double the amount of fish, there remains an enormous gap to be closed between 76,000 tons of fish and 42,000,000 tons of crops plus millions of stock animals.

The very low economic efficiency of utilization of phosphate in the sea may be illustrated by the following figures.

McCance & Shipp (1933, table A) published large numbers of analyses of phosphorus in cooked fish flesh which were surprisingly uniform, around 0·25% of the weight of cooked edible flesh. Allowing a 20% loss in weight on cooking, this becomes about 0·2% of the raw flesh, a figure confirmed by a few analyses of raw fish fillets in their table H. The offal from the fish, mainly head, bones and gut will contain more phosphorus than the flesh, as was shown by the analyses of whitebait and sprats which were cooked whole. Though the fish curing or processing factory will be likely to convert its offal into agricultural fertilizers, domestic offal and that from retail shops will go to the rubbish bin. Only phosphate in fish flesh is of value to the domestic consumer. The significant ratio is thus:

$$\frac{\text{Phosphorus in fish flesh}}{\text{Wet weight of whole fish}}.$$

If two-thirds of the wet weight of the fish is considered to be edible flesh, this ratio will be $2/3 \times 0{\cdot}2\%$ wet weight of whole fish $= 0{\cdot}13\%$. This figure, though not very accurate, is a sufficient measure of the usable phosphorus recovered from the sea in fish and may be combined with figures for total landings of marketable fish to give a measure of the efficiency of recovery of phosphorus from the sea. Data for the years 1925–37 have been extracted from the *Bulletin Statistique* for areas VIID+VIIE (English and French Channel ports). The area of the English Channel is about 82,100 sq.km. and its average depth about 72 m. Its volume is therefore about $5{\cdot}9 \times 10^{12}$ cu.m. To derive a round figure for the phosphate available for plant growth, the winter maximum for station E1 has been assumed to represent an average figure for the whole of the Channel, enabling the total phosphate content of the Channel to be calculated for each year. The ratio of edible phosphorus in marketable fish to the available stock of phosphorus in the water has then been calculated.

Year	Weight of fish landed (tons)	Edible phosphorus in fish landed (tons)	Phosphorus available for growth in Channel (tons)	Usable phosphorus landed as percentage of phosphorus available for growth (%)
1925	81,000	115	111,000	0·104
1926	79,000	103	137,000	0·075
1927	74,000	96	100,000	0·096
1928	71,000	92	126,000	0·073
1929	95,000	123	126,000	0·098
1930	58,000	75	—	—
1931	76,000	99	95,000	0·104
1932	88,000	114	86,000	0·133
1933	95,000	123	97,000	0·127
1934	93,000	121	88,000	0·137
1935	65,000	85	77,000	0·110
1936	79,000	103	86,000	0·120
1937	76,000	99	88,000	0·112

Thus, during many years of intensive fishing, each year 0·1 % of the available stock of phosphorus has been recovered in a form usable by man. Though the fertility of the water might well be increased rather more than in proportion to the amount of fertilizer added, the prospect of dumping phosphate in a body of water such as the Channel appears most unattractive. Even if the efficiency of recovery could be increased ten times, the yield would still be much less than could be got from the soil dressed with the same amount of fertilizer.

As matters now stand, very large amounts of nutrients are being poured into the sea, the great sink, as sewage from coastal towns and by way of the rivers from inland towns and farms fertilized and unfertilized. Phosphorus is a very precious commodity which in not so many years will become very scarce. The scale on which phosphorus even now is being dissipated to the sea is more than the world can afford. In years to come the cry will be for more methods for recovering phosphorus from the sea, not for putting it in.

Millennia ago, nomadic man, realizing that hunting was an uncertain and wasteful method of gaining a livelihood, changed to an economically far more efficient, pastoral and agricultural existence. Fishing remains as the major hunting pursuit still followed by civilized man. If it is to become more productive it must cease to be feral and become pastoral. To that extent I agree with Ritchie. But an efficient exploitation of the soil—or the sea—demands full control of weeds and pests which otherwise take an undue share of any plant nutrients added. Until fishing becomes pisciculture, and weeding of unwanted algae and control of unwanted pests, i.e. competitive predators and parasites, take their due place, fishing can never be more than ruthless and non-selective hunting.

The writer wishes to express his indebtedness to Mr E. Ford and Mr G. A. Stevens for stimulating discussions and pertinent criticism.

SUMMARY

Landings of the spurdog, *Squalus acanthias*, at Mevagissey reflect the changes in phosphate at the neighbouring International Hydrographic Station E 1.

It is suggested that commercial landings of rays and skates ought to follow major changes in nutrient content of the overlying water with a time lag of some years. Fishery statistics for English Channel ports support this view. The dependence of the bottom in-fauna upon the fertility of the overlying water is also discussed.

Proposals to improve commercial fisheries by artificially fertilizing considerable areas of the open sea are critically examined. Such artificial enrichment is considered to be grossly uneconomic unless fishing becomes pisciculture in which weeding of unwanted algae and control of unwanted pests—competing predators and parasites—is undertaken.

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THE ESTIMATION OF PHOSPHATE AND OF TOTAL PHOSPHORUS IN SEA WATERS

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(Text-figs. 1–11)

CONTENTS

The Estimation of Phosphate

Description of an Absorption Meter

During an investigation of the total phosphorus (present as dissolved phosphate, dissolved organic phosphorus compounds, microplankton and suspended detritus) in samples of sea water, it became necessary to estimate phosphate in solution by an objective method. Estimation by eye of the molybdenum blue formed in the Atkins-Denigès method of analysis appeared to vary in precision from day to day; moreover, it was quite unsatisfactory in waters made turbid with added bacteria or other organisms.

Discussion with Dr W. R. G. Atkins, F.R.S., indicated that any method was to be avoided which measured the change in intensity of illumination falling on a photoelectric cell because very small voltage changes would have a considerable effect on the intensity of the light source. Such an instrument would not only need calibration, but this might change with time as the characteristics of the cell altered. The desiderata were defined as:

(1) The use of balanced photoelectric cells, the light falling upon them being kept constant except for fluctuations in intensity of the light source, which are not easily avoided.

(2) Fluctuations in the intensity of the light source to have no effect upon the null point.

(3) Any change with time in the characteristics of the photocells to become at once obvious and correctable.

(4) Readings in units directly proportional to the light absorbed by the molybdenum blue formed on adding stannous chloride to the liquid, and not based on a calibration curve.

It was also desired that no more than 70 c.c. of liquid should be sufficient for a measurement to be made.

A simple instrument has been developed which meets these requirements (Fig. 1). The observation tube T is filled with water and reagents; the slide, carrying lamp and photocell P_2, is then moved until no current flows through the galvanometer. The distance of lamp from P_1 is measured and the pointer on the slide set to read the *virtual* distance of L to P_2 on a logarithmic scale.

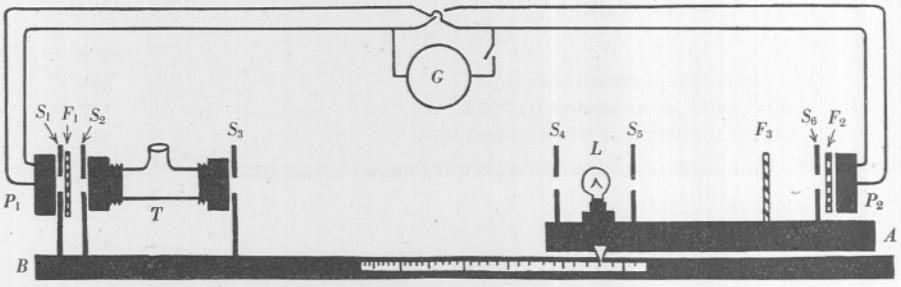

Fig. 1. Photoelectric meter. A, slide on which lamp and P_2 are mounted. B, base, 120 cm. overall length, on which P_1 and T are mounted, carrying scale showing logarithm of virtual distance of lamp from P_1. F_3, neutral filter made from two glass plates with smoked faces. G, galvanometer 483 ohms resistance, 2 sec. period, 200 mm. per μA. deflexion. L, lamp, car headlight 48 W. 12 V. with coiled filament in shape V, *circa* 4 mm. from corner to corner, held in bracket which is earthed, and operated through a dimming switch. P_1, P_2, selenium photocells, 23 mm. aperture, made by Evans Electroselenium Ltd., Bishop's Stortford. F_1, F_2, red light filters, Ilford 608 Spectrum. S_1 to S_6, light stops, diameter of aperture $\frac{1}{2}$ in. in S_1, $\frac{17}{32}$ in. in S_2, $\frac{7}{16}$ in. in S_3, $\frac{1}{2}$ in. in S_6. T, observation tube, glass $\frac{7}{8}$ in. internal diameter 15 cm. long with ground ends against which windows are held, having a thin coat of vaseline between the ground glass and window surfaces; the windows are removable for cleaning.

Since the 15 cm. observation tube is filled with liquid with refractive index *c.* 1·4, the virtual distance is some 4·3 cm. less than the actual distance. Thus if the actual distance were 80·0 cm. when no deflexion was shown on the galvanometer the virtual distance would be 75·7 cm. and the reading (or log d_0) 1·8791.

Stannous chloride is then added, the liquid replaced in the tube, and when the blue has fully developed the slide is moved towards P_1, until no current flows through the galvanometer. The virtual distance of lamp from P_1, or log d_1, is then shown by the pointer.

The increase in absorption of light passing through the 15 cm. column, due to the formation of molybdenum blue, is

$$\frac{d_0^2}{d_1^2} = 2\,(\log d_0 - \log d_1) \quad \text{or} \quad 2\Delta,$$

and the extinction coefficient of the molybdenum blue formed in the solution

$$E_{\text{cm.}} = \log_{10} \frac{\text{incident light}}{\text{transmitted light}} \text{ per cm. length of column}$$

$$= \frac{2\,(\log d_0 - \log d_1)}{15} \quad \text{or} \quad \frac{\Delta}{7{\cdot}5}.$$

The value of Δ is a direct measure, strictly proportional to the increase in blue colour in the solution, brought about by adding the reductant.

The value of the extinction coefficient needs further qualification. It changes with the wave-length of light, and therefore with both the composition of the red light transmitted by different filters and with the sensitivity of the photocell within this wave band. It is different if the same red filter is used with a different kind of photocell, or the eye, and it is different if the same photocell is used with another red filter which transmits light of different spectral composition.

Readings of $\log d_0$ and $\log d_1$ can be made to within 0·0005 on the scale; thus the instrumental error in values of Δ does not exceed $\pm 0{\cdot}001$. The colour formed in sea water due to the presence of 1 mg. phosphate-P per m.^{3} requires a movement of *c.* 0·0040 on the scale ($\Delta = 0{\cdot}0040$). Hence the instrumental error does not exceed that attributable to $\pm 0{\cdot}25$ mg. P per m.^{3}

Adjustment of the absorption meter is carried out as follows. The lamp filaments and stops S_3, S_2 and S_1 are carefully aligned with the slide in its nearest and farthest extreme positions, by observing the shadows cast by the stops with the light filters and photocells removed. The observation tube is inserted and the annularity of the light ring cast on S_1 checked by inspection.

The photocells and light filters are replaced and the slide moved until no current flows through the galvanometer. After the light has been on for several minutes the slide is readjusted to zero current (or null point) position and the light dimmed by the dimming switch. The galvanometer will show a deflexion as the light dims, coming back to zero as the light fades out. This appears to be caused by the two photocells differing in either their 'change in sensitivity with illumination', or in the speed with which they attain constant potential. When due to the former it can be remedied.

The sensitivity of a selenium cell changes if the illuminated area on its surface is moved. The 'change in sensitivity with illumination' likewise changes, but to a lesser extent.

To remedy the change in null point with change in intensity of light source, it was sufficient to move the stop S_6 in the vertical plane. This allowed the two photocells to be matched with respect to this characteristic. This having been done, slowly dimming the light source had no effect on the null-point position. Once matched the photocells appear to remain matched. In another similar instrument, an alternative method was used. A resistance was interposed

between the galvanometer and one of the photocells; this also necessitated altering the neutral filter F_3.

Two further properties of selenium photocells require consideration. If one cell is illuminated much more brightly than the other for more than a few seconds, it will fatigue and require a short time to return to equilibrium. There is no need to let this happen, because after the tube has been refilled with another sample, the lamp is turned on slowly through the dimming switch while the slide is being moved towards the new null-point position.

After the photocells have been in the dark for some time (as when the apparatus has not been in use), they require a short period to attain a full response after the light is switched on. In consequence the null point changes during the first 1 or 2 min. However, if the cells are illuminated (equally) for 10 min. and the light is then switched off for a short period, the response is more rapid when the light is switched on again. The null point steadied in 30–40 sec. and remained steady for several hours in a dust-free room.

Owing to this property of the cells the following procedure was adopted. After use, the observation cell is filled with water, replaced, and the slide set at null point, the lamp being then switched off. When next required the lamp is switched on about 10 min. before starting a series of measurements and only switched off for the short periods required for changing the contents of the observation tube.

After the initial alignment and matching of the photocells, the strict linearity of the readings was checked. This is necessary to make sure that (*a*) alignment is perfect at all positions of the lamp and (*b*) there is either no reflexion from the walls of the tube, or that the very small amount of reflected light follows the inverse square law.

The tube was filled with water and reagents and log d_0 noted. Several neutral glass filters were inserted between F_1 and S_2, one at a time, and the d_1 values noted. Then two or more were inserted, and Δ for the combination was found to equal the sum of the Δ's observed for each separately.

A further check was made to be sure the simple theory involved in calculating the virtual distance was obeyed.

The tube was withdrawn and the slide moved until no current flowed through the galvanometer. The *actual* distance between lamp and photocell (d_a) was measured in cm. A neutral filter having an optical density of about 0·4 was then inserted between F_1 and S_2, the slide moved to zero current position, and the actual distance between lamp and photocell (d_b) measured.

The optical density of this neutral filter for the particular wave band of red light and photocell used equals

$$2 (\log d_a - \log d_b).$$

The filled tube was then replaced, the slide moved until no current flowed, and log d_c, being the log of the *virtual* distance of lamp from photocell, read. The neutral filter was again inserted, the slide moved to the new zero deflexion position, and log d_e noted

$$2 (\log d_a - \log d_b) \text{ equalled } 2 (\log d_c - \log d_e).$$

This evidence indicated that the instrument obeyed the simple theory involved. It also satisfied the other initial desiderata.

On the Formation of Molybdenum Blue

When stannous chloride is added to sea water containing ammonium molybdate and sulphuric acid, either no colour is formed, colour is formed only when phosphate is present, or colour is formed when no phosphate is present.

The conditions under which these eventualities take place are shown in Fig. 2.

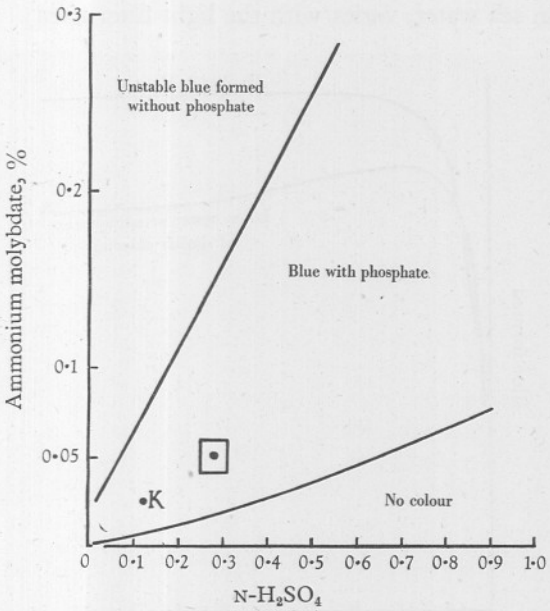

Fig. 2. Diagram showing the effect of varying concentrations of sulphuric acid and molybdate on the formation of blue in sea water, from data by Gripenberg (1929) and Kalle (1934). The point within the square represents the conditions investigated in this communication, the point marked *K* the conditions investigated by Kalle.

The following observations relate to the formation of molybdenum blue in sea water and in very dilute solutions of phosphate to which had been added:

(1) Sulphuric acid, making the solution *c.* 0·28 N with a pH of 0·82.

(2) 0·05% ammonium molybdate,

and had been reduced by the addition of at least 0·00045% $Sn^{\cdot\cdot}$.

This corresponds to the addition of 2 c.c. of Atkins-Denigès reagent to 100 c.c. of sea water.

On the addition of stannous chloride, the colour develops rapidly (Fig. 3) and is followed by a relatively slow fading, which becomes still slower as fading progresses.

With phosphate in distilled water colour development is less rapid, and the depth of colour, due to the presence of the same amount of phosphate, is greater than in sea water or salt solution.

In distilled water and in sea water, both the rate of colour development and the final depth of colour produced are greater with increasing temperature, and are greater if more stannous chloride is used, up to but not beyond a limiting quantity.

The blue substance produced in distilled water has a different light-absorption band to that produced in sea water (Buch, 1929; see also Cooper, 1938, who finds that the 'salt error', or ratio of colour produced in distilled water to that produced in sea water, varies with the light filter used).

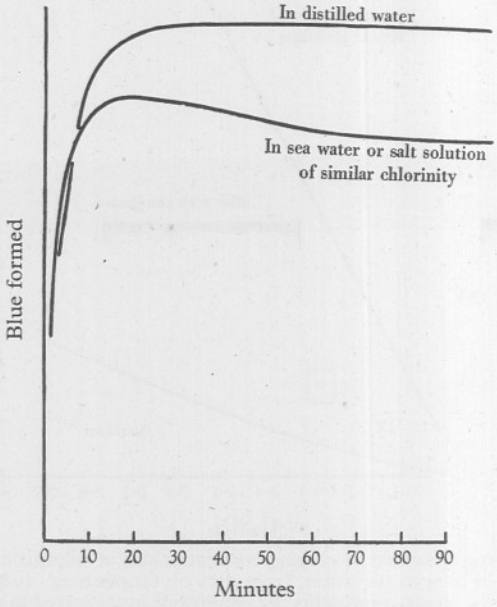

Fig. 3. Diagram showing rate of formation and subsequent fading of blue, due to the same concentration of phosphate in distilled and in sea water, at 10° C.

These blue substances have colloidal properties. The molybdenum blue formed by mixing trivalent molybdenum with molybdate has been seen to consist of aggregates or micelles of very different sizes (Schirmer, Andrieth, Gross, McClellan & Seppi, 1942) when viewed under an electron microscope. It seems reasonable to assume that the subsequent fading of the blues produced in acid water or acid salt solutions is due to aggregation of colloidal particles or micelles.

A study of these variables which affect the production of the blue substance in sea water containing phosphate was made in order to decide upon a reasoned procedure in carrying out analyses.

Variation in acidity. The effect of relatively small variations in acidity and in molybdate concentration upon the quantity of blue formed in sea water

containing phosphate are shown in Fig. 4. It is seen that a 1% increase in acid decreases the colour by about 0·5%.

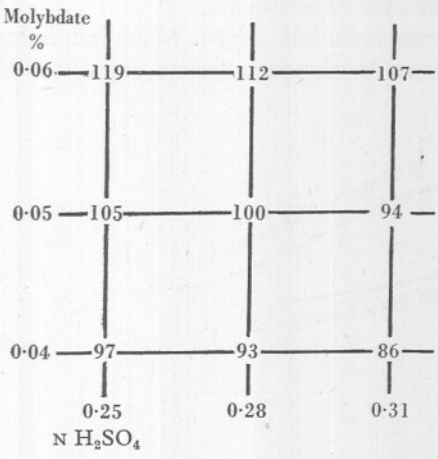

Fig. 4.

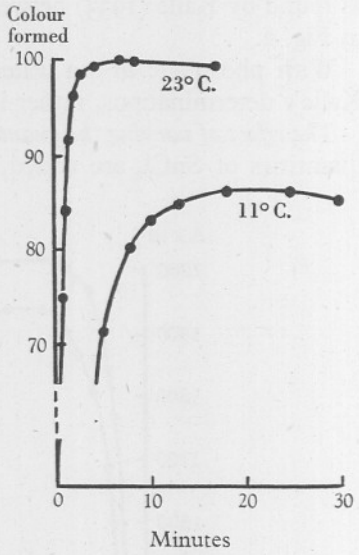

Fig. 5.

Fig. 4. Effect of varying concentrations of sulphuric acid and of molybdate on the blue formed in sea water containing phosphate. The colour produced with 0·05% ammonium molybdate and 0·28 N-acid is shown as '100'.

Fig. 5. Diagram showing the rate of colour formation and the amount of blue developed in the same sea water at 11 and 23° C., after addition of the same quantity of stannous chloride.

Time taken for ionic equilibrium to be attained after adding the acid molybdate to sea water. The following experimental data show that a period of some 3 min. is taken for equilibrium to be attained:

Stannous chloride added	Intensity of blue formed
At once	>161
After 1 min.	124
3	100
6	99·4
26	100·5
60	100
3 hr.	100
24	95·6

The effect of temperature. Increasing the temperature increases the velocity of the reactions involved. The maximum colour is developed sooner and the interval of time during which the colour is within ±0·5% of its maximum value becomes shorter. (This interval is also shorter if the quantity of stannous chloride is reduced.) Increasing the temperature also increases the amount of blue colour developed (Fig. 5).

With phosphate in distilled water the increase in colour was found to be 0·96% per rise of 1° C. within the range of 8–20° C. This is the same value as found by Kalle (1933) between 5 and 30° C. under the condition indicated in Fig. 2.

With phosphate in sea water the increase was 1·2% per rise of 1° C. in Kalle's determinations, rather less in my own experiments.

The effect of varying the quantity of stannous chloride added. When increasing quantities of $SnCl_2$ are added, the colour developed increases rapidly until

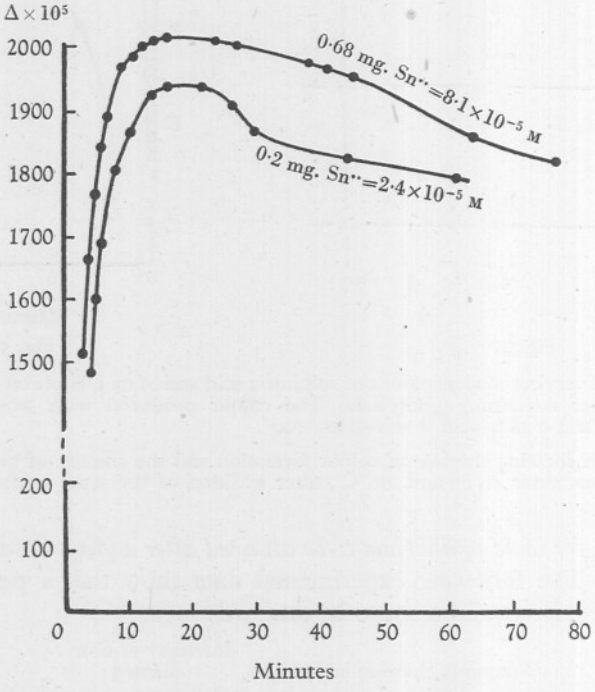

Fig. 6. The formation and fading of blue developed in a sea water, enriched with 40 mg. phosphate-P per m.³ at 10·5° C. Upper curve shows the course with 0·68 mg. Sn·· added to 70 c.c. of liquid, lower curve with 0·2 mg.

about 12 times more Sn than the phosphate-P present has been reached (Gripenberg, 1929). Thereafter there is a slow increase in colour developed. With considerable additions the development of blue in phosphate solution becomes disproportionately greater than in sea water.

The present investigation has been confined to additions varying between 0·2 and 0·8 mg. Sn·· to 70 c.c. of liquid—initial concentrations lying between 2·4 and $9{\cdot}6 \times 10^{-5}$ molar Sn·· and not exceeding $0{\cdot}15 \times 10^{-5}$ molar phosphate.

Within this range the increase in colour due to each additional 0·1 mg. Sn·· added amounts to approximately 1%.

Increasing the stannous chloride also increases the velocity of colour formation, and, in addition, delays the onset of fading (Fig. 6).

On mixing with sea water to which acid and molybdate have been added, the stannous ions are rather soon oxidized. By adding N/100 iodine after intervals and back-titrating with thiosulphate, it was possible to follow the fall in concentration of stannous ions (Fig. 7).

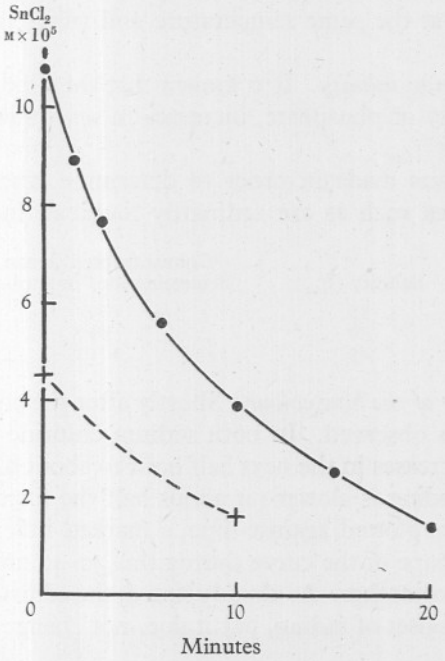

Fig. 7. Diagram showing the rate of oxidation of stannous chloride added to sea water containing 2% of Atkins-Denigès reagent.

The effect of particulate matter in suspension and of the products of organic decay. The presence of either bacteria or of kaolin in sufficient quantity to make the water just visibly cloudy does not interfere with reasonably exact measurement of the blue formed on adding stannous chloride.

Series of estimations have been made with a sea water in which bacteria had grown after the addition of asparagin in sufficient quantity to raise its absorption coefficient in red light by $E_{cm.}$ 0·0036. The effect of this particulate matter was to reduce the blue colour formed on adding stannous chloride by 4%.

A further series of estimations have been made with a natural sea water just visibly cloudy with kaolin, which had entered the sea after heavy rains. There was sufficient in suspension to increase the absorption coefficient of red light of the water by $E_{cm.}$ 0·009. This had the effect of reducing the blue colour formed by 5%.

In another experiment a natural sea water was incubated for several days with a considerable quantity of added plankton organisms until they had decayed. After passing through filter paper the water showed no marked cloudiness. Determinations of the increment in blue colour developed due to the addition of 20 mg. phosphate-P per m.^{3} were made in order to find out whether organic decay products affected the production of blue colour. The increment found ($\Delta = 0{\cdot}0832$) at 15° was the same as that in unpolluted sea water ($\Delta = 0{\cdot}0830$) at the same temperature and using the same quantity of stannous chloride.

The effect of varying salinity. It is known that the blue colour formed, due to the same quantity of phosphate, increases in sea waters when the salinity falls below about 25 °/$_{oo}$.

An experiment was made in order to determine whether there was any variation at salinities such as are ordinarily found in inshore and open sea waters:

Salinity (°/$_{oo}$)	Colour formed (mean of determinations in triplicate)
35	100
30	99·5
25	101·8

The rate of fading of the blue colour. Shortly after the colour has reached its maximum, fading is observed. In both sodium chloride solution and in sea water the colour decreases in the next half hour to about 94 % of its maximum value. Thereafter fading is slower (at about half the former rate). When the red light absorbed is plotted against time, a marked inflexion in the curve is apparent, and the shape of the curve during this 30–40 min. differs somewhat from one sea water to another. As already stated, the addition of more stannous chloride delays the onset of fading, but it does not change the general form of the curve.

These observations led to the following hypothesis. Immediately after formation, the molecules aggregate to form micelles and these continue to aggregate—growing larger and fewer. The rate of aggregation, quick at first, slows later. As the molecules aggregate and gradually grow larger, the blue colour fades. The light absorbed by the solution not only becomes less but moves from the red towards shorter wave-lengths. On inspection the fading blue solutions are seen to become both less intense and to acquire a blue-green hue. Addition of 0·05 % of gum arabic, acting as a protective colloid, smooths out the inflexion in the curve as the blue fades while greater additions delay the inception of fading.

That aggregation would cause fading seems certain on considering the situation in reverse, for if particles of a slowly dissolving dye are suspended in water the colour increases as the particles dissolve.

This hypothesis provides an explanation of observed characteristics in the rate at which the blue colour develops.

On the rate at which blue develops. As a first approximation, the light absorption (Δ_t) was taken as proportional to the quantity of phosphate which had reacted at time t during the reaction, since the maximum colour developed (Δ), when the rate of development and fading balance each other, has been found proportional to the phosphate present at the commencement.

Then $\Delta - \Delta_t$ is a measure of the unreacted phosphate concentration at time t; if the logarithm of this is plotted against time an almost linear relation is found. It is almost but not quite linear with phosphate in distilled water, salt solution or sea water.

This indicates that of the several ions taking part in the reaction all but one—phosphate—are in excess. The apparent velocity approaches that of a monomolecular reaction controlled by the concentration of unchanged phosphate ($\Delta - \Delta_t$) present at any moment.

The velocity will tend to depart somewhat from the course of a monomolecular reaction because (*a*) the concentration of stannous chloride is falling throughout the reaction (Fig. 7) and also because (*b*) fading of the blue, due to aggregation of molecules and then further aggregation of the micelles so formed, is not likely to follow the course of a monomolecular opposed reaction.

With regard to (*a*), it is seen from Fig. 6 that increasing the initial concentration of reductant increases the reaction velocity. It would be expected that if the oxidation of stannous chloride by atmospheric oxygen throughout the period of blue formation could be retarded, this would have the same effect. Experiment confirmed this. The addition of sulphite (0·01 M) increased the rate of blue formation. Hence the quantity of stannous chloride present is not in sufficiently gross excess throughout to allow the monomolecular reaction to proceed entirely unaffected.

With regard to (*b*), if fading is most rapid immediately after the formation of blue and does not continue at a rate proportional to the intensity of blue colour during the early part of the reaction (which it does not do, *vide supra*), Δ_t is more than proportional to the phosphate which has taken part in the reaction at time t. In consequence the apparent velocity calculated from this first approximation (Δ_t = phosphate which has reacted at time t) should (i) be greater than the mean velocity early in the reaction while the major part of the blue is being formed, (ii) dwindle for a short period after the initial rapid development of most of the blue while this is most rapidly aggregating, and (iii) indicate a slight rise in apparent velocity towards the end of the reaction after the greater part of the blue has been formed and has had time to undergo its early aggregation. Experiment shows that this is so.

The velocity constant for a monomolecular reaction was calculated for successive intervals of time during the formation of blue:

$$k\,(\text{apparent}) = \frac{2{\cdot}3}{t_2 - t_1} \log_{10} \left(\frac{\Delta - \Delta t_1}{\Delta - \Delta t_2} \right).$$

The following is illustrative.

Time (min.)	Δ_t or light absorbed	Interval (min.)	k (apparent)
0	0	—	—
0·935	0·0900	0·933	0·769
1·45	0·1130	0·517	0·604
1·935	0·1285	0·483	0·586
2·47	0·1410	0·533	0·575
3·25	0·1530	0·783	0·540
3·915	0·1595	0·667	0·504
6·0	0·1710	2·083	0·587
8·0	0·1742	2·0	[0·55]
10·0	0·1752	—	—
13	$0·1758 = \Delta$	—	—

A number of such experiments have been made and the value of the 'apparent velocity constant' plotted as in Fig. 8. A fall in the value followed by a slight rise was always found, before the value finally fell to zero at the point where maximum colour is attained. These phases during the course of the formation of blue are typical of the reaction in sea water, in salt solution and in distilled water.

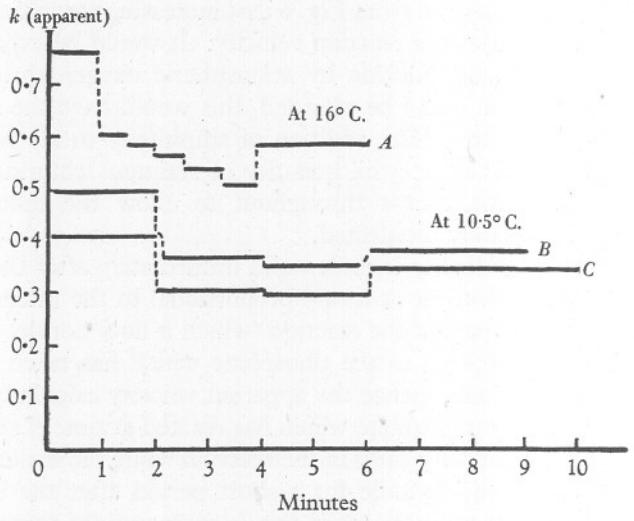

Fig. 8. The value of the apparent reaction velocity during intervals while blue was being formed in phosphate-enriched sea water. *A* at 16° C. and $6·3 \times 10^{-5}$ M-$Sn^{\cdot\cdot}$; *B* at 10·5° C. with $8·1 \times 10^{-5}$ M-$Sn^{\cdot\cdot}$; C at 10·5° C. with $2·4 \times 10^{-5}$ M-$Sn^{\cdot\cdot}$.

The addition of a protective colloid hinders the aggregation of the electronegative blue micelles, thereby increasing the rate of colour formation. Gelatin, electropositive at this acidity, proved more potent than arabic. Both substances have a secondary effect, reducing the total colour developed possibly because they form complexes with the molybdic acid. Citric, oxalic

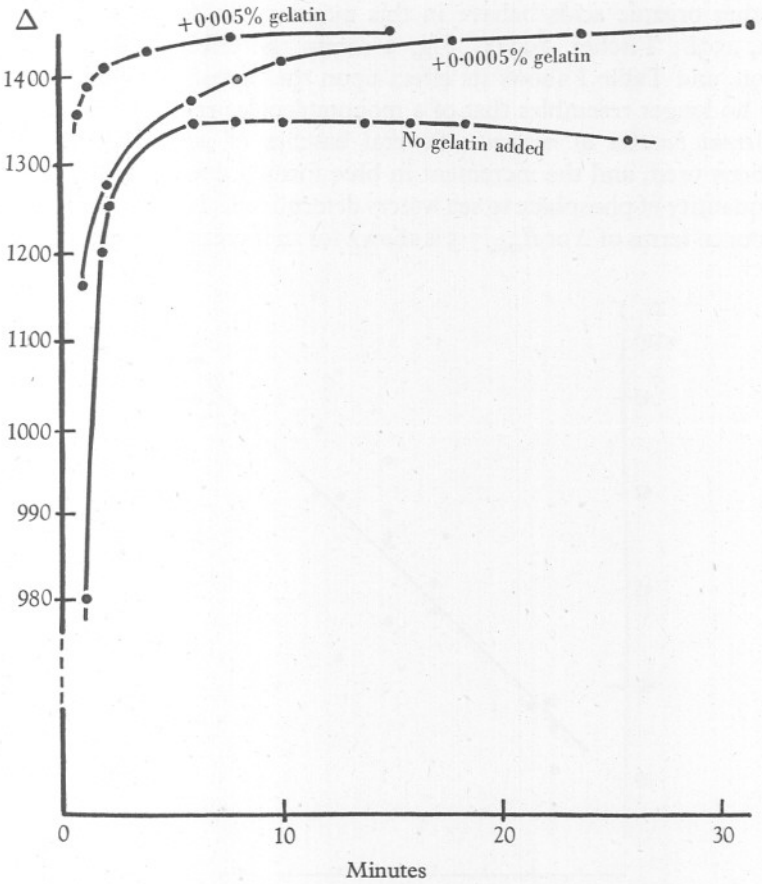

Fig. 9. The effect of a protective colloid on the formation of blue in a phosphate-enriched sea water.

TABLE I. INFLUENCE OF GELATIN ON THE APPARENT VELOCITY CONSTANT IN SEA WATER AT 16° C.

No addition		With addition of gelatin	
Interval (min.)	k (apparent)	Interval (min.)	k (apparent)
0 –0·92	1·4	0 –0·90	1·75
0·92–1·28	1·2	—	—
1·28–1·75	0·9	0·90– 2	0·334
1·75–2·15	0·44	—	—
2·15–4	1·0	2 – 4	0·260
4 –6	0·6	4 – 6	0·194
Maximum colour reached in 10 min.		6 – 8	0·174
		8 –12	0·10
		12 –24	0·11
		Maximum colour reached in 30 min	

and other organic acids behave in this manner (Kalle, 1935; Berenblum & Chain, 1938; Tischer, 1934). Fig. 9 shows the effect of gelatin upon the reaction, and Table I shows its effect upon the 'apparent velocity constant', which no longer resembles that of a monomolecular reaction.

Different batches of reagent. Several batches of acid molybdate reagent have been used, and the increment in blue formed due to the addition of the same quantity of phosphate to sea waters determined. In Fig. 10 the increment in colour in terms of Δ or $E_{cm.}/7{\cdot}5$ is shown for an increase of 1 mg. phosphate-P

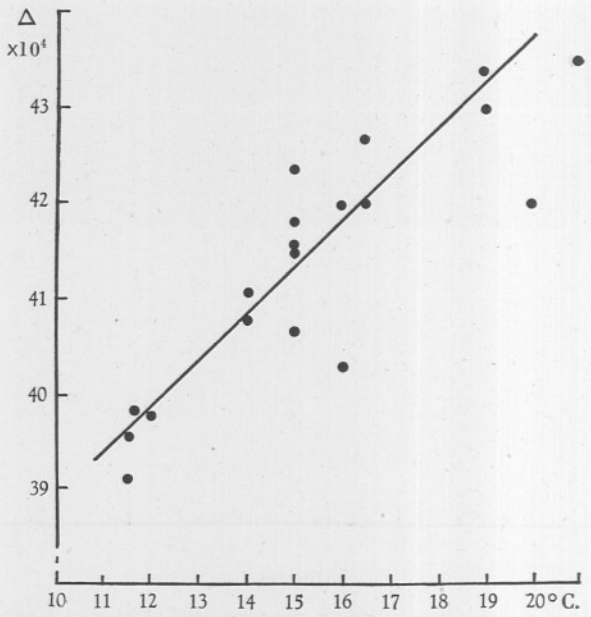

Fig. 10. The points show the increment in Δ due to the addition of 1 mg. phosphate-P per m.^{3}, obtained with different batches of reagent and different sea waters on different dates. The line shows the temperature relation as found by Kalle, being an increase of 1·2% per degree rise in temperature. The quantity of stannous chloride added varied between 0·35 and 0·44 mg. $Sn^{\cdot\cdot}$ to 67 c.c. of sea water plus 3 c.c. of reagents.

per cm.^{3} as found on a number of occasions. This *increment value* appears to be almost constant, when the same quantity of stannous chloride has been used, and provided that the effect of temperature is taken into consideration—an increase of 1·2% per rise of 1° C. The line in the diagram is drawn to show this temperature relation.

Since it has been noticed that the rate of colour formation and fading, at the same temperature, etc., differs slightly between one sea water and another, and since particles in suspension affect the colour formation, a precise agreement would not be expected. The majority of the values lie within about $\pm 2\%$ of the expected, which is greater than the probable experimental error.

Hence there appears some reason to suppose that sea waters may be met with which contain a substance which slightly inhibits the formation of blue.

Linear relation between phosphate present and colour formed. A number of experiments have been made which show proportionality. The following series of observations is illustrative:

	Δ observed		
With distilled water	0·0032		
	0·0032		
	0·0024	Reagent-blank = 0·003Δ mg. P per m.³ calculated*	
With filtered sea water	0·0120	2·20	
	0·0115	2·09	
			Increase
Do. + 5 mg. phosphate P per m.³	0·0320	7·1	5·1
	0·0335	7·5	5·4
Do. + 10 mg. phosphate P per m.³	0·0535	12·4	10·3
	0·0533	12·3	10·2
Do. + 15 mg. phosphate P per m.³	0·0735	17·2	15·1
	0·0735	17·2	15·1
Do. + 20 mg. phosphate P per m.³	0·0910	21·6	19·5
	0·0900	21·5	19·3
	0·0905	21·4	19·3

* Being Δ observed less the reagent-blank, divided by the increment value for the temperature of the experiment, 14° C., this increment value being 0·00408Δ per mg. P per m.³.

Successive reactions. If, after adding the stannous chloride and allowing the colour to develop fully, phosphate (in 0·28 N-H_2SO_4) is added within the next few minutes there is a second development of blue. The amount formed in this second reaction is slightly less than the increment due to adding the same quantity of phosphate initially. This lesser formation of colour appears due to some of the stannous chloride having been oxidized during the first reaction.

However, if, at the same time as adding the phosphate, a second addition of stannous chloride is made (about two-thirds of the quantity originally added) the amount of blue which develops is nearly equal to the increment when the same amount of phosphate is added initially. For instance:

Using filtered sea water at 18° C., the following readings were obtained on the photoelectric meter.

	log cm. virtual distance lamp to photocell	
	Sample A	Sample B
67 c.c. sea water + acid molybdate + Sn	1·8465	1·8470
After adding 20 mg. phosphate-P per m.³ and more Sn	1·7590	1·7600
Increment due to addition	0·0875	0·0870
After adding a further 20 mg. phosphate-P per m.³ and more Sn	1·6740	1·6760
Increment due to addition	0·0850	0·0840
67 c.c. sea water + 20 mg. phosphate-P per m.³ + acid molybdate and Sn	1·7620	1·7610
Increment over mean value of 1·8467 for water without added phosphate	0·0847	0·0857

Procedure adopted. (1) To 67 c.c. samples of sea water in 100 c.c. flasks were added 3 c.c. of an acid molybdate solution made by dissolving 6·5 g. of ammonium molybdate in 500 c.c. of water and 110 c.c. of concentrated sulphuric acid. This acid molybdate solution attains ionic equilibrium within 48 hr. and thereafter undergoes little or no change when stored in hard glass, but changes in contact with ground glass. It is equivalent to the Atkins-Denigès reagent diluted with an equal quantity of water. The addition is made most conveniently by means of a simple automatic pipette (Fig. 11) while swirling the sea water, in order to avoid the possible formation of chlormolybdate (*vide infra*).

(2) The sea-water samples plus reagent are each transferred to the observation tube of the photoelectric meter, the d_0 readings obtained, and the liquids returned to the flasks.

(3) Three drops of a solution of stannous chloride in dilute hydrochloric acid, containing between 0·3 and 0·45 mg. $Sn^{\cdot\cdot}$, are then added while swirling each sample of the sea water plus reagent. Each sample is returned to the observation tube and the d_1 reading taken when the colour formation reaches a maximum. The time interval, after adding the reductant, during which 99·5 % of the maximum colour is present, varies with temperature at

11° C.	14–23 min.
14° C.	12–18 min.
16° C.	11–15 min.
23° C.	5–14 min.

The difference between d_1 and d_0 or Δ is thus obtained for each sample, and the temperature noted. Before calculating the phosphate content it is necessary to subtract the value for the reagent-blank.

(4) The observation tube is very thoroughly washed to rid it of any trace of stannous ions, and the operations repeated using the same reagent and stannous chloride solution with distilled water. With distilled water the time interval after adding reductant during which 99·5 % of the maximum colour is present is longer than with sea water at

9° C.	45–145 min.
15° C.	15– 45 min.
20° C.	10– 30 min.

The value found for Δ for each sample of sea water minus the value of Δ for distilled water (the reagent-blank) is then directly proportional the phosphate content of the sea-water sample.

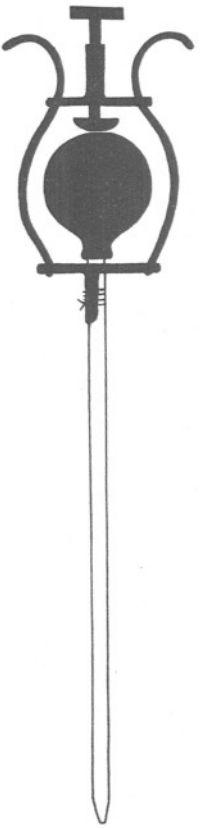

Fig. 11. Simple automatic pipette used for adding reagent while shaking contents of flask, showing rubber ball on glas pipette and plunger wit limited travel adjusted t suck in and deliver t required quantity.

(5) The increment value for unit addition of phosphate-P per m.^{3} may be fo by enriching samples with 20 mg. phosphate-P per m.^{3}. This is conveniently don adding 0·3 c.c. of a solution containing 4·5 mg. phosphate-P per l. of 0·28 N-H_2 to 67 c.c. samples of filtered sea water by means of a Krogh syringe pipette.

Alternatively, the increment may be determined by adding phosphate additional reductant after the d_1 reading has been obtained (p. 351).

The values of phosphate content obtained by the phosphomolybdate method of estimation include any *arsenate* which may be present in the water. Rakestraw & Lutz (1933) found between 7 and 24 mg. As per m.^{3} in various samples of sea water, and if all this were present as arsenate it would cause the formation of blue colour equivalent to 3–10 mg. phosphate-P per m.^{3}. That at least some of the arsenic present in natural sea waters is in the form of arsenate seems probable, because if small quantities of sodium arsenite (20–40 mg. As per m.^{3}) are added to sea water it oxidizes to arsenate, the rate of oxidation varying between one sea water and another.

The values of phosphate found also include any particles of inorganic phosphate suspended in the water which dissolve in the 0·28 N-acid. Turbid inshore waters may contain considerable quantities. Thus a water collected off the Suffolk coast after filtration and addition of the acid reagent was found to contain 3·7 mg. phosphate-P per m.^{3}; but, when the acid reagent was added before filtering, 10·5 mg. per m.^{3} were found. In the clear transparent waters of the English Channel, Cooper (1948) finds evidence of particulate phosphate in suspension varying hand in hand with the quantity of particulate iron present.

In consequence, it is to be expected that subsamples from the same sea water may vary in their phosphate content. In clear waters from the English Channel variations exceeding ± 1 mg. phosphate-P per m.^{3} appear to be rare.

With subsamples of a filtered water, on the other hand, the variation appears to lie within or almost within the instrumental error, $\pm 0{\cdot}25$ mg. P per m.^{3}.

The Estimation of Organic Phosphorus Compounds in Solution

Several attempts to estimate the total phosphorus in sea-water samples culminated in the successful methods devised by Kalle (1935) and by Redfield, Smith & Ketchum (1937).

The present investigation aimed at modifying this latter method to obtain a quicker and simpler technique. For survey purposes it had the disadvantage that the estimation involves several processes taking time; it also involved subtracting rather high and variable reagent blank-values.

Preliminary experiments, using visual colorimetry, showed that autoclaving at 135–140° C. for 5–6 hr. in 0·28 N-H_2SO_4 completely hydrolysed such compounds as nucleic acid, phospho- or glyceroproteins dissolved in sea water. It also completely hydrolysed the phosphoric esters in diatoms.

In control experiments using distilled water instead of sea water, the reagent-blank values were high and variable. It became apparent that phosphate leached out of the glass flasks into the 0·28 N-acid during autoclaving at 140° C., even after the flasks had previously been cleansed by heating in concentrated sulphuric acid. Finally, 100 c.c. silica flasks were used. These

give consistent reagent-blank values equal to the values obtained without autoclaving.

During the cooking any arsenite was oxidized to arsenate; by adding a small quantity of sodium sulphite before heating, this was not only prevented but added arsenate was almost completely reduced to arsenite. These latter observations were confirmed after the method of phosphate estimation had been more fully developed.

Experiment showing the effect of adding arsenate to an aged and filtered sea water. Samples of the water, with and without the addition of 44 mg. arsenate-As per m.^{3}, were acidified to 0·28 N with sulphuric and 0·15 % $Na_2SO_3.7H_2O$ added. After autoclaving for 5 hr. at 140° C. and cooling, the volumes were made up to the original volumes and an acid molybdate solution added to give 0·05 % ammonium molybdate.

	Phosphate found mg. P per m.^{3}
Water only	7·6, 6·7, 6·7
Water and arsenate	7·0, 6·9

The photoelectric estimation of molybdenum blue allowed determinations to be made in water turbid with bacteria. This, in turn, allowed the question whether hydrolysis of the organic phosphorus was complete to be attacked by another and more exact method.

When sea water is enriched with asparagine (20 mg. per l.) and the bacteria allowed to proliferate, the inorganic phosphate is used up by the bacteria, which are singularly rich in phosphorus, having a C/P ratio of about 22. When phosphate was added to such a slightly turbid water and then estimated, the increase in phosphate due to this addition found by experiment was 4 % less than when the same quantity of phosphate was added to a control in which bacteria had not developed. Following this preliminary experiment a sea water containing 14½ mg. inorganic phosphate per m.^{3} was enriched with asparagine. Ten silica flasks were charged with this, five being incubated until all the dissolved phosphate had been used by bacteria while the other five were acidified and stored in the cold. Then, after hydrolysis at 140° C., the resulting phosphate (including the equivalent of any arsenic present) was estimated (Table II).

TABLE II. PHOSPHATE, IN MG. P PER M.^{3}, FOUND IN FLASKS

After storage without development of bacteria	After incubation and utilization of 14·5 mg. P per m.^{3} of dissolved phosphate by bacteria
22·6	21·6
22·75	21·7
22·2	21·0
22·4	21·2
22·6	21·6
Mean 22·51 ± 0·149	Mean 21·42 ± 0·165 + 4 % = 22·28 ± 0·172

Hence at least 93 % of the phosphorus in the bacterial tissue had been reconverted to phosphate in solution. Assuming that the bacterial cells, after cooking, reduced the colour formation by 4 % as they did in the previous experiment, virtually all the phosphorus in the bacterial tissue was reconverted to phosphate.

In the earlier experiments it was found that the use of ammonium molybdate solutions in distilled water led to high reagent-blank values; furthermore some anomalous results were obtained. This necessitated a study of the inter-ionic changes which take place in solutions of ammonium molybdate during storage and on their mixing with acidified sea water. The following observations were made.

Formation of silicomolybdate. On storing aqueous solutions of ammonium molybdate in glass bottles, their use resulted in high reagent-blank values, less so if the molybdate was dissolved in dilute sulphuric acid, and not at all if dissolved in 3 N or 6 N-acid.

No such progressive increase in the reagent-blank values resulted from the use of a 1·65% solution of ammonium molybdate in 0·56 N-H_2SO_4 stored in waxed bottles.

Presumably silicomolybdate ions were formed during storage in glass and these were reduced to silicomolybdate blue by stannous chloride. The reduction takes place more rapidly than that of phosphomolybdate; another difference is that the same amount of blue is formed in sea water as in distilled water. In consequence the 'reagent-blank' resulting from the presence of silicomolybdate is not subject to salt error.

Experiment. A 1·65% solution of ammonium molybdate in 0·56 N-H_2SO_4 was used which had been stored in a glass bottle for 2 months, its reagent-blank having meanwhile risen to a high value while the liquid had acquired a faint yellow tint.

With filtered sea water the blue formed after adding stannous chloride was the same, whether the reductant was added a few minutes or 18 hr. after the molybdate solution. This showed that the silicomolybdate ions were stable in sea water containing acid and molybdate at the customary concentration and acidity. The silicomolybdate blue developed more rapidly (80% within 50 sec. at 10° C.) than the phosphomolybdate blue develops from the reduction of phosphomolybdic acid at the same temperature (80% in *c.* 4·5 min.) and its subsequent rate of decay was less.

A further experiment was made with the same stored reagent in order to find whether the presence of a large quantity of silicomolybdate ions would interfere with the estimation of phosphate.

A filtered sea water was found to contain 7·0 mg. phosphate-P per m.^{3}, using a reagent with very low reagent-blank. The following estimation was then made using the glass-stored acid molybdate solution.

	Colour developed
With distilled water (duplicate)	118·1 117·9 } reagent-blank
With filtered sea water (duplicate)	145·2 144·8
With same + 20 mg. phosphate-P per m.^{3}	222 222

Increase in colour due to addition of 20 mg. P/m.^{3} = 77·0
Colour developed in filtered sea water (145) less reagent-blank (118) = 27·0
Phosphate content of filtered sea water = $\frac{27{\cdot}0 \times 20}{77{\cdot}0}$ or 6·8 mg. phosphate-P per m.^{3}

Silicomolybdate ions are formed on addition of a silicate to a relatively strong (1·65%) solution of ammonium molybdate in water or dilute sulphuric acid, but they are not formed if small quantities of silicate are added to 0·05% molybdate in 0·28 N-H_2SO_4. Thus they are not formed at room temperature *subsequent* to the addition of the molybdate reagent in phosphate determinations.

Some evidence was obtained of the formation of silicomolybdate while a 5% solution of ammonium molybdate mixes slowly with the acidified sea water, but not if a 1·65% solution in dilute sulphuric is used.

Formation of chlormolybdate. Evidence was obtained suggesting that within a limited range of acid and of molybdate concentration chloromolybdic ions may be formed in the presence of chloride. The supposed chloromolybdate is reduced by stannous chloride to a blue; the chlormolybdate is unstable at greater dilution in sea water made 0·28 N with sulphuric acid, slowly reverting to molybdate. The above conclusions rest on the following observation.

(*a*) When sulphuric acid was added to sea water without shaking, so that the upper levels in the flask were less acid, and molybdate then added, the flask being later shaken before adding reductant, over twice as much blue colour developed than when the water and acid had been thoroughly mixed prior to the addition of the molybdate.

(*b*) A solution was made containing 1·67% ammonium molybdate and 3½% sodium chloride in 0·28 N-sulphuric acid. After it had stood for 4 days, its addition to acidified sea water in the customary proportions followed 15 min. later by stannous chloride led to the formation of a strong blue colour. If the reductant was added 30 min. after the acid-salt-molybdate solution, the blue colour formed was 25% less. The experiment suggested that chlormolybdate ions had formed in the acid-salt-molybdate solution, and on adding this to sea water made 0·28 N with sulphuric acid, these ions slowly reverted.

The foregoing observations led to the adoption of the following procedure in estimating the total phosphorus in a water sample—phosphorus present as phosphate in solution, as organic phosphoric esters in solution, in microplankton and in organic detritus, and as phosphate present as inorganic phosphate particles.

Procedure adopted for estimation of total phosphorus in water samples. To 67 c.c. of sea water in 100 c.c. silica flasks are added (i) exactly 1 c.c. of concentrated sulphuric acid which has been diluted with an equal volume of water, (ii) 4 drops of a saturated solution of sodium sulphite. The flasks are then covered and heated for 5–6 hr. in a pressure cooker at between 30 and 40 lb. per sq.in. After cooling the volume of each is made up to 68 c.c. with distilled water, the contents being thoroughly mixed. Then, while the contents are being swirled in the flask, 2 c.c. of a solution containing 6·6 g. of ammonium molybdate in 400 c.c. of water plus 6 c.c. of concentrated sulphuric acid are added. This addition is conveniently made by means of an automatic pipette (Fig. 11); the use of a burette or any contact with ground glass is to be avoided. The molybdate solution is stored in a waxed bottle, and appears to attain interionic equilibrium within 48 hr. after being made.

The d_0 and d readings are taken. From the Δ values so obtained the phosphate contents are calculated after subtracting the reagent-blank.

These values are subject to a small, almost insignificant, correction because the acidity is reduced by about 4% as the sulphur dioxide formed is driven off during cooking. This reduction in acidity causes 2% more colour to be formed: therefore the calculated values of phosphate content are subject to a correction of −2%.

The estimations of phosphate by this (or Redfield's) method will not include the equivalent of any arsenate present in the water.

Variable quantities of microplankton organisms and of particulate phosphate are likely to occur in subsamples of the same sea water. Some data showing the variability encountered in offshore English Channel waters are given in the following.

(i) Sea water collected near the Eddystone Lighthouse, 30 September 1946, at a depth of 2 m., contained an appreciable quantity of particulate matter. Estimation of phosphate in seven subsamples showed the presence of 12·0, 12·6, 12·6, 12·6, 12·7, 12·8, 12·8 mg. phosphate-P per m.^{3}.

Estimation of total phosphorus in seven subsamples showed the presence of 16·1, 16·1, 16·1, 16·5, 16·5, 17·0, 17·0 mg. P per m.^{3}.

(ii) Subsamples of raw sea water collected 10 miles south of the Eddystone at a depth of 2 m. on 5 December 1946 were found to contain 12·9, 12·9, 13·2, 13·4, 13·6, 14·0 mg. phosphate-P per m.^{3}, and 15·7, 17·3, 17·65 mg. total P per m.^{3}.

Storage of sea-water samples. Early in this investigation it was found that when sea water is stored in glass bottles, the quantity of total phosphorus in solution and suspended particles decreases. This might be expected, since there is a rapid development of bacteria firmly attached to the glass surface during storage in small vessels and since bacteria are singularly rich in phosphorus. However, when sea water, collected during the summer when the organic phosphorus in solution is at a maximum, was saturated with chloroform, which hinders but does not entirely stop bacterial growth, or was acidified (0·28 N) and stored for a few days, there was also a reduction in total phosphorus. A clean glass surface adsorbs organic matter when in contact with sea water (Harvey, 1941; ZoBell & Grant, 1943), and presumably some organic phosphorus is lost by adsorption during storage. I am indebted to Dr J. F. Danielli for the information that a glass surface may adsorb a layer, some 50 Å. thick, of certain proteins or nucleic acids. This suggests that storage in small glass vessels could reduce the concentration of organic matter in solution materially.

It was therefore essential to find some method of storing samples without loss of organic phosphorus by attachment to the storage bottle. Experiment indicated, as far as the methods of estimation permitted, that this can be done by means of a simple device. Before filling with a measured quantity of sea water, the storage bottles were 'baited' with a small quantity of recently precipitated aluminium hydroxide, in order that the adsorbable organic matter and any epiphytic bacteria might attach to the large surface exposed by this finely divided and reactive solid rather than to the glass, and a few drops of chloroform were also added to hinder the proliferation of bacteria. After

storage the sulphuric acid was added to the storage bottle; this quickly dissolved the alumina, unseating the adsorbed organic matter and attached bacteria. The whole contents of the bottle were then poured into the silica reaction flask.

Later a suspension of thorium carbonate was substituted for aluminium hydroxide as bait, because the latter becomes less rapidly soluble in sulphuric acid after more than a week or 10 days' storage.

Water collected 22 July 1946 near Eddystone Lighthouse, was found to contain:
Filled direct into flasks: 14·0 mg. P per m.^{3} (mean of 4 estimations).
After storage in glass bottles: 10·5 mg. P per m.^{3} (mean of 4 estimations).
After storage in 'baited' bottles: 14·7 mg. P per m.^{3} (mean of 4 estimations).

Water collected 17 August 1946 near Eddystone Lighthouse:
Filled direct into flasks: 16·3 mg. P per m.^{3} (mean of 6 estimations).
After storage in baited bottles: 16·9 mg. P per m.^{3} (mean of 8 estimations).

Water collected off Eddystone Lighthouse 17 September 1947:
Filled direct into silica flasks: 17·0, 16·7, 16·4, 15·7, 15·4, 15·0, 14·8, 14·7, 14·2 mg. P/m.^{3} (mean 15·54).
Stored 4 days in bottles baited with thorium carbonate: 17·8, 16·1, 15·6, 15·6, 14·8 mg. P/m.^{3} (mean 16·0).
Stored 4 days in glass bottles in dark: 16·4, 13·9, 13·6, 13·6, 12·7 mg. P/m.^{3} (mean 14·0).

Results obtained by using this technique, with the object of investigating the cycle of phosphorus in the sea, its changes with time, depth and place, will be the subject of a further communication. Yet it seems desirable to include a series of data, typical of several such, from clear offshore water of moderate depth in summer (Table III).

TABLE III. PHOSPHORUS IN OFFSHORE WATER

Position, 3 miles south of Eddystone Lighthouse. Depth, 67 m. 23 July 1947.

	mg. P per m.^{3}		
Depth in m.	Inorganic*	Total	Organic†
5	2·3	10·2	7·9
10	2·2	11·6	9·4
20	5·0	12·3	7·3
30	6·5	12·6	6·1
40	7·4	11·8	4·4
60	6·8	13·1	4·3
65	6·8	14·2	7·4
35 cm. above sea floor	8·4	14·7	6·3
6 cm. above sea floor	8·8	24·0	16·2
6 cm. above sea floor (200 yd. distant)	7·9	18·0	10·1

* Including the equivalent of any arsenate present. † By difference.

I would wish to acknowledge many most helpful conversations with Dr W. R. G. Atkins, F.R.S., and Dr L. H. N. Cooper, F.R.I.C., who also carried out a number of check estimations by means of a Pulfrich photometer during the early part of the investigation.

Summary

A simple photoelectric meter is described which allows the molybdenum blue formed in sea water, due to the presence of phosphate, to be estimated within that due to $\pm 0{\cdot}25$ mg. phosphate-P per $m.^3$.

The effects of concentration of acid, molybdate, reductant, temperature and suspended particles on the rate of formation, fading and amount of molybdenum blue, formed in sea waters containing phosphate are detailed.

Intramolecular changes taking place during storage of molybdate solutions, and while being mixed with acidified sea water, have been investigated.

The hydrolysis of organic phosphorus compounds in acidified sea water at 140° C., and the prevention of arsenate formation, are described.

Procedures, resulting from these investigations, for the estimation of phosphate, and of total phosphorus, are described.

The growth of bacteria and the physical adsorption of organic phosphorus compounds in solution on the walls of glass vessels used for storage of sea water have been investigated, and a method of prevention evolved.

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FURTHER EXPERIMENTS ON THE FERTILIZATION OF A SEA LOCH (LOCH CRAIGLIN). THE EFFECT OF DIFFERENT PLANT NUTRIENTS ON THE PHYTOPLANKTON

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(Text-figs. 1–10)

Introduction

In 1942 and 1943 an experiment was made on the effect of adding nutrient salts to an isolated body of sea water. The experiment, which was initiated by Dr F. Gross of Edinburgh University and financed by Imperial Chemical Industries, Billingham Division, was carried out in Loch Craiglin, a small

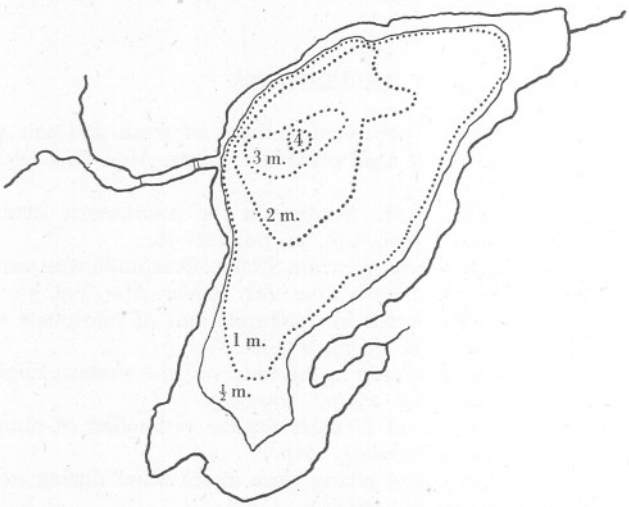

Fig. 1. Sketch-map of Loch Craiglin showing approximate depth contours. Scale 15 cm. = 1000 m.

salt-water loch about eighteen acres in area and 70,000 m.^{3} in volume opening off one of the arms of Loch Sween on the west coast of Argyll. It is connected with Loch Sween by a narrow channel and the entrance and exit of water could be partly controlled by a dam. Loch Craiglin (Fig. 1) is shallow, most of it being less than 2 m. in depth, although there is a small area up to 5 m. deep opposite the mouth of the channel. The bottom is mostly soft mud but round the edge there are patches of sand or sandy mud. A small burn enters at the north-eastern end.

The plankton and hydrographic results from the first two years' work have already been described (Marshall, 1947; Orr, 1947) and it was shown that the addition of nutrients sometimes caused an immediate increase in the phytoplankton. In the early work the effect of the nutrients was assessed during visits made at about fortnightly intervals. The present paper deals mainly with a series of experiments in which the effect of added nutrients, both on the phytoplankton and on the hydrographic conditions, was followed from day to day.

We should like to express our thanks to Imperial Chemical Industries for their support, and also to our colleagues Dr F. Gross, Prof. J. E. G. Raymont, Dr D. T. Gauld and Mr S. R. Nutman for help in taking samples.

Methods

The methods of chemical analysis and for sampling the plankton were the same as those described in the earlier papers.

There was considerable interference with the accuracy of the estimations of phosphorus and nitrogen compounds, both because of the richness in phytoplankton organisms and the presence of free sulphide in the deep water. To avoid damage to the glass electrode pH readings were not taken at 3 m. when hydrogen sulphide was present.

During the fertilization experiments the number of μ-flagellates in all water samples was counted daily on a haemocytometer slide without concentration. However, the fluctuations from day to day were so great that although some of the increases in numbers may be related to fertilizations, others were not and it is felt that a further study of the methods of counting and of the normal seasonal and diurnal variations in numbers is needed before any conclusions can be drawn. The numbers were, however, high when compared with counts from elsewhere, varying from a few hundred to several thousand per mm.^{3}

Pump samples were taken on each visit in 1944 and once during each fertilization in 1945. The zooplankton on the whole was rather poor except for rotifers, and as might be expected showed no direct relation to fertilization. *Oithona* (chiefly *O. nana*) was the most abundant of the copepods.

Fertilizers were distributed by mixing up the solids with a large excess of loch water in a rowing boat and baling out the supernatant liquid into the loch. This was repeated until all had been distributed. It was spread as evenly as possible by rowing across the loch in a series of parallel lines while baling, but owing to wind and weather it was not always possible to keep accurate lines. Most of the nitrogen compounds dissolved readily but the superphosphate was not easily soluble and some of it certainly went to the bottom as solid.

Fertilization Experiments

During the whole of the autumn and winter of 1943–44 the loch was rich in phytoplankton, mainly dinoflagellate, although there was a short outburst of diatom growth found on 26 November and 11 December. The dinoflagellate plankton consisted mainly of *Peridinium triquetra* and *Gymnodinium* spp., the diatoms of a minute form, *Chaetoceros simplex*, which Miss M. V. Lebour kindly identified.

The numbers of dinoflagellates continued to rise at the surface and at 1 m. throughout December 1943 and January 1944 (Figs. 2, 3). A fertilization was made on 18 January with the usual quantities of sodium nitrate (142 lb.) and superphosphate (44 lb.) which is sufficient to raise the nitrogen and phosphorus from zero to well above normal winter sea values. The plankton was not examined again until 31 January when diatoms (minute *Chaetoceros* sp.) were very abundant and dinoflagellates had increased once more. They were almost entirely *Peridinium triquetra* and at the surface had reached a figure of 2350 per ml.

By 9 February diatoms had almost disappeared but dinoflagellates were still increasing. *P. triquetra* now numbered 3500 per ml. at 0 and 1 m. and 1125 per ml. at 2 m. A fertilization was made on 11 February using nearly three times the usual quantities of sodium nitrate (336 lb.) and superphosphate (112 lb.) and the plankton was sampled on 19 February. The *P. triquetra* had increased to over 6000 per ml. at the surface and 1730 at 3 m. but diatoms were practically absent. Seventeen days later on 7 March, *P. triquetra* had reached its maximum in the surface layer, 8165 per ml., but was scarce in deeper water and diatoms were still absent.

To find the amount of photosynthesis going on, samples of the phytoplankton-rich surface water were suspended in bottles at different depths on 20 February with similar controls kept in the dark. In spite of the enormous numbers of dinoflagellates, however, the oxygen production in these bottles over 24 hr. was negligible at all depths.

The numbers of dinoflagellates remained high, over 1000 per ml., until the middle of June although the species present altered as time went on. On 9 April *Gymnodinium* spp. outnumbered *Peridinium triquetra*, though both were abundant. On 27 April this change had gone still further and *Gymnodinium* spp. had risen to 2435 per ml. at the surface, while *Peridinium triquetra* had declined to 130 per ml. *Gymnodinium* remained predominant in May and June but at the beginning of July dinoflagellates had decreased to figures of 30 or 40 per ml. At the end of June there was a sudden and short-lived increase in diatom numbers, again the minute *Chaetoceros* sp.

The number of 30 or 40 dinoflagellates per ml. would be considered very high in normal sea plankton; it is low only in comparison with the extraordinarily high figures throughout the winter and spring in Loch Craiglin. The

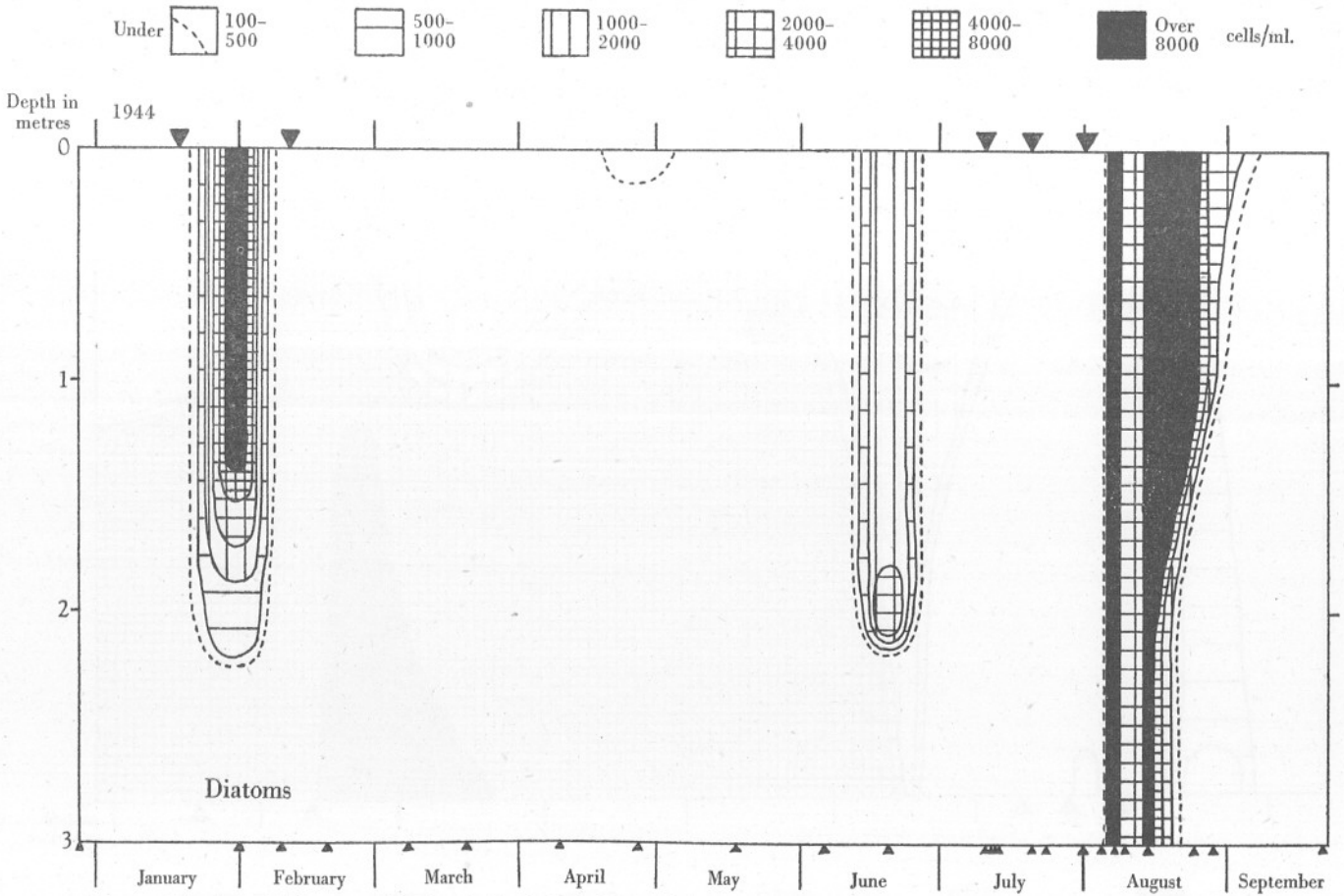

Fig. 2. Number of diatoms at Station I, Loch Craiglin, during 1944. Large triangles above show dates of fertilizations; small triangles below show dates of sampling.

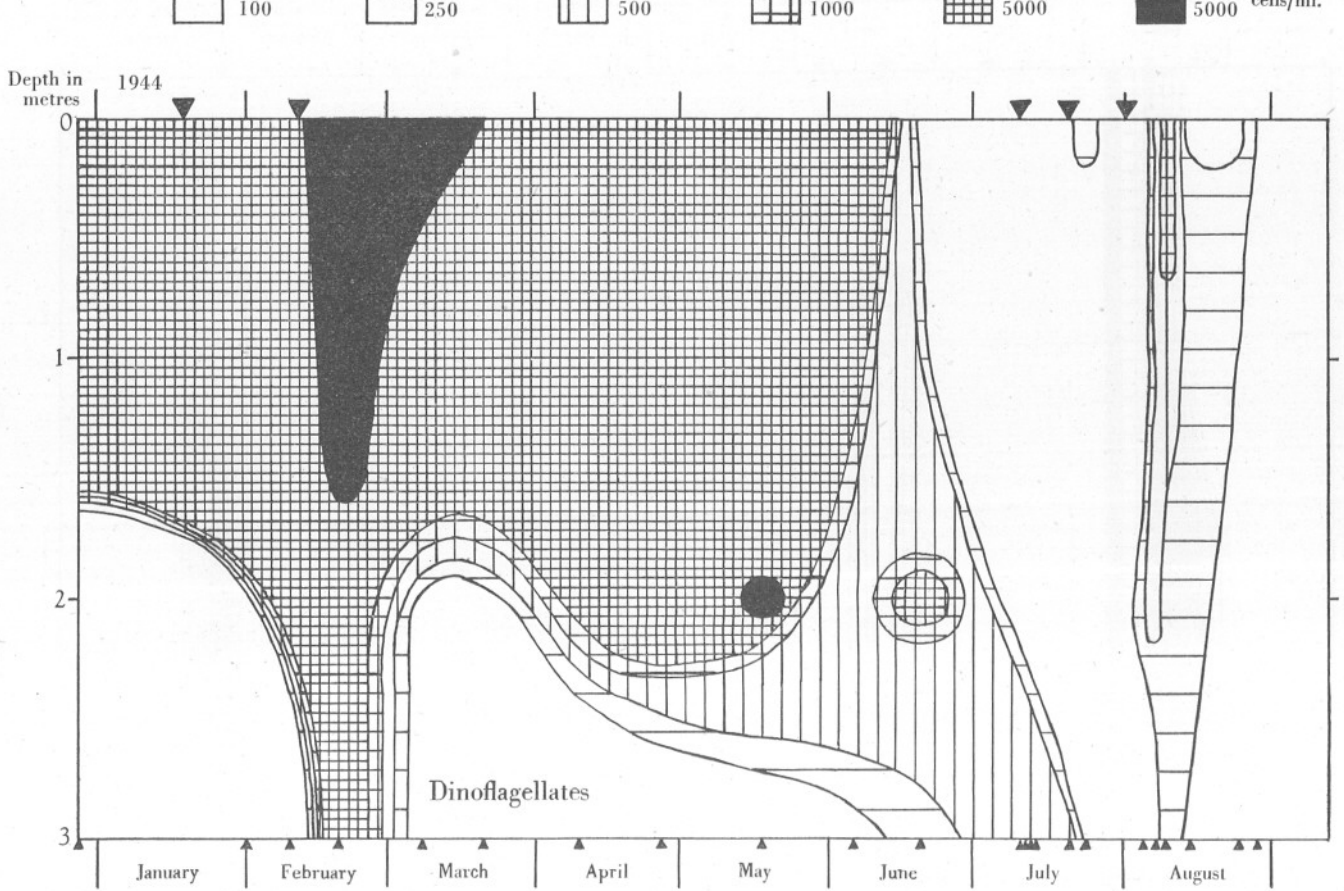

Fig. 3. Number of dinoflagellates at Station I, Loch Craiglin, during 1944.

most abundant species, *Peridinium triquetra*, usually has a summer maximum and it is a form which, under favourable conditions such as in the polluted waters of the inner Oslo Fjord (Braarud, 1945), is capable of increasing until it forms a 'waterbloom' or 'red water'. The maximum number recorded elsewhere, however, is a little below 3000 per ml.; in Loch Craiglin it rose to over 8000 per ml. on two occasions and remained above 2000 per ml. for weeks on end. The water in the loch had during this period a distinct brownish red tinge.

There are several interesting points about these winter fertilizations. The first of them, on 18 January, was followed by a great increase of diatoms but dinoflagellate numbers, very high already, showed no comparable increase. On the other hand, the second fertilization on 11 February was followed by a decided increase in dinoflagellate numbers but had no effect on diatoms although the fertilizer added was nearly three times the usual amount. As a general rule in the open sea dinoflagellates follow diatoms in their times of maximum abundance and they are said (Barker, 1935) to be able to use lower concentrations of nutrients than the former. It is therefore surprising to find an increase of diatoms (19 June) following immediately upon the dying away of dinoflagellates.

The plankton in Sailean More, the open arm of Loch Sween with which Loch Craiglin is connected, showed no comparable changes; dinoflagellates were scarce throughout and, although there was a great rise in diatom numbers in April (over 2000 per ml. at 1 m. on 27 April), the form was a *Leptocylindrus* sp. The minute *Chaetoceros* sp. so characteristic of Loch Craiglin did not appear in Sailean More.

A curious feature of the spring of 1944 in Loch Craiglin was that the phytoplankton remained rich throughout the spring months when, in other years, fertilizations at that time had little or no effect on the phytoplankton because the attached vegetation seemed to have first call on the nutrients.

In July and August a series of fertilizations was begun and each was followed up by sampling at short intervals, usually 1 or 2 days (Figs. 4–6). The first fertilization was made with superphosphate alone, the second with nitrate alone and the third with ten times the usual quantity of both.

During these months there was a rich vegetation, consisting partly of algae (chiefly *Enteromorpha* and *Cladophora*) and partly of *Zostera* and *Ruppia*, round the shores and this interfered considerably with the experiments.

On 10 July, 66 lb. superphosphate was added. This caused a rise in soluble phosphate chiefly at the surface which lasted for only 2 or 3 days. The pH and percentage oxygen saturation rose after the fertilization, in agreement with the utilization of the soluble phosphate, though the highest figure for oxygen (150% saturation) was at 1 m. At the surface equilibrium of oxygen with the air seems to be reached rapidly. Nitrate was present down to 2 m. and although it was only in small quantities it does not seem probable that it was a limiting factor.

This fertilization had no visible effect on the phytoplankton. Dinoflagellates were present in fair numbers but diatoms were absent. This suggests that

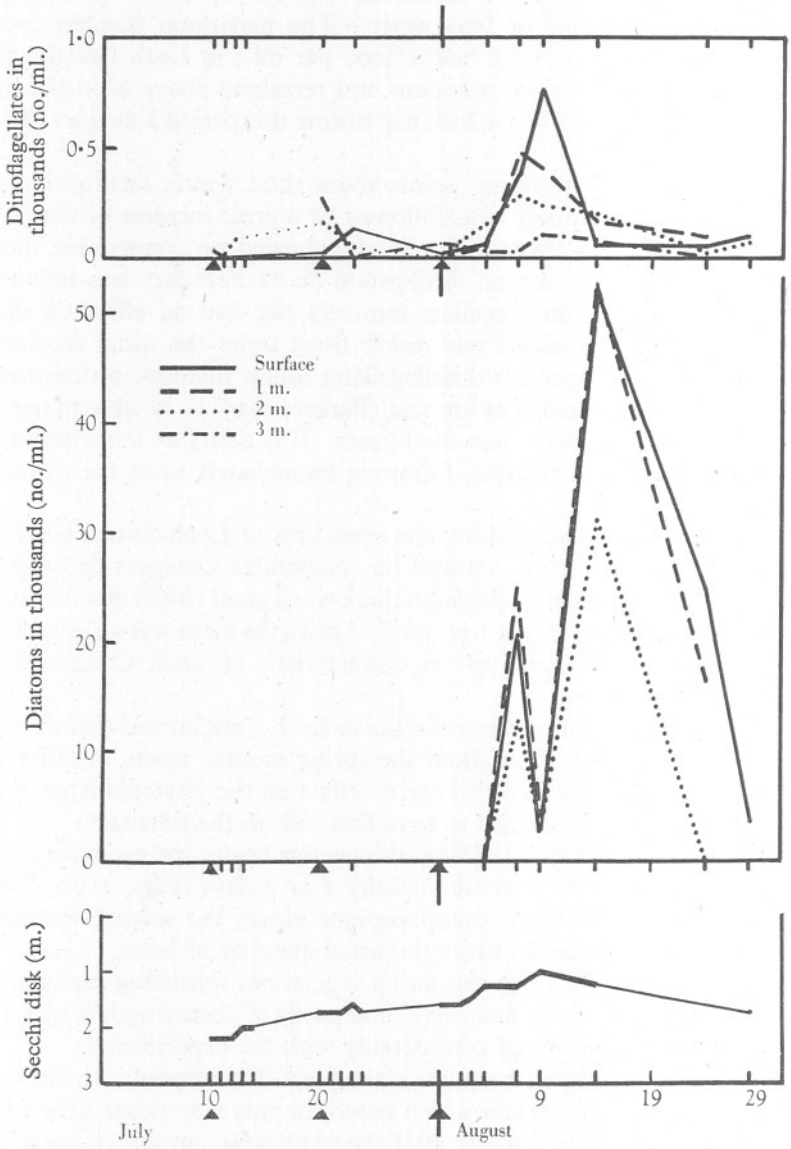

Fig. 4. Effect on the phytoplankton and the transparency of the water of fertilizations during July and August 1944. Triangles below show dates of fertilizations.

the vegetation round the shore and in shallow water was absorbing all the nutrients.

On 20 July, 141 lb. sodium nitrate was added. This caused a rise in nitrate-N at all depths. Values remained high for 3–4 days but after a week were back to normal. Percentage oxygen saturation and pH were high except in deep water; the values were affected by slight salinity changes caused by an influx of water from Sailean More.

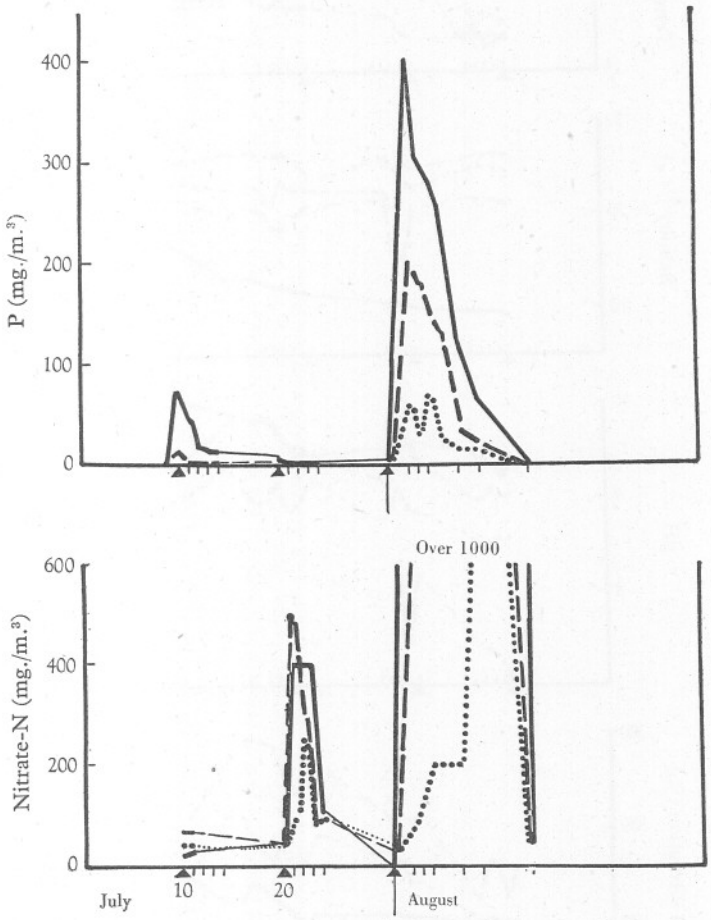

Fig. 5. Changes in nutrient salt concentrations during fertilizations in July and August 1944. Depths marked as in Fig. 4.

Although there was apparently a slight increase of dinoflagellates (naked forms, mainly *Gymnodinium* spp. and *Oxyrrhis marina*), this is probably not significant, since the numbers actually fell at 1 m. from 20 to 23 July.

The fertilization with about ten times the usual quantity of nutrients (1344 lb. sodium nitrate and 448 lb. superphosphate) was done on 1 August. This raised the dissolved phosphate up to 400 mg./m.³ at the surface and to high

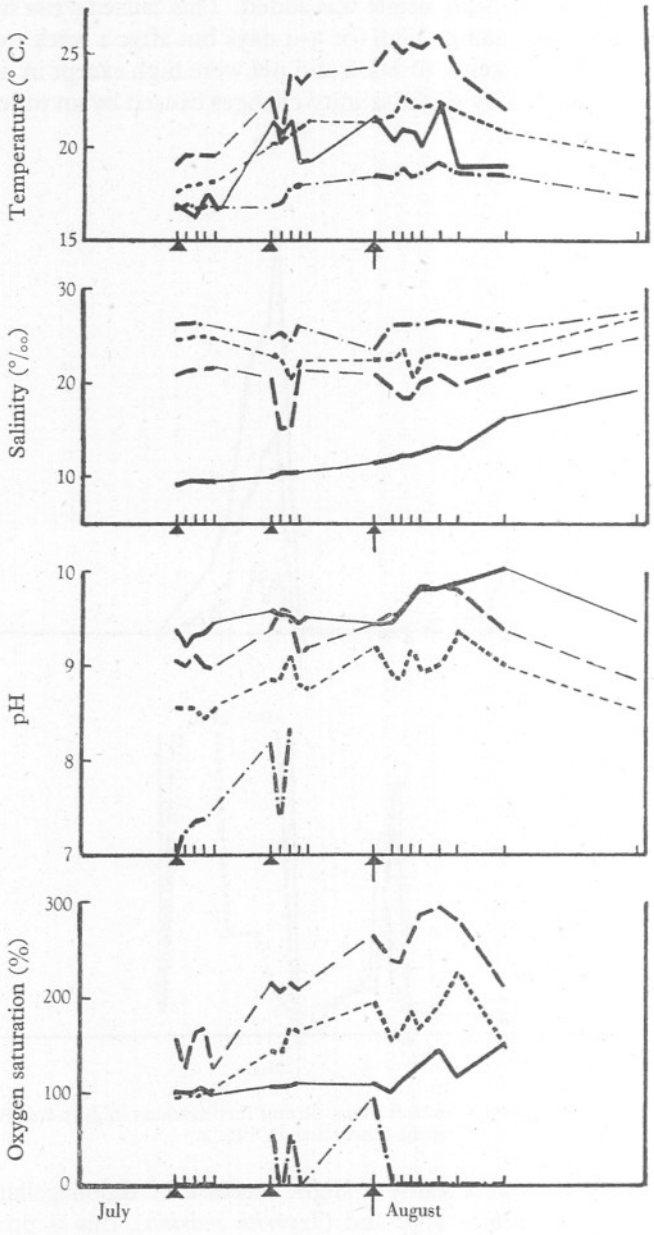

Fig. 6. Hydrographic and chemical changes during fertilizations in July and August 1944. Depths marked as in Fig. 4.

values in deeper water also. Utilization was rapid and in less than a fortnight values were back to normal. A rise in pH and percentage oxygen saturation coincided with the utilization of the soluble phosphate. Nitrate-N, very high immediately after fertilization, fell to normal within a fortnight.

Phytoplankton was very poor on the day of fertilization and for several days after, but on the seventh day after fertilization, diatoms were present in large numbers at all depths. There was a curious and unexplained fall in numbers (not confirmed by Secchi disk readings) on 9 August, but on the 14th the numbers were very high indeed (just over 50,000 cells/ml.) and thereafter fell rapidly. Three species took part in the increase, *Chaetoceros simplex*, a minute Naviculid and the triradiate form of *Nitzschia closterium*. Dinoflagellates also became abundant. Their numbers rose from 31 July to 4 August and again from the 4th to the 9th after which they fell. *Exuviella marina* was the commonest form, but *Gymnodinium* spp. also increased.

Compared with the first two fertilizations this one did produce a large increase in the phytoplankton, but only after a lapse of 4 days for dinoflagellates and of 7 days for diatoms.

The results of these three fertilizations suggest that only when the needs of the attached algae have been satisfied can the phytoplankton utilize any remaining nutrients. During the nitrate fertilization, however, the very low phosphate may have been a limiting factor. It is also possible that some of the nutrients are adsorbed by the mud or by non-living material suspended in the water. This would explain their very rapid disappearance.

The above experiments, as well as earlier work in this loch (Marshall, 1947; Orr, 1947), indicate that when shore-living algae are growing, fertilization does not result in an increase in phytoplankton except when very large quantities of nutrients are used.

During the winter the attached algae are absent or dormant but there is still enough light to permit photosynthesis in the shallow water of Loch Craiglin. A series of experiments was therefore carried out during the winter months to find whether other forms of inorganic phosphorus and nitrogen besides nitrate and superphosphate could be used by the phytoplankton (Figs. 7–9). The following combinations were tested:

(1) Superphosphate and sodium nitrate as normally.
(2) Superphosphate and ammonium sulphate.
(3) Superphosphate and ammonium nitrate.
(4) Mono-ammonium phosphate and ammonium sulphate.

The salts used were all available commercially as fertilizers, but were nevertheless of high quality.

Ammonium salts were chosen both because of the biological interest of testing whether ammonia-nitrogen could be used directly by the phytoplankton and because it is one of the commonest artificial fertilizers applied to land and so available in large quantities. Ammonium nitrate is used as an explosive and

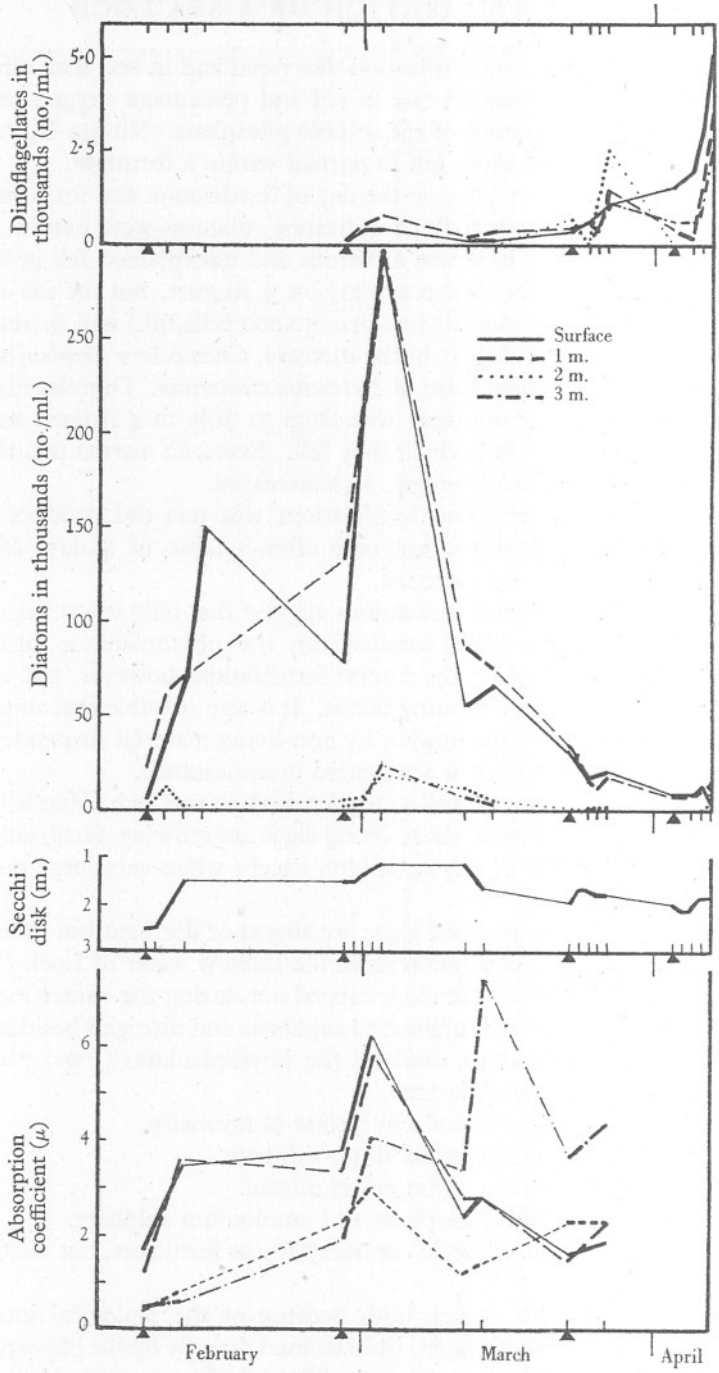

Fig. 7. Effect on the phytoplankton and the transparency of the water of fertilizations during February to April 1945.

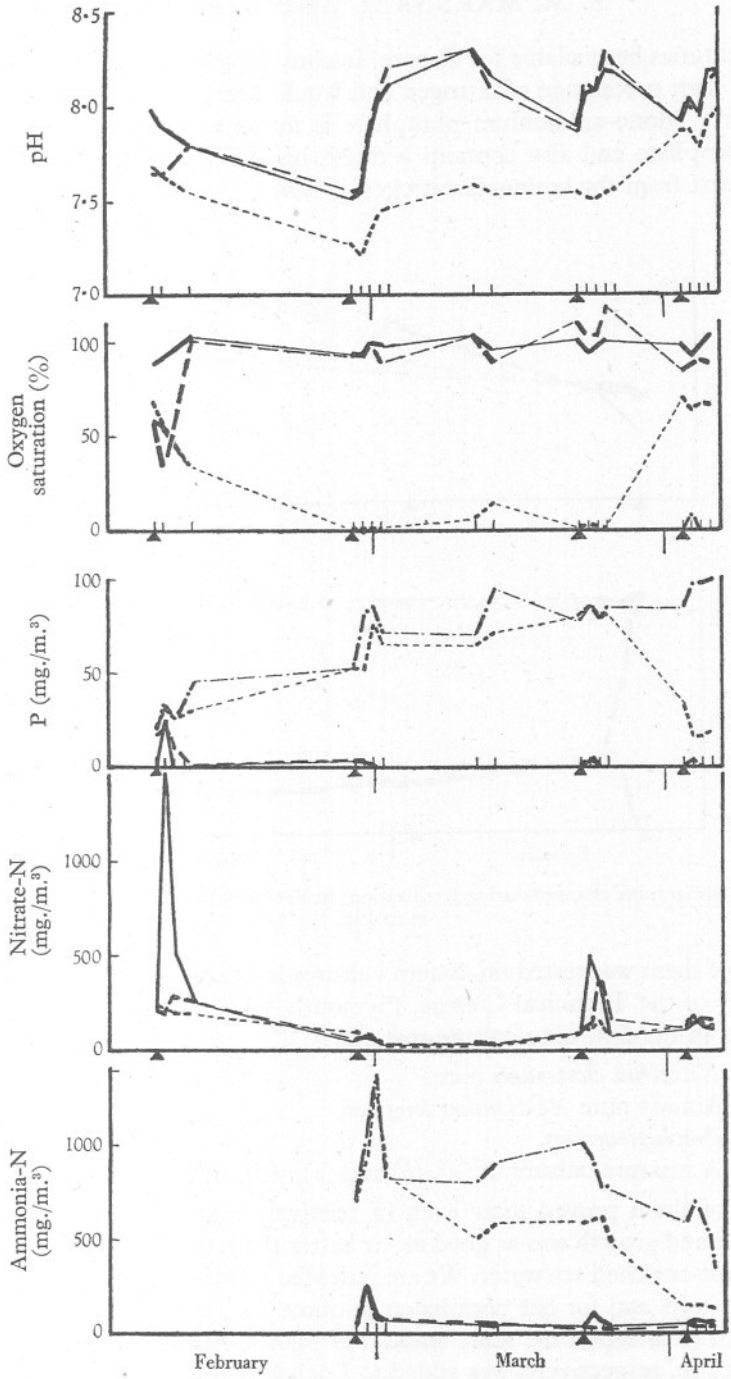

Fig. 8. Changes in nutrient salts and other chemical factors during fertilizations in February to April 1945. Depths marked as in Fig. 7.

might at times be available for disposal in considerable quantities. In addition it has a high percentage of nitrogen and would therefore be less expensive to transport. Mono-ammonium phosphate is much more readily soluble than superphosphate and also contains a much higher percentage of phosphorus quite apart from the ammonia-nitrogen present.

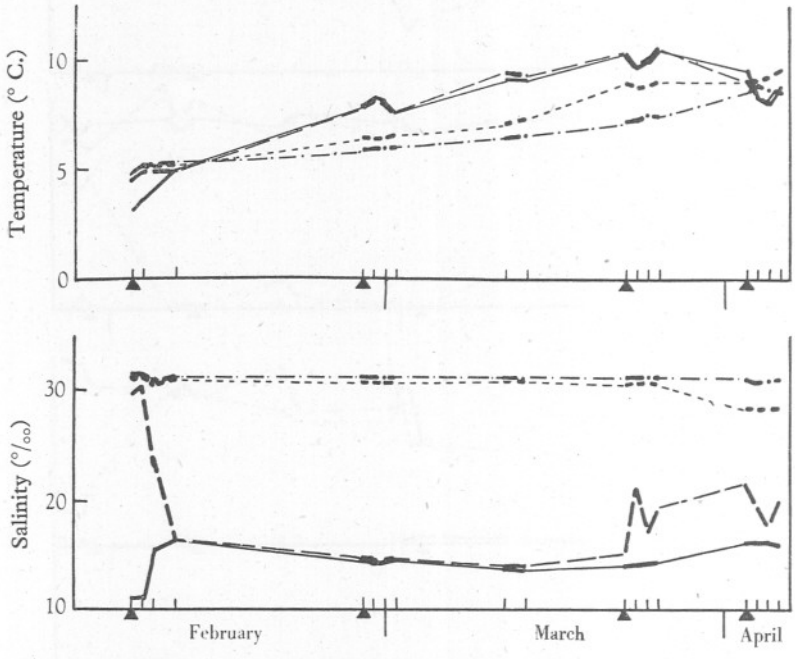

Fig. 9. Hydrographic changes during fertilizations in February to April 1945. Depths marked as in Fig. 7.

Each of them was tested on diatom cultures in the laboratory by Miss F. A. Stanbury of the Technical College, Plymouth, who used them as a source of nutrients in the following culture material:

(*a*) *Nitzschia closterium* pure.
(*b*) Almost pure *Achnanthes longipes*.
(*c*) *Amphipleura* sp.
(*d*) A mixture culture of (*a*), (*b*) and (*c*), with other forms.

None of them proved toxic even in relatively high concentrations (up to 25 mg./l.) and growth was as good as, or better than, that in controls in 'aged' or nutrient-enriched sea water. We are indebted to Miss Stanbury for carrying out these tests and for her permission to quote the results.

In each fertilization the same amount of phosphorus and nitrogen, namely 3·4 and 23 lb. respectively, was added to Loch Craiglin. This was roughly the same as in the normal fertilizations with sodium nitrate and superphosphate

and was enough to raise the concentration to well above average winter sea values.

During the winter the dam was open to allow interchange with the main loch outside, and aeration of the deep water was fairly good. The dam was closed on 5 February 1945 and the normal amounts of fertilizers added that afternoon in the form of sodium nitrate and superphosphate. Phosphate was initially high at 1, 2 and 3 m. and after the fertilization rose at all depths. The day following fertilization, however, soluble phosphate dropped to zero at the surface and 3 days later was absent at 1 m. Nitrate gave winter values at the surface before fertilization and increased afterwards only at the surface. The following day it fell abruptly at the surface and 2 days later values were only slightly above normal winter concentrations.

During the course of the experiment changes in salinity showed that the surface and 1 m. layers had mixed. This affected the pH and percentage oxygen saturation values at these depths.

Diatoms (*Chaetoceros simplex*) were abundant in the loch before fertilization (5300 cells/ml. at the surface and 22,500 cells/ml. at 1 m.), and increased afterwards to 149,000 per ml. at the surface on the 11th. Dinoflagellates were scarce and showed no significant change. This enormous production affected the transparency of the water considerably. The Secchi disk which initially was visible down to $2\frac{1}{2}$ m. was visible only to $1\frac{1}{2}$ m. on the 9th. Measurements of the absorption coefficient with the Pulfrich absorptiometer showed a rise from 1·7 and 1·3 at 0 and 1 m. on the 5th to 3·5 and 3·7 on the 9th. In deeper water there was little change (Fig. 7).

On 26 February the loch was fertilized again with 42 lb. superphosphate and 110 lb. ammonium sulphate. In spite of this addition no phosphate appeared at the surface or 1 m. although there was a rise below this at 2 and 3 m. where the phosphate value had in any case remained high since the previous fertilization. This immediate disappearance of added nutrients was found on several occasions and is not understood. The rapidity suggests that absorption either by the phytoplankton and fixed algae, or else adsorption by dead particulate matter, is responsible.

The added ammonium sulphate did cause an increase, though a small one, in the ammonia present at the surface and 1 m. as well as a greater increase at 2 and 3 m. Nitrate remained low. All the nutrients had fallen to their original level in 2 days.

At no other time was a fertilization made when phytoplankton was initially so abundant. Although numbers had fallen at the surface from 11 February, they had risen at 1 m. and at both these depths were very high indeed (77,000 cells of *Chaetoceros simplex* per ml. at the surface and 133,000 at 1 m.). These high numbers rose still further on succeeding days; on 28 February there were over 200,000 and on 2 March nearly 300,000 cells/ml. down to 1 m. At 2 and 3 m. numbers were much lower (18,000 and 24,000 cells/ml. respectively).

Dinoflagellates were relatively unimportant but did show a slight increase. *Oxyrrhis marina* was the most abundant species.

Accompanying the great increase in diatoms there was a sharp rise in pH in the surface layers from 7·6 to 8·3, and there was a slight effect even at 2 m. The change, however, in percentage oxygen saturation was small. The transparency of the water, which was initially the same as at the end of the previous fertilization, showed an abrupt decrease, especially at the surface and 1 m. By 1 March the water was very turbid indeed, the absorption coefficient being 6·2 and 5·8 at 0 and 1 m. respectively.

The results of this fertilization confirm the findings in the laboratory of Harvey (1933) and ZoBell (1935) that ammonium salts can be used directly by the phytoplankton.

In Loch Craiglin (of which the greater part is less than 2 m. in depth) photosynthesis can go on down to the bottom even in winter providing there is a sufficiency of plant nutrients, and the mere closing of the dam might be enough to start off a phytoplankton increase by cutting down interchange with the outside loch and enclosing, in winter, nutrient-rich water. In an attempt to test this, the dam was left open from 2 to 11 March. It was closed that day and samples were taken then and 3 days later. The hydrographic data show, however, that there had been very little interchange with outside owing to poor tides and there was also no significant change in the phytoplankton in the 3 days although it had decreased greatly from the maximum on 2 March. The experiment therefore failed in its object.

On 22 March a fertilization was made using 42 lb. superphosphate and 67 lb. ammonium nitrate. The added phosphate had, as after the previous fertilization, disappeared before samples were taken the following day. Ammonia and nitrate, however, showed an increase at the surface which lasted for 2 days. Diatom numbers had fallen still further from 14 March and continued to do so in spite of the fertilization, although there were still between 10,000 and 30,000 per ml. present at the surface and 1 m. Dinoflagellates on the other hand showed a distinct increase at the surface. The form causing it was a *Gymnodinium* sp. and *Oxyrrhis marina* was numerous on the 26th at 2 and 3 m.

That appreciable photosynthesis had taken place was shown by the abrupt rise in pH at the surface and 1 m. immediately following fertilization. The attached algae were noted as just beginning to grow and, since the rapid rise in pH and the disappearance of the nutrients are much more than could be accounted for by the comparatively small numbers of diatoms and dinoflagellates present, it is probable that the attached algae were responsible. This is confirmed also by the transparency measurements which show only a slight decrease when compared with previous fertilizations.

On 2 April a fertilization was made using 12·4 lb. mono-ammonium phosphate and 103 lb. of ammonium sulphate. Neither the phosphate nor the ammonia

showed any appreciable increase when the loch was sampled the following day. Hydrographic conditions were not very stable but there was a marked rise in pH. Diatom numbers had fallen still further since the last fertilization and remained steady at about 10,000 cells/ml. *Chaetoceros simplex* still predominated although *Thalassiosira nana* was also numerous. Dinoflagellates had increased at the surface and decreased at 1 m. since the last visit but fertilization resulted in a marked rise at both depths, up to nearly 5000 and 3400 cells/ml. at 0 and 1 m. respectively. *Gymnodinium* spp. were again mainly responsible. On this occasion the Pulfrich absorptiometer was not available and the transparency was measured by the Secchi disk only; it showed a slight decrease. In the earlier fertilizations there was a good agreement between the phytoplankton and the Secchi disk readings (Fig. 4).

By now, April, the attached algae were obviously competing successfully with the phytoplankton for nutrients and the testing of the remaining nitrogen salts (ammonium chloride and urea) was deferred till the following winter. The algae remained rich during the summer of 1945 and were still abundant in the autumn so that it was not till January 1946 that it was considered safe to do further fertilizations.

The loch was then in a condition very different from what it had been in 1945. Phytoplankton was very scarce with numbers of only 10 or 20 cells/ml. as against the thousands of the previous year. In addition plant nutrients, though not abundant, were present in sufficient quantity to allow plant growth. As in 1944–45, the dam had been opened during the autumn months and was closed at the end of December so that the great difference in the quantity of plankton in the 2 years cannot be explained thus.

A fertilization was made on 10 January with 42 lb. superphosphate and 112 lb. ammonium chloride and another on 23 January with 42 lb. superphosphate and 56 lb. urea. At neither time was there any increase in the phytoplankton, nor was there any important increase in the amounts of nutrients in the water although there was a short-lived rise in soluble phosphorus at the surface after the second fertilization.

At the time of the first fertilization the loch level was unusually high and it fell rapidly during the following days; it was estimated that the loss during the period of this fertilization amounted to about a third of the total volume of Loch Craiglin. Some of the fertilizer must undoubtedly have been lost in this way and it may partly account for the lack of any increase in nutrients. During the second fertilization there was no appreciable change in loch level.

The reason for the failure of these two fertilizations to produce a phytoplankton increase is not known, but several suggestions can be made. Compared with 1945 there was an insignificant number of diatoms present initially and although the shore algae appeared to be negligible, the proportion of diatoms to attached algae may still have been too small for the diatoms to compete successfully for the nutrients. On the other hand, the fact that

nutrients were present before fertilization seems against this explanation. The considerable fall in loch level may have carried away both the nutrients and the phytoplankton.

The weather was unfavourable for diatom growth; during the first experiment the loch was frozen over several times and the light thus cut down; during the second it was overcast, wet and stormy. In view of the success of Miss Stanbury's tests which were made on these fertilizers also, it seems unlikely that they were at fault, though she noted a delay in diatom growth with urea as a source of nitrogen when compared with the others.

It must be admitted, however, that none of these explanations is very convincing and that there may be other factors, of which nothing is known, playing a more important part.

Diurnal variation. In shore pools there is normally a considerable diurnal fluctuation in hydrographic conditions and it was thought that this might be true in Loch Craiglin also. In conjunction with an experiment on the vertical migration of *Euglena* (see below), samples of the water were taken at a series of depths every 4 hr. over 24 hr. in August. The weather was calm and bright. During the period of the experiment a high tide in Sailean More caused an influx of open loch water which would affect the results. There was an appreciable diurnal temperature range at the surface which was still noticeable at 1 m. The changes in pH value and percentage oxygen saturation are, however, not related to light and dark at Station I as was expected. Samples taken close inshore where *Ruppia* was growing luxuriantly did show a rise in pH during the day up to nearly 10. A point of interest was the peak in oxygen saturation at 4 p.m. at 2 m. which may be related to the abundance of *Euglena* at that depth.

Occurrence of Euglena. Occasionally in Loch Craiglin very large numbers of a species of *Euglena* (kindly identified by Prof. F. E. Fritsch as *E. proxima*) were present. These were comparatively large (about 70μ long) and might be present in such numbers that the water samples were bright green. Although holotrophic they never appeared in numbers at the surface and were usually most common at or near the boundary between oxygenated and oxygen-free water. They were often found richest in samples smelling of hydrogen sulphide but the numbers fluctuated widely from one depth to another and from day to day so that they apparently occupied a rather narrow zone. They occurred both in winter and in summer although the highest numbers (40,000–54,000 per ml.) were in summer.

Because of this peculiar depth distribution it was thought that their light requirements might be low and that they might migrate towards the surface at dusk and dawn. A series of pump samples was therefore taken at four depths at 4-hr. intervals, from 8 a.m. on 4 August to 4 a.m. on 5 August 1944.

To ensure that the whole water column was sampled fairly evenly, the mouth of the hose in taking each sample was rapidly raised and lowered between

0 and 1, 1 and 2, 2 and 3 or 3 and 4 m. until an adequate amount (about 7 l.) had been taken from each depth. This was well shaken and a subsample drawn off and preserved. The results (Fig. 10 and Table I) were unexpected and are not easy to explain. At no time did the *Euglena* appear in numbers in the water above 2 m. They were always most numerous between 2 and 3 m. except at 4 a.m. when there were slightly more between 3 and 4 m. than between 2 and 3 m. During the course of the experiment there was a slight increase in total numbers at 12 noon but a steady decrease thereafter so that at 4 a.m. there were only a little more than a third of the maximum number.

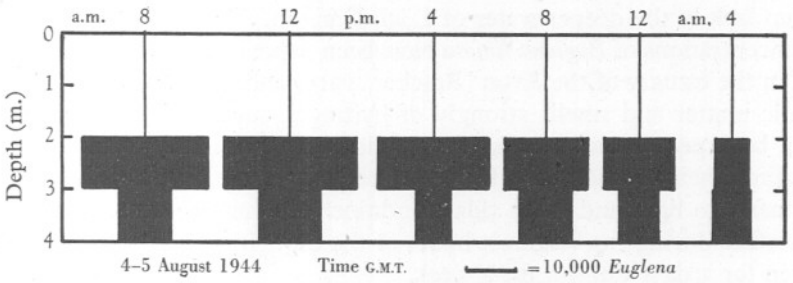

Fig. 10. Vertical distribution of *Euglena proxima* over a 24-hr. period 4–5 August 1944.

TABLE I. DIURNAL VARIATION IN NUMBERS OF *EUGLENA* (NO./ML.)

Depth in m.	Time					
	8 a.m.	12 noon	4 p.m.	8 p.m.	12 midnight	4 a.m.
0–1	25	Lost	10	25	50	25
1–2	5	5	0	5	15	15
2–3	24,500	26,000	21,500	16,000	13,000	6,250
3–4	10,500	12,000	7,000	10,000	7,500	7,500

The Euglenids obviously do not carry out any appreciable diurnal vertical migration but it is difficult to understand the variation in numbers. They may occur in swarms and a small difference in position may make a large difference in numbers.

Since in spite of their green colour they live so deep in the water, an opportunity was taken when they were numerous of measuring their respiration and photosynthesis. On 23 July 1944 a large sample of water from 3 m. containing 22,400 *Euglena*/ml. was thoroughly stirred to oxygenate it and the changes in oxygen content both in light and dark measured over a period of 4 hr. in the afternoon (12.15–4.15 p.m. G.M.T.) at a depth of 1 m. Samples for the initial oxygen content were drawn off and the samples for light and dark exposure put in bottles, the latter enclosed in opaque black cloth. The bottles in the light showed an oxygen rise of about 0·31 ml. O_2/l. whereas those in the dark showed a consumption of 1·83 ml. O_2/l. This is equivalent to a total oxygen production of 0·024 ml. oxygen per hour per million *Euglena*. It is

obvious that although this *Euglena* does carry on photosynthesis, the oxygen thus produced even at a depth of 1 m. is much exceeded by that used up in respiration. Over the whole day, at the depth where they normally occur, photosynthesis must play only a very small part in their metabolism. This was confirmed by an experiment done the previous day when samples of water from each depth (0, 1, 2 and 3 m.) were shaken well to oxygenate them and the oxygen utilization in the dark measured after 24 hr. The utilization was small at the surface (0·14 ml./l.), slightly greater at 1 and 2 m. (0·18 and 0·38 ml./l.) and rose suddenly to 1·74 ml./l. in the 3 m. sample which was slightly green with *Euglena*. The *Euglena* undoubtedly are at times partly responsible for the oxygen lack in the deeper water of Loch Craiglin.

Concentrations of *Euglena limosa* have been described on the surface of tidal mud in the estuary of the Avon (Bracher, 1919) and, since this mud is rich in organic matter and smells strongly of hydrogen sulphide when disturbed it might be expected that the *Euglena* would behave similarly to those in Loch Craiglin. Their reactions are, however, very different. *Euglena limosa* is very responsive to light and when tidal conditions are suitable comes up to lie on the surface during the brightest hours of the day. It is able to survive lack of oxygen for 3 days but not for a week.

Summary and Conclusions

Loch Craiglin cannot be considered as merely an isolated basin of Loch Sween. Its shallowness, the great growth of vegetation round the shore and the fluctuations in salinity make it atypical. On the more complete isolation of the loch with the making of the dam, these conditions led to a lack of circulation, a consequent development of hydrogen sulphide in the deep water and very high pH values near the surface. One could not therefore expect the plankton to be very similar to that in the outside loch (see, however, Marshall, 1947). Unfortunately there are no records of seasonal variation before fertilization was begun. In spite of these drawbacks Loch Craiglin was a convenient and manageable area for small-scale experiments on fertilization.

The experiments may be divided into two groups, those which were not followed up in detail and those in which the plankton and hydrographic results were followed from day to day. The former, made during the earlier stages of the work, are mainly described in the previous papers and led to the conclusion that the richness in phytoplankton of Loch Craiglin in 1943 was probably caused to a large extent by fertilization. Those of the second group, described above, were made to test special points and different fertilizers. Two experiments made during the summer of 1944 had apparently no effect on the phytoplankton but a third in which a very large excess of fertilizer (ten times the normal quantity) was added, gave a good increase after an initial lag. At that time the attached algae were abundant and their needs apparently had to be satisfied before any nutrients could be used by the phytoplankton.

The remaining experiments, which were therefore made during the winter, showed that commercial ammonium sulphate, ammonium nitrate and mono-ammonium phosphate could replace the sodium nitrate and superphosphate used hitherto. Two further experiments in the following spring using ammonium chloride and urea did not, however, prove effective. All the salts used had been found in laboratory tests by Miss F. A. Stanbury to be suitable for diatom growth, and the reason for the failure of these two in the loch is unknown.

The utilization of the nutrients was extremely rapid even in winter but it is probable that their disappearance is not entirely caused by the phytoplankton or bottom-living algae but partly by adsorption on the bottom mud or by suspended matter. On one occasion it may have been caused by a serious fall in loch level.

The zooplankton which during the first year of fertilizations was richer in Loch Craiglin than in the outside loch diminished in the second year and remained poor thereafter in spite of, or perhaps because of, the intensive fertilizations. The dense phytoplankton and other vegetation raised the pH at times to levels dangerous to animal life.

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STRUCTURE, TADPOLE AND BUD FORMATION IN THE ASCIDIAN *ARCHIDISTOMA*

By N. J. Berrill

From the Plymouth Laboratory and McGill University, Montreal

(Text-figs. 1–6)

Archidistoma is a genus created for a species discovered and described briefly by Garstang in 1891 as *A. aggregatum*. It has been found at different times only in the Plymouth region, the original locality, attached to rock surfaces in a few feet of water. Recently, however, it has been found near Cape Hatteras on the eastern American coast (Van Name, 1945), suggesting either harbour to harbour transportation by ship bottom, or that its inconspicuous appearance has obscured a wide distribution.

This form is of considerable interest as it represents a type less specialized in zooid structure, nature of tadpole, and method of budding than any merosomatous ascidian except the diazonids. Diazonids are oviparous and have the simpler development and tadpole associated with small eggs, although there is a more elaborate branchial sac correlated with the relatively large size of the mature zooid.

Colony

The colony consists of a brownish tough matrix forming a thin encrustation on stones and shells, with individual zooids (a few millimetres long), partly embedded in the test but for the most part extending freely in clumps usually of three or four. Sand grains and round particles adhere to or become buried within the test, making this form the most inconspicuous of ascidians.

Structure of Zooid

The zooid is divisible into a thorax and a long slender abdomen (Fig. 1). There is no postabdomen, nor is there hypertrophy of the ventral test vessels, thereby segregating *Archidistoma* as a type from that of the synoicids (polyclinids) and the clavelinids respectively. The relatively great length of the oesophageal region of the abdomen also marks it off from the didemnids and *Distaplia*.

Both branchial and atrial siphons are well developed and are independent, the atrial siphon having six unmodified lobes like those of the branchial siphon. This is correlated with the absence of true zooid systems and is undoubtedly a primitive feature.

The thorax is short and wide, as is the contained endostyle. Tentacles are simple and about thirty in number. There are three rows of straight stigmata,

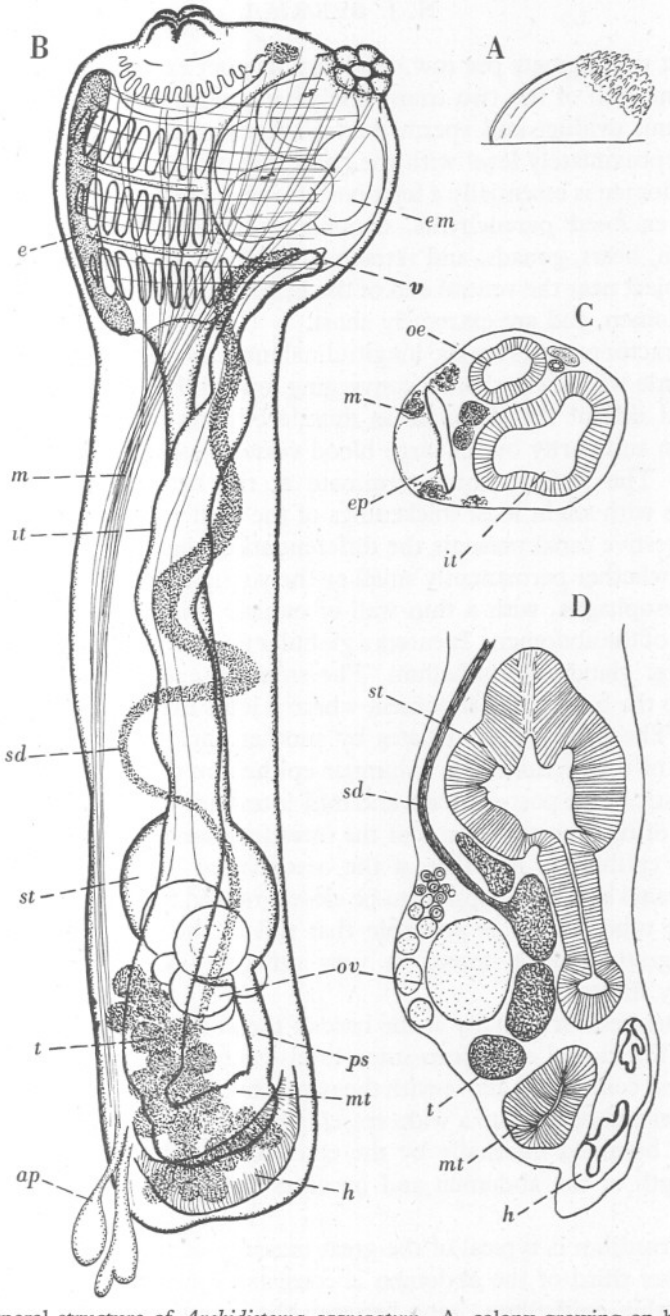

Fig. 1. General structure of *Archidistoma aggregatum*. A, colony growing on bivalve shell, about natural size; B, mature zooid from left side showing organization of thorax and abdomen; C, cross-section through upper part of abdomen showing epicardium; D, longitudinal section of lower part of abdomen showing relationship of gonads and heart to digestive tube. *ap*, ampulla; *e*, endostyle; *em*, embryos in atrial cavity; *ep*, epicardium; *h*, heart; *it*, intestine; *m*, retractor muscle; *mt*, mid-intestine; *oe*, oesophagus; *ov*, ovary; *ps*, post-stomach; *sd*, sperm duct; *st*, stomach; *t*, testicular follicle; *v*, distal ends of oviduct, sperm duct and rectum opening together into base of atrial chamber.

with about ten stigmata per row. A dorsal languet extends into the branchial cavity from each of the two transverse vessels separating the stigmata rows. The rectum, oviduct and sperm duct all terminate well short of the atrial siphon, approximately level with the basal row of stigmata (Fig. 1B).

The abdomen is essentially a long posterior extension of the epidermal body wall and enclosed parenchyma, containing the U-shaped digestive canal, epicardium, heart, gonads, and retractor muscles. The ventral ampullary test vessels project near the ventral end of the heart close to the posterior extremity of the abdomen, and are extremely short.

The retractor muscles are the longitudinal muscles spread somewhat fanwise in the mantle wall of the thorax, converging toward the base and passing down the ventral side of the abdomen as muscle bundles separated partly by the epicardium and partly by the large blood vessel passing from the heart to the endostyle. The muscle fibres terminate at the extreme abdominal tip in association with slight local thickenings of the epidermis.

The digestive canal presents the differentiation characteristic of most small ascidians, whether permanently small or the young of larger forms. A narrow straight oesophagus, with a thin wall of cubical epithelium, descends to the lower part of the abdomen. It enters a globular thick-walled stomach composed of columnar glandular epithelium. The stomach extends as a narrow post-stomach to the base of the abdomen, where it joins the almost horizontal mid-intestine. The latter communicates by another narrow constriction with the lower end of the rectum. The columnar epithelium of the stomach is equally characteristic of the post-stomach and mid-intestine. The rectum, which forms the whole of the ascending limb of the intestine, has a thinner wall consisting of cubical epithelium like that of the oesophagus. The various divisions of digestive canal as a whole appear to be closely related to the locations within the abdominal tube, and it is probable that this is the primitive arrangement, similar digestive canals found in very different locations having become secondarily shifted.

The heart lies horizontally at the base of the abdomen, just below the mid-intestine. The dorsal end opens into vessels branching over the region of the stomach and connecting above with the parenchymal blood channels in general. The ventral end opens into a wide vessel lying between two bundles of muscle fibres and bounded internally by the epicardium. This vessel passes up the whole length of the abdomen and becomes the subendostylar vessel in the thorax.

The epicardium is typical of the great majority of merosomatous ascidians. In the lower third of the abdomen it consists of a single cavity lined by an extremely thin epithelium, lying between the digestive canal and the ventral aortic blood vessel. It ends blindly posteriorly, but anteriorly above the level of the stomach, the right and left divisions, detectable even when fused, become separate from one another and extend as a pair of sacs to the base of the thorax.

There they end blindly as a pair of horns immediately beneath the floor of the pharynx (from which they were originally derived).

The gonads also occupy the position characteristic of most enterogonid ascidians, namely, in the general vicinity of the intestinal loop between the stomach and the mid-intestine. The small spherical testicular follicles extend farther posteriorly to the level of the heart, that is, as far as they can go, and do not extend quite as far anteriorly as the ovary which reaches the lower part of the stomach. To a significant extent, the ovary, or at least the relatively large ova, are surrounded by the follicles of the testis. The oviduct follows the rectum to open alongside the anus into the base of the atrial cavity. The sperm duct does the same except that it takes a somewhat more tortuous course. The arrangement both of the gonads and their ducts is undoubtedly primitive and common to the majority of ascidians.

Sexual Reproduction

Eggs pass up the oviduct and undergo their development as far as the active tadpole stage within the atrial cavity, taking up so much space there that the branchial sac may become pushed very much to one side. Fertilization probably occurs within the oviduct, although this is not known for certain. Eggs are of 0·23 mm. diameter and are extremely yolky, closely resembling those of the synoicids and likewise undergoing epibolic gastrulation.

Considerable increase in size occurs during the development of the embryo, although at the time of liberation of the tadpole a mass of relatively large yolky endodermal cells still remain more or less unutilized, lying between the pharynx and digestive canal, alongside the epicardium. The paired peribranchial ectodermal invaginations fuse to form the single median atrial siphon while yet in the embryonic phase.

The tadpole larvae (Fig. 2) have a short free-swimming period, on an average of about 2 hr. Their organization is interesting in several ways, being a generalized type compared with those of the synoicids, didemnids, clavelinids and holozoans.

The larval structures are rather large relative to the size of the trunk. The tail is long, the sensory vesicle large, and the permanent organs occupy practically all the remaining space, as though there had been a restriction of the surface area of the epidermis. The sense organs are typical, though individually relatively large, and consist of a unicellular otolith and an ocellus bearing three unicellular lenses (Fig. 3 B). The tail, as in all viviparous (large-egged) enterogonid ascidians, has undergone a 90° torsion, so that the tail fin is horizontal and the nerve, no longer dorsal, runs along one side (Fig. 3 C). It has the universal number of approximately forty notochord cells, while the muscle cells, in a relatively advanced state of differentiation, are arranged in series as three bands down each side of the notochord. Anteriorly the three adhesive organs project on long slender stalks, in triangular arrangement, each organ consisting of a central adhesive papilla and a marginal epidermal cup or sucker.

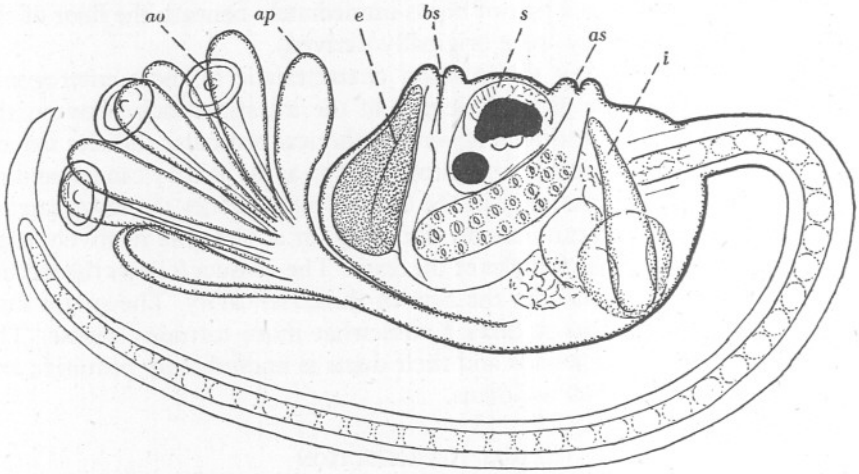

Fig. 2. *Archidistoma aggregatum*. Tadpole larva, with eight ampullae and three adhesive organs anteriorly, a relatively large sensory vesicle, and three rows of perforated stigmata on each side. *ao*, adhesive organ; *ap*, ampulla; *as*, atrial siphon; *bs*, branchial siphon; *e*, endostyle; *i*, intestine; *s*, sensory vesicle.

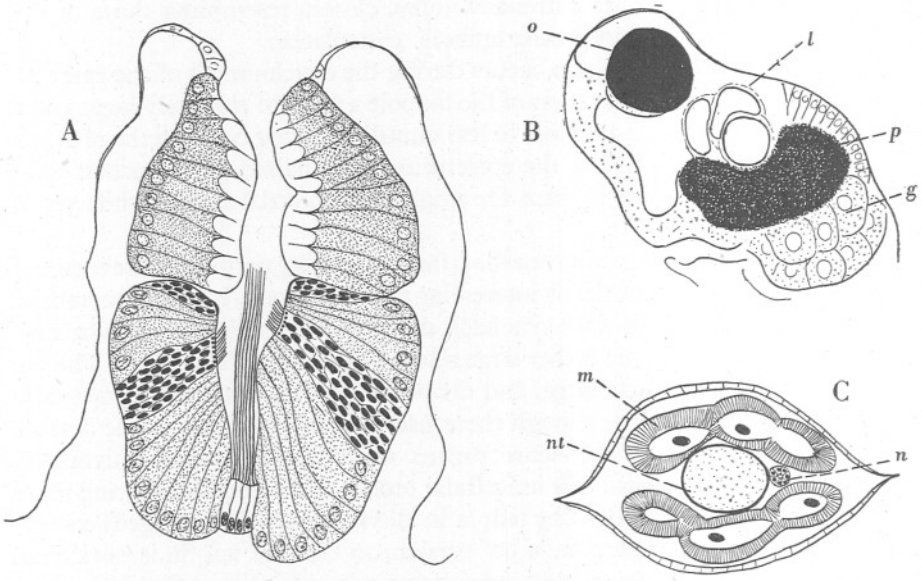

Fig. 3. *Archidistoma aggregatum*. A, section through endostyle of adult, showing lateral bands of large glandular cells alternating with bands of small ciliated cells, and a median ventral band with very long flagella; B, sensory vesicle of tadpole with unicellular otolith, and typical three-lens ocellus; C, section through tail, showing notochord, neural tube, and lateral muscle bands of three cell-rows (highly differentiated). *g*, ganglion cells; *l*, lens cells; *m*, muscle cell; *n*, nerve tube; *nt*, notochord; *o*, otolith; *p*, pigment obscuring retinal cells.

The larval endostyle is relatively large and lies vertical to the main axis. The digestive tube is fairly well differentiated, while the peribranchial sacs, already fused mid-dorsally to form the atrial siphon, are united with the pharyngeal wall on each side and bear each three rows of perforate, definitive stigmata. There are eight or nine stigmata in each row, approximately the same as in fully grown zooids.

From the mid-ventral surface of the trunk of the tadpole, corresponding to the future posterior end of the individual zooid, a slender epidermal process grows out and forwards. From the anterior end of this there extends not only the three adhesive organs described above, but eight club-shaped ampullae, arranged in a ring around the more centrally placed adhesive organs.

After metamorphosis the ventral process expands and grows as the stalk of attachment into which extend the digestive canal, epicardium and heart. The anterior ampullae form an irregular organ of attachment, the adhesive organs having a transient function only.

Budding

During the winter, and to a very slight extent in summer months, growth of the colony occurs by budding. The method of bud formation (Figs. 4–6) is simple in the extreme, is found in other ascidians only in the diazonids, and is strikingly reminiscent of *Phoronis*.

The thorax slowly resorbs, a common phenomenon among ascidians. Associated with this, large cells or trophocytes, heavily laden with acquired food reserves, migrate posteriorly and congest the abdomen. They are similar to those that reach and congest the postabdomen of synoicids and the stolonic ampullae of the clavelinids, but here they are confined to the abdomen owing to the absence of any postabdominal extension and the lack of hypertrophy of the test vessels respectively.

Local growth activity of the epidermis then constricts the long abdomen into several oval masses, one constriction usually occurring below the stomach and two above. Only the epidermis plays an active part in the process, the inner tissues (epicardium, both limbs of the digestive canal, etc.) being passive and cut through by the invaginating outer layer.

The process of constriction is a local annular growth of epidermis inwards, the cells of the invading ring not only proliferating but exhibiting a change from cubical to columnar form. The inner end of each cell becomes greatly extended by a large vacuole. A similar vacuolated condition is typical of epicardial cells, at least in fragmenting abdomens. In both the vacuolar region is away from the exposed epithelial surface.

Regeneration occurs subsequently. In this process it is the epidermis that has the relatively minor role, producing only the epidermis of the new outgrowths. The internal structures, both anterior and posterior, are developed

from the proliferating ends of that section of the epicardium contained in the isolated piece. The new branchial sac and gut loop thus formed are fitted on to

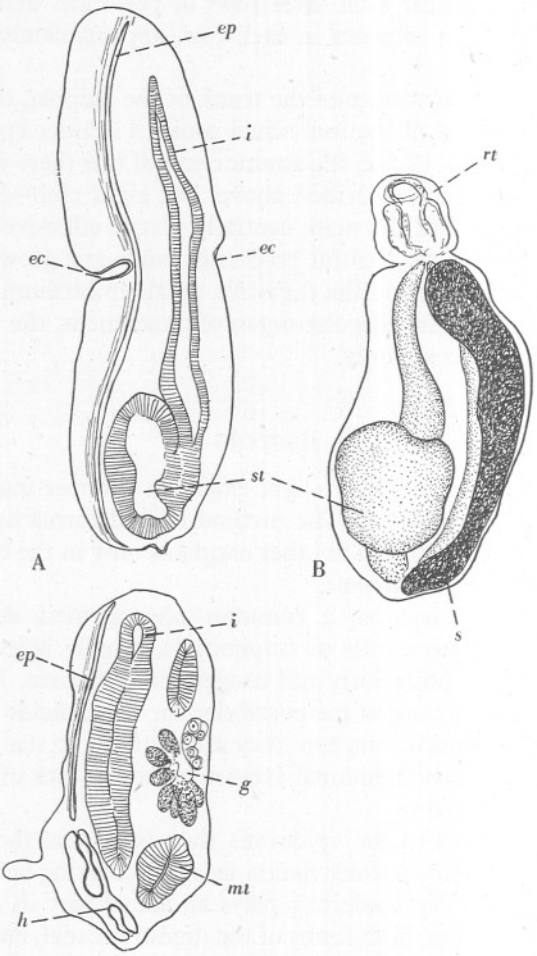

Fig. 4. *Archidistoma aggregatum*. Strobilation and regeneration. A, longitudinal section through strobilating abdomen, showing complete transverse division immediately below stomach, and incomplete constriction in middle part of anterior fragment (thorax resorbed); B, fragment containing original stomach etc., and part of distended sperm duct, regenerating new thorax from upper end of epicardium. *ec*, epidermal constriction; *ep*, epicardium; *g*, gonad; *h*, heart; *i*, intestine; *mt*, mid-intestine; *rt*, regenerating thorax; *s*, original sperm duct; *st*, stomach.

the surviving part of the original digestive canal. The food-laden cells produced during the resorption process become progressively smaller and more numerous, yielding their reserves in nourishing the growing tissues.

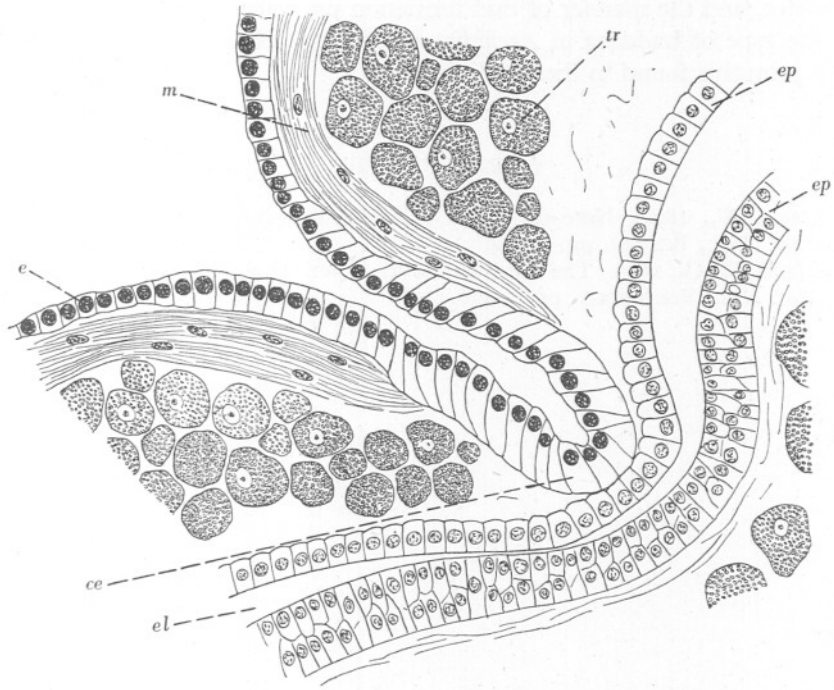

Fig. 5. *Archidistoma aggregatum*. Higher magnification of epidermal constriction shown in Fig. 4, showing epidermis cutting in through muscle and trophocyte layers and impinging on epicardium. *ce*, enlarged epidermal cells of constricting region; *e*, normal epidermis; *el*, lumen of epicardium; *ep*, lining cells of epicardium; *m*, retractor muscle; *tr*, trophocyte.

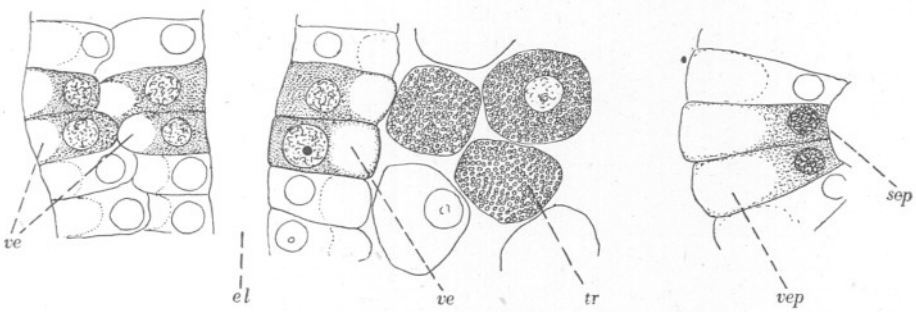

Fig. 6. Enlarged view of constricting epidermal cells and activated epicardial cells (× 1000), both showing vacuolization of the cells on the side away from the outer surface of the body and the epicardial cavity respectively. *el*, epicardial lumen; *sep*, outer surface of epidermis; *tr*, trophocyte; *ve*, vacuole of epicardial cell; *vep*, vacuole of epidermal cell in constricting region.

In so far as the general structure of the zooid appears to be comparatively primitive, and the manner of bud formation undoubtedly as simple as it can be, the type of budding in *Archidistoma* may reasonably be regarded as the most primitive found in the ascidians.

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THE DEVELOPMENT, MORPHOLOGY AND BUDDING OF THE ASCIDIAN *DIAZONA*

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(Text-figs. 1–7)

Diazona is represented in European waters only by *Diazona violacea* Savigny. It is a compound ascidian forming massive colonies of spectacular size and appearance. In many ways it is the most interesting of all ascidians, for in its adult structure it straddles two commonly accepted orders and in itself is a strong argument against such a division; it is the only oviparous and small-egged compound ascidian, two features undoubtedly primitive; and its manner of budding is the simplest and probably is the basic type for the group as a whole. Only fragmentary descriptions of the morphology and reproduction exist, and a more or less complete account of the various stages of the life cycle may be of some value. The family Diazonidae includes, in addition to *Diazona* itself, the genera *Tylobranchion* of subantarctic regions, and *Rhopalea* of Mediterranean and northern waters. In its entirety *Diazona* appears to link with such divergent forms as *Ciona* on the one hand and *Archidistoma* on the other. The fact that *Diazona* is obtained by dredging in relatively swift offshore waters and lives poorly in an aquarium probably accounts for the existing unsatisfactory state of knowledge of most of its phases. Most of what is known concerns asexual reproduction; and attention has been given, at various times, primarily to the process of regeneration, rather than bud formation, for example by Della Valle (1884), Caullery (1914), Oka (1906) under the name *Aphanobranchion*, and by Salfi (1926).

The material of the present account was collected at various times in the Plymouth area from the Mewstone and Eddystone grounds.

General Structure

Diazona is usually found as large massive colonies exceeding in bulk all other known compound ascidians, both in aggregate and individual. All but the anterior ends of the zooids are embedded in a massive firm gelatinous matrix of tunicin. The individual zooid represents a combination of characters that is unique (Fig. 1). It is merosomatous, that is, the body is divided into thorax and abdomen, the abdomen lying posterior to the thorax and joined to it by a relatively narrow oesophageal region. A slender vascular process descends from the posterior part of the abdomen far into the common test, branching and bearing a number of blind ampullary sacs. The vessels remain narrow,

never becoming enlarged as in *Clavelina* (they contain an afferent and efferent vascular current). There are independent branchial and atrial siphons, and there is no tendency for individuals to become arranged in systems.

The thorax is relatively large, though seldom expanded in specimens in aquaria, and has sixty to seventy rows of stigmata in each branchial wall. Vascular papillae grow into the branchial cavity from the inner wall and bear

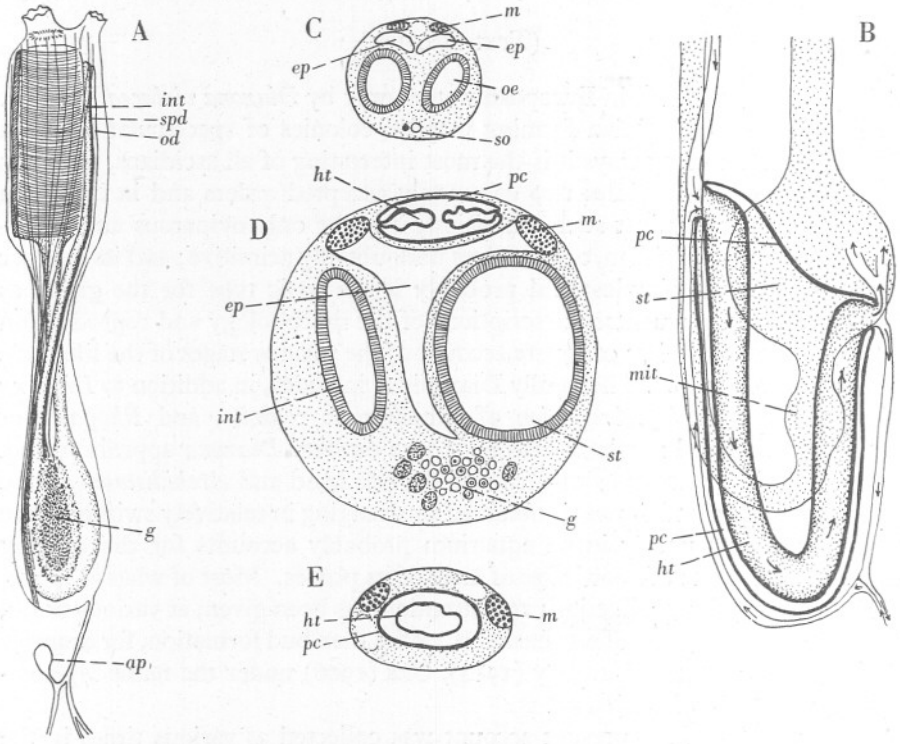

Fig. 1. Morphology of *Diazona violacea*. A, complete mature zooid; B, heart, pericardium and digestive canal, reconstructed from serial sections; C, cross-section through oesophageal region, showing paired epicardial sacs; D, section at level of stomach, showing fused epicardia and heart; E, section posterior to digestive canal and epicardium, showing heart and terminal region of heart. *ap*, ampulla of stolonic vessel; *ep*, epicardium; *g*, gonad; *ht*, heart; *int*, intestine; *m*, muscle; *mit*, mid-intestine; *od*, oviduct; *oe*, oesophagus; *pc*, pericardium; *so*, sperm duct and oviduct; *spd*, sperm duct; *st*, stomach.

the inner longitudinal vessels. Secondary papillae are absent, and the branchial sac is not otherwise complicated. Longitudinal muscles extend the length of the thorax and converge in the oesophageal region into a narrow band on the ventral side. Posteriorly in the abdomen the band is divided ventrally by the pericardium, and the two bundles become separately inserted at the base of the abdomen. White reflecting pigment cells congest the endostyle, peripharyngeal bands, and the dorsal lamina, making the combined system very

conspicuous. The dorsal lamina is basically composed of a languet projecting into the branchial cavity corresponding to each of the sixty to seventy transverse blood vessels separating adjacent rows of stigmata.

The abdomen contains, in addition to the longitudinal muscles, the loop of the digestive canal, heart, epicardium, and the reproductive organs. The form of the digestive canal is obscure in whole specimens but is readily seen in regenerating forms and from serial sections. The long narrow oesophagus expands into a globular stomach. Posteriorly the stomach constricts and then the canal expands again to form the mid-intestine, which opens into the base of the loop, the intestine proper or rectum. This last ascends the abdomen and thorax to open close to the atrial siphon.

The pericardium and heart occupy the lower half of the abdomen on the ventral side. The pericardium is a simple sac, blind at the base, extending higher on the left than the right side, and wider laterally than dorso-ventrally. The heart is a deep invagination of the pericardial wall extending the whole length of the lateral margin, from the high left shoulder of the pericardium to its base and along the right side to the region of the stomach, forming a deep V-shaped tube. In a functional sense the invagination is closed to form a deep tube of contractile cardiac tissue. Morphologically it remains continuous with the pericardium and the cardiac lips merely approximate to form the so-called 'raphe' of the heart. The vessels connected with the two ends of the heart are similar to those of other merosomatous forms and of *Ciona*. Entering the high ventral end are two vessels, one of which extends through the oesophageal region and becomes the sub-endostylar vessel. The other passes posteriorly and enters the narrow stolonic vessel together with a similar vessel from the dorsal end of the heart. The two channels thus formed are separated by a mesenchymatous septum. The dorsal end of the heart is also supplied with vessels from the stomach region and the blood sinuses of the body as a whole, of blastocoelic character.

The epicardia for the most part are fused to form a median chamber, as in all other merosomatous ascidians except *Euherdmania* and the Didemnidae. They end blindly just beneath the posterior wall of the branchial sac, one on each side, and extend as separate tubes through the oesophageal region, lying between the digestive canal and muscle band. Near the stomach fusion occurs to form a single large cavity apposed ventrally to the pericardium, with a lateral horn reaching the muscle band on each side, and a median dorsal extension between the descending and ascending limbs of the digestive canal. Posteriorly it ends blindly at a level intermediate between the end of the pericardium and the bend of the gut.

The gonad is a large hermaphrodite gland consisting of a centrally situated ovary with numerous and relatively small eggs, with numerous peripheral testicular follicles, the whole mass lying across the loop of the digestive canal posterior to the oesophagus, on the left side. Both sperm duct and oviduct are

fairly straight and accompany the rectum to open near the anus just below the atrial siphon. This proximity, together with the small size of the eggs, assures a condition of oviparity.

Sexual Reproduction and Development

Sexual maturity is reached in July and lasts into September. Eggs are liberated in fair number and develop outside of the colony. They are very similar to those of the Ascidiidae, both in size (0·1 mm.), and in the character and number of the outer and inner follicle cells (Fig. 2A). The outer cells are typical, neither exhibiting the flattening out over the chorion as in viviparous forms, nor specialized as 'floats' to the extent found in *Ciona intestinalis*, *Corella parallelogramma* and *Ascidiella aspersa*.

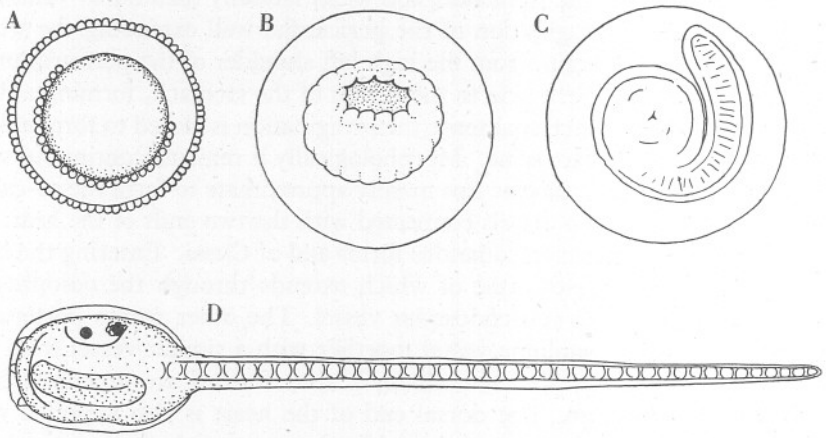

Fig. 2. *Diazona violacea*, development of tadpole. A, egg; B, gastrula; C, embryo; D, tadpole.

At 16° C., gastrulation occurs after about 5 hr., and is enbolic as in most ascidian eggs of this size (Fig. 2B). The transparency of the egg is indicative of a relatively small amount of yolk. The tadpole (Fig. 2D) hatches by digestion of the chorion after 26–27 hr. at the above temperature. Tadpoles at first are positively heliotropic, and the free-swimming period is normally from 20 to 24 hr. In general, the tadpole larva is much like that of the above three forms, both in size and appearance. The endostyle is more or less horizontal at this stage. The sensory vesicle contains the usual single-celled otolith and 3-lens ocellus. The three adhesive papillae are simple. There are about forty notochord cells in the tail, and the cuticular tail fin is virtually absent.

The process of metamorphosis is prolonged (Fig. 3). After fixation by the adhesive papillae, the tail absorbs and the anterior end of the trunk grows out as the preoral lobe, as in *Ciona*. Rotation occurs, and after 10 or 11 days at 16° C., the siphons are contractile and the heart is beating. The characteristic

rythmical reversal of heart beat is exhibited from the beginning. The cilia of the gill slits beat actively about 16 days after fixation. During this period the preoral lobe grows as the stalk attaching the young Diazonid individual to the substratum. Finally it is a vertical stalk as long as the trunk proper, divided basally into two or three wide anchoring lobes. As in *Ciona*, *Corella* and

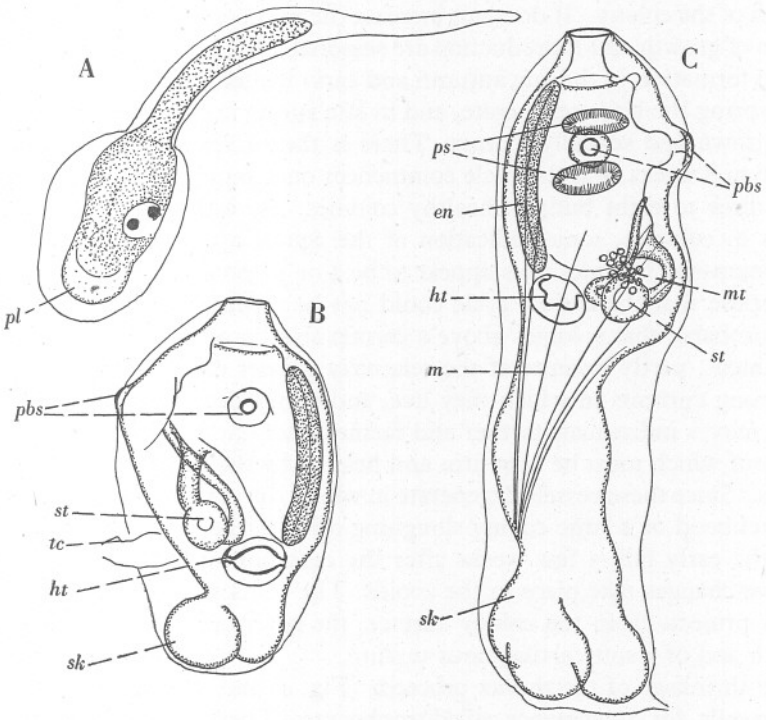

Fig. 3. *Diazona violacea*, post-larval development. A, metamorphosing tadpole with preoral lobe. B, stage 9 days later with active heart and siphons but no cilia; C, stage 5 days later (at 16° C.) fully active, with paired peribranchial siphons. *en*, endostyle; *ht*, heart; *m*, muscle; *mt*, resorbed muscle cells of tail; *pl*, preoral lobe; *pbs*, peribranchial siphons; *ps*, protostigmata; *sk*, stalk; *st*, stomach; *tc*, tunic remnant of tail.

Ascidia, at this stage, there is a single branchial siphon and in place of a median-dorsal atrial siphon there are a pair of very widely separated peribranchial siphons, extending laterally from the body wall. Each connects with a peri-branchial sac associated with two large protostigmata. The digestive canal lies more or less horizontally immediately beneath the branchial sac, as in *Ciona*, and at this stage shows no inclination to descend into the stalk. The heart is simple and lies directly between the base of the endostyle and the bend of the intestine. A group of longitudinal muscles extends from the trunk into the

stalk, and when contracting causes the ascidian to bend in an arc about the junction of stalk and trunk. An avoidance reaction is strongly suggested.

This is the latest stage to which the oozooid has been raised, and there is a remarkable resemblance to the equivalent stage of *Ciona*.

Asexual Reproduction

Asexual reproduction or budding concerns entirely the establishment and growth of the colony. It does not increase the number of colonies. The various phases of growth and reproduction are seasonal. A colony undergoes a process of bud formation in the late autumn and early winter. During late winter and early spring the buds regenerate, and in late spring and early summer become fully grown and sexually mature. There is then a sexual breeding season of from 6 to 8 weeks, and the cycle commences once more. Each zooid produces from three to eight buds in healthy colonies. By estimating the number of zooids in colonies, some indication of the age of a colony can be obtained. Maximum-size colonies thus appear to be 4 or 5 years old. There is no reason to suppose that the annual cycle could not be repeated indefinitely, but it is most probable that colonies above a certain size become dislodged from their attachment, partly because of the relatively greater exposure to the action of the strong currents in which they live, and partly because increase in colony size removes individuals farther and farther from the base of attachment, the matrix of which must be kept firm and healthy by the activity of their stolonic vessels. Since these vessels degenerate in winter during the process of budding, the likelihood of a large colony sloughing off must be great.

In the early fall, a few weeks after the cessation of sexual breeding, progressive changes take place in the zooids. The thorax of each shrinks, and no longer projects from the colony surface, the latter becoming finally perfectly smooth and of a soft cartilaginous texture.

The shrinkage of the thorax proceeds (Fig. 4) and is due to the activity of certain cells, for convenience called trophocytes. These cells, of obscure origin, derived probably from blood cells, appear throughout the thorax and grow in size as they accumulate within themselves a pseudovitellus or protein reserve. They probably arise in the same way as, and are homologous with, the cells described by Spek (1927) from living vitally stained tissues of *Clavelina*. A comparable histological study of resorption in *Diazona* along such lines would be of interest. Their appearance or growth occurs simultaneously with a shrinkage or autolysis of the differentiated thoracic structure, and is followed, as in *Clavelina* and many other forms, by a migration of the trophocytes towards the posterior end of the zooid. The cells are too large to enter the narrow stolonic vessels (which in *Clavelina* are large and become congested), and it is the abdomen and oesophageal region that becomes distended with them. The thorax survives for a while as a faint ghost of itself, but finally disappears entirely. The trophocytes form a nutritive reserve, but they

neither take an active part in the formation of buds nor contribute to new structures apart from the maintenance of the nutritive medium in which such structures develop. Bud-formation is essentially a process of epidermal strobilation or constriction. As the trophocytes withdraw from the anterior regions, transverse constrictions develop that cut deep into the internal tissues. The narrow oesophageal 'neck' is involved to a varying extent, the abdomen from the posterior end to just anterior to the stomach not at all. The constrictions for the most part occur in the intermediate zone anterior to the stomach. As far as can be determined the constrictions appear and develop simultaneously, finally

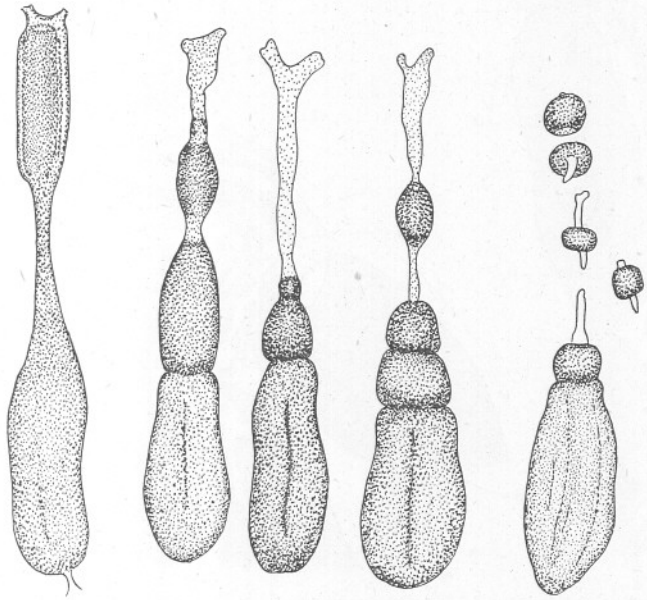

Fig. 4. *Diazona violacea*. Five zooids illustrating stages in resorption, posterior congestion by trophocytes, and strobilation.

isolating masses more or less at the same time, with the exception of the most posterior constriction. This last constriction may commence later than the more anterior, but it is also possible that the belated separation of the bud is due to the greater growth of the process. Anyhow, it is a typical condition for four or five more or less spherical masses to be isolated in series immediately anterior to, and in line with, the posterior part of the abdomen, with the proximal bud regenerating from the anterior end and still in process of constriction posteriorly. Finally, this bud too is isolated and the large abdominal remnant regenerates anteriorly. Frequently a mass may undergo isolation in the anterior oesophageal region, the region immediately posterior to it evacuated, and the posterior strobilating part connected with it by 'ghost' tissue. It raises the question of the relative roles of epidermis and internal tissues.

Two phenomena require elucidation—the fact of constriction itself, and the virtual spherical shape of the constricted mass. Sectioned material confirms the conclusion derived from direct observation on intact constricting zooids, that there is necessarily a real growth of epidermis in order to produce the deep folds (Figs. 5, 6). In the narrow band of the constriction itself the epidermal cells are unusually large and show every appearance of extensive growth, pressing against, and in effect dividing, the trophocyte mass, actually cutting through the longitudinal muscle, epicardium, and limbs of the digestive canal.

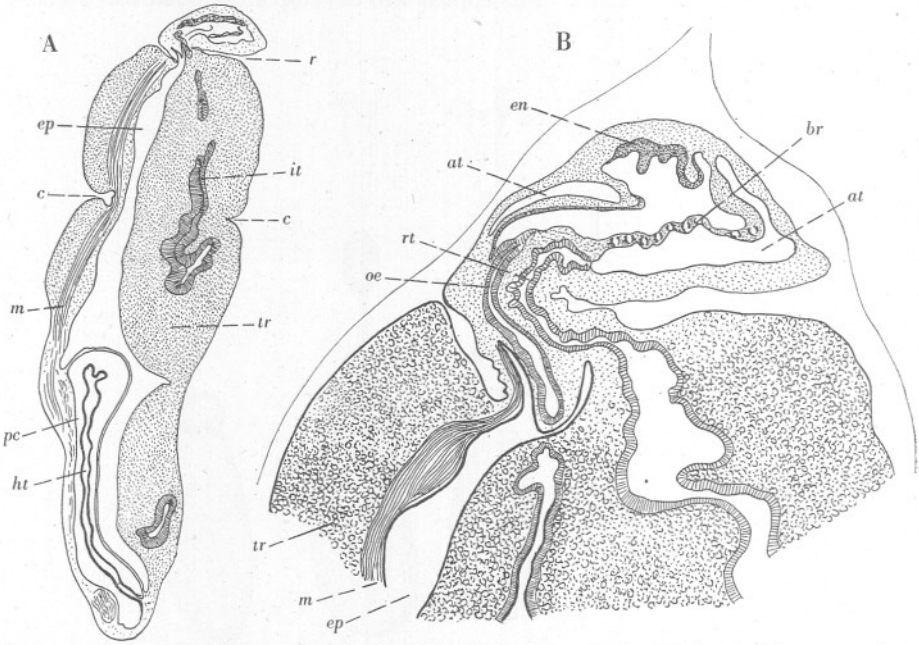

Fig. 5. *Diazona violacea*. Longitudinal sections through strobilating and regenerating abdomen, showing anterior regeneration of thorax from horns of epicardia, a mid-abdominal constriction, and the persistence of the heart and other structures. *ct*, atrial cavities; *br*, branchial wall; *c*, constriction; *en*, endostyle; *ep*, epicardium; *ht*, heart; *it*, intestine; *m*, longitudinal muscle; *oe*, new oesophagus; *pc*, pericardium; *r*, rectum; *rt*, new rectum; *tr*, trophocytes.

The epidermal tissue grades gradually from the actively growing tissue at the cutting surface back to the inactive tissue at the original surface of the zooid, indicating a slow recovery of normal cell size and appearance with the cessation of growth. All of the internal tissues are purely passive, playing no part in the process of constriction itself.

When constriction nears completion and later until regenerative processes modify the initial shape, a bud is practically spherical. This appears to be due to a freedom of movement of the large trophocytes, so that the shape of the whole contained mass of them conforms to the action of surface forces, as would a mass of small greased balls within a contracting envelope.

Salfi (1926) discusses the process of bud production in *Diazona* in terms of Child's 'physiological isolation', but whatever this may be it does not seem to apply here. Resorption of the thorax is a common phenomenon among compound ascidians and is generally followed by its regeneration from the oesophageal region or from the abdomen. In *Diazona* this process is combined with that of strobilation of the anterior abdominal and oesophageal region. The posterior abdominal region regenerates anteriorly in the usual way. Strobilation itself is something clearly distinct from 'physiological

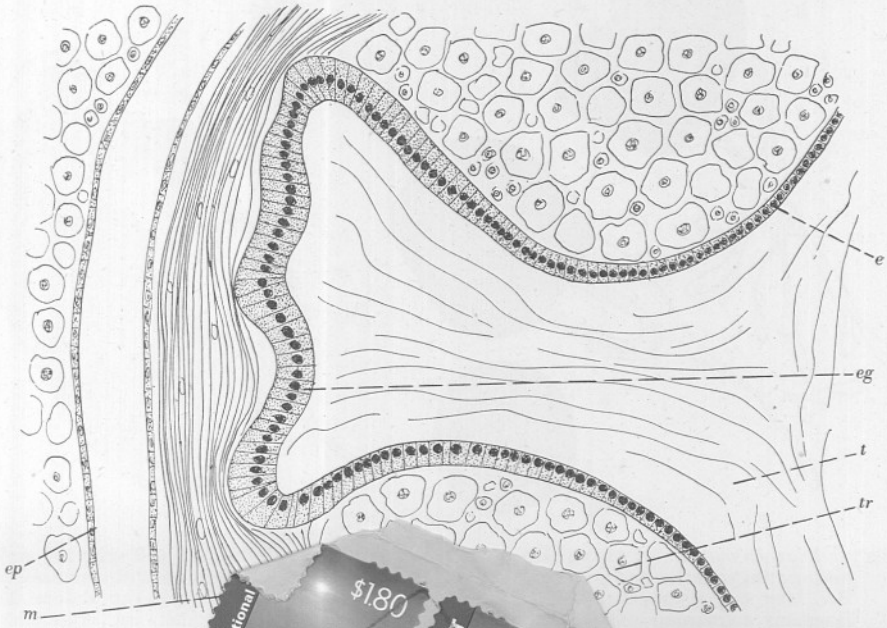

Fig. 6. *Diazona vio* minal constriction, showing active epiderma gh passive internal tissues. *e*, normal epi striction; *ep*, epicardium; *m*, longitudin roove; *tr*, trophocytes.

isolation'. on of buds by otherwise intact or that physical forces are at least a pa e residual region, constrictions appe a the long axis approximately the same as idth of the strobilating region is determined with the degree of trophocyte congestion, the e gly suggests an effort to produce spheres, as a liquid cylind erical drops as a result of surface-acting powers. If there is any truth to this, then it implies that the differential growth of the epidermis which is the actual constriction is a local response to physical forces related to surface tension.

The phenomena of reconstitution have been studied by Salfi in some detail. His observations are more or less confirmed, namely, that the fragments of the epicardium, longitudinal muscles, and descending and ascending limbs of the digestive canal survive, and that regeneration at the anterior and posterior surfaces is due almost entirely to epidermal growth forming the new epidermis, and the epicardium the internal structures (Fig. 7).

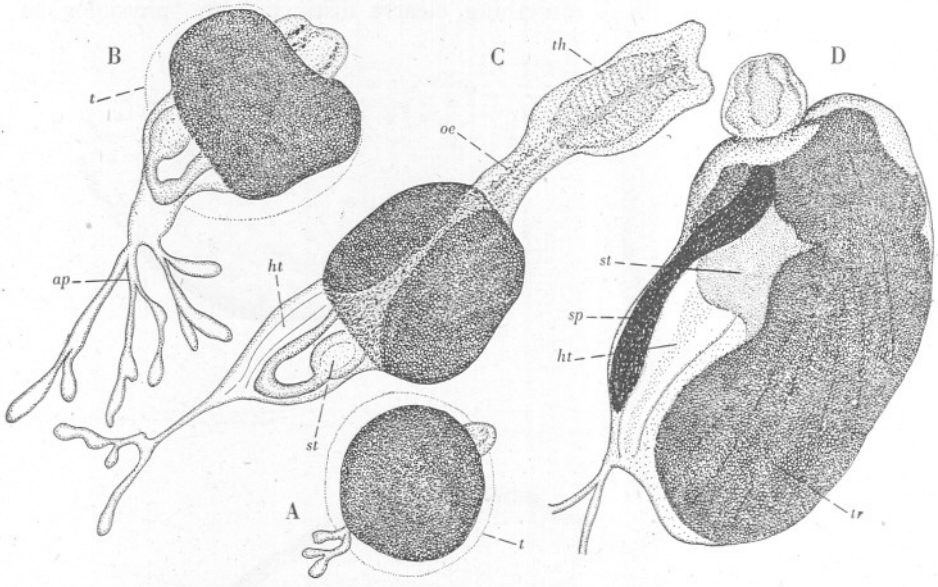

Fig. 7. *Diazona violacea*. Isolated buds in process of regeneration. A, bud still spherical and just starting anterior and posterior regeneration (dotted line indicates zone of new tunic); B,C, later stages of regeneration; D, anterior regeneration from large posterior abdominal fragment containing old heart, stomach, distended sperm duct, etc. *ap*, ampullary stolonic vessels of test; *ht*, heart; *oe*, oesophagus; *st*, stomach; *sp*, sperm duct; *t*, new test substance; *th*, new thorax; *tr*, trophocytes.

The new thorax, including the branchial sac, atrial sacs, ganglion, and thoracic part of the intestine, but excluding the epidermis, arises from tissue formed by the proliferating anterior end of the epicardium. The new intestine becomes joined to the remnant of the old. There is some indication of cellular replacement within the old sections of the digestive tube even though there is continuity in gross appearance. The regenerating branchial sac develops about seventeen rows of stigmata, the number varying somewhat with the size of the bud, but always a number far less than that typical of mature adults. Additional rows are added after the reconstituted zooid becomes functional, during renewed growth. The early stages of thoracic morphogenesis, other than the general epicardial origin, has not been determined. Posteriorly regeneration is a little more complex in origins though not in structures produced.

The epidermis grows out and branches terminally to form clusters of stolonic vessels. As far as can be determined from somewhat limited material, the two posterior ends of the digestive canal fragments unite and the loop grows posteriorly keeping pace with the epidermal outgrowth, and at an early stage shows differentiation into stomach, post-stomach, and mid-intestine regions. The new pericardium and heart appear to develop from the posterior extremity of the epicardium, as in *Aplidium* (Brien, 1925). Unlike the majority of ascidian buds, therefore, reconstitution of the new zooid is fundamentally a typical regeneration process from anterior and posterior surfaces, rather than either reorganization or a virtual complete development.

With continued growth of the structures thus established, the opaque mass of the original bud gradually becomes progressively transparent and eventually forms an elongated oesophageal region of the reconstituted individual. This is complete in early spring, and growth to sexual maturity occupies a succeeding 2 or 3 months.

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THE ECOLOGY OF THE AMPHIPODA OF THE SOUTH OF THE ISLE OF MAN

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(Text-figs. 1, 2)

CONTENTS

Introduction

An ecological survey of the Amphipoda on the shores of the Port Erin district was begun in November 1939. After a few months the work was interrupted by the war, but was recommenced early in 1946. In the later period the survey was extended to include the offshore grounds.

The object of the survey was in general to revise the records of the Amphipoda of the area and to obtain an estimate of the relative abundance of the species present, and in particular to provide additional information about the habitat of each species. It was thought that valuable results would be obtained by intensive collecting over a restricted area.

The majority of the records previously published were made by A. O. Walker over 50 years ago, but a few additions have been made since (Moore, 1937). It is interesting to note that of 121 species previously known 114 were collected during 1946–47. The remaining seven have each been recorded on only one occasion and are probably very scarce. An additional thirty-five species are here recorded for the first time (Appendices I and II). Some of these additions are due to recent taxonomic revisions such as that on

Marinogammarus (Sexton & Spooner, 1940), but the majority are the result of more intensive and specialized collecting of the order. The total number of species now known from the area is 156.

The taxonomy of the Amphipoda has been fairly well worked out in British waters. Although certain genera would repay revision there are probably very few species occurring around the British Isles that have not been described, except perhaps in the deep water to the west of Ireland. During the course of this work not one individual was obtained that could not be referred to an existing species.

It is not possible in a survey covering a large number of species to work on strict quantitative lines. On most types of shore exact quantitative methods are difficult to achieve and offshore they can only be attained on suitable ground by the use of the bottom-sampler which is not an ideal instrument for the collection of small Crustacea. However, in the course of a large number of shore collections and dredgings a fair estimate can be made of the relative abundance of the species found. Often the numbers were greater than had been expected.

Information on the habitats of amphipod species is generally limited, except for those of a few genera which have been recently revised and those of intertidal sands and of estuaries. The more important papers published with reference to this aspect are those of Reibisch (1905–6), Crawford (1937*a*, *b*, *c*), Elmhirst (1931), Southern (1915) and Watkin (1941). Lists of species from various habitats are given by Chevreux & Fage (1925) and Stephensen (1929). Pirlot (1932*a*, *b*, *c*) discusses the influence of a burrowing mode of life and a tendency towards parasitism upon the mouthparts of Amphipoda.

The Area under Investigation

The area within which this survey was made included the coastline within a radius of 10 miles from Port Erin. Some stations were worked more intensively than others, but collections were made at sufficient localities to be representative of the whole shore. The offshore work was mainly carried out in depths of less than 40 m. within a few miles of Port Erin Bay. Some hauls were taken in depths down to 84 m. at distances up to 10 miles from Port Erin, but the deeper water cannot be considered thoroughly investigated.

A description of the shores near Port Erin with charts is included in Moore (1937) and the closer offshore grounds are dealt with by Jones (1940). It may be added that the sea bottom slopes gradually to a depth of about 45 m. at a distance of roughly 6 miles from the coastline and thereafter more steeply down to about 85 m. in a further 2–3 miles. In depths of 45–80 m. the bottom consists for the most part of muddy sand, the silt-content increasing with the depth. In over 80 m. the deposit is clay or mud.

Collecting Methods

On the shore all likely habitats were searched, the amphipods found being placed in tubes and taken to the laboratory for examination. Notes were made on the spot of the abundance or otherwise of species that could be recognized, and each part of the collection was referred to the nearest tidal level.

On rocky facies the specimens were obtained by turning over stones and boulders and searching among algae. Pools were investigated and the fauna collected with a hand net. A net was used for collecting among algae at the edge of the tide. *Laminaria* holdfasts were removed complete and searched for animals in the laboratory.

Sandy beaches were investigated by sieving $\frac{1}{4}$ sq.m. samples, and by collecting specimens found under stones and among algae at low-water mark.

Particular attention was paid to fresh-water streams emptying over the beach and to the Silverburn, a small river with its estuary at Castletown.

On the offshore grounds all types of gear were used except the bottom-sampler, in particular the following: (i) naturalist's dredge with 2 ft. 6 in. mouth and a bag of heavy $\frac{1}{2}$ in. mesh; (ii) scallop dredge with 3 ft. mouth and a bag lined with shrimp netting; (iii) small otter trawl; (iv) two dredge frames, 1 ft. 6 in. and 2 ft. 6 in. across respectively, each with a bag made of sacking material; (v) D-net with canvas bag; (vi) coarse tow-net attached to the back of the otter trawl.

The first two, the naturalist's dredge and the scallop dredge, were not very successful, as part of the contents was washed out, and often only those species were obtained which were clinging to hydroids, etc., attached to stones and shells. The small otter trawl (iii) suffered from the same disadvantage and could be used only on smooth bottoms: it was useful, however, for collecting loose algae. The dredge frames with sacking (iv) were the most useful instruments. They can be used on any type of ground. They fill up almost immediately and bring up the deposit intact so that a fairly true estimate can be made of the habitat of animals found among the contents. It is necessary to attach a heavy weight to the rope a few feet in front of the dredge, and it is an advantage to have the mouth bowed out so that it will dig into the substratum. The D-net (v) was not successful: it could only be used on sand or mud and merely skimmed the surface. This may have been due to faulty construction, but its use is limited. The coarse tow-net (vi) was the most successful instrument for collecting numbers of species and individuals but it has the following disadvantages: it must be towed over a long distance and can only be used in conjunction with a trawl or dredge, and it does not provide any indication of the type of bottom over which it has travelled. It is therefore necessary to sample the deposit by other means, or to use the tow-net only on ground which is already known. The silk is easily torn on rough ground.

It is probable that a net such as that described for catching bottom plankton by Russell (1928), with slight modification to stir up the bottom, would be an excellent instrument for capturing Amphipoda, but I have had no opportunity of using it.

Wherever possible the whole of the contents of the dredge or other instrument were brought back to the laboratory and searched for amphipods. I consider it essential to adopt this procedure as it is usually impossible to look through the material thoroughly in a small boat. With the fine-meshed dredge the most satisfactory method of obtaining the specimens is to place the contents in shallow dishes with sufficient sea water just to cover them. The deposit is stirred and the dish gently tilted from side to side. Most species of amphipod are eventually caught in the surface film, though the process may take a long time. Fine sand and mud may be passed through sieves but it is difficult to sieve coarse deposits and many small forms will be missed.

The numbers of each species were recorded for each haul. The bottom deposits were occasionally analysed by sieving and weighing the separated constituents but most were graded by visual observation. Positions at sea were ascertained by means of bearings taken on prominent landmarks with a prismatic compass, and soundings were taken with a line.

A list of hauls is given in Appendix III (p. 436).

A representative collection of the species obtained is held at the Marine Biological Station, Port Erin.

The Amphipoda of the Area

There follows a systematic list of species obtained with locations and habitats. Offshore collections made during 1946 are summarized in Table I (p. 416). Under each species, where possible, a short general statement of habitat is given, compiled from my observations in conjunction with previously published work. A number of records made during 1947 from bottom-sampler hauls but not tabulated are included.

I have not included geographical range except where this is extended, as it is adequately dealt with by Chevreux & Fage (1925), Norman & Scott (1906), Stephensen (1929 and 1935–42), and Tattersall (1913).

The division of hauls into sections according to habitat in Table I was carried out from notes of the contents of the instrument used and from previous knowledge of the area. When two different types of ground were traversed in one haul, as happened with the otter trawl and tow-net, I have placed the hauls in a mixed section. Allocation to one section is sometimes arbitrary, but I have been guided by experience and any error is likely to be small.

Tidal levels are referred to by their usual abbreviations (H.W.S., H.W.N., M.T.L., L.W.N. and L.W.S.). A prefixed E. denotes 'extreme'.

Abbreviations for place-names include I.O.M. for Isle of Man, P.E. for Port Erin, and P.StM. for Port St Mary.

The terms 'shallow', 'deep', etc., as applied to the bathymetrical range of a species, refer only to the littoral system, i.e. depths of less than 200 m.

Gammaridea

LYSIANASSIDAE

Acidostoma obesum (Bate): usually in muddy sand at moderate depths. I.O.M.: 59–75 m., muddy sand, few.

Euonyx chelatus Norman: in moderately deep water; possibly always associated with *Echinus esculentus*. I.O.M.: 36–46 m., in dredge hauls with *Echinus*, few.

Nannonyx goësi Boeck: mainly at and near low-water mark among algae, and occasionally on hydroids in deeper water. I.O.M.: intertidal, in *Laminaria* holdfasts, P.StM. Ledges and Outer Harbour, few. Offshore: 60 m., on hydroids, one.

N. spinimanus Walker: no reliable estimate can be made of its habitat as the records of its occurrence are too few. I.O.M.: on *Maia squinado*, L.W.S., Langness Point, one.

Lysianassa plumosa Boeck: on coarse or mixed grounds in moderate depths. I.O.M.: 11–55 m., particularly on mixed grounds, fairly common.

Perrierella audouiniana (Bate): mainly in sponges in moderately deep water, but may occasionally be found on hydroids or algae. I.O.M.: 33–82 m., on hydroids and in sponges, few.

Orchomene humilis (Costa): usually in shallow water on algae or hydroids. I.O.M.: intertidal, in *Laminaria* holdfasts, Poyllvaaish, few. Offshore: 22–33 m., on algae and hydroids, few.

Socarnes erythrophthalmus Robertson: most abundant in fine gravel in shallow water but may also be found on fine sand. I.O.M.: intertidal: in fine sand at low water, P.E. Bay, few. Offshore: 2–18 m., in stony gravel and fine sand, abundant.

Hippomedon denticulatus (Bate): burrowing in sand of all grades, usually in fairly shallow water. I.O.M.: 9–33 m., fine and coarse sand, few.

Scopelocheirus crenatus Bate: usually on muddy sand or muddy gravel in moderate depths. Carnivorous and probably acts as a scavenger. I.O.M.: 27–82 m., particularly on muddy sand, few.

Tryphosa sarsi Bonnier: on sandy bottom in shallow water. Probably feeds on sponges and acts as a scavenger. I.O.M.: intertidal: on sponges at L.W.S., Chapel Bay, few. Offshore: 4–9 m., on muddy sand and abundant on dead fish.

Tmetonyx similis (G. O. Sars): has a wide bathymetrical distribution and seems usually to occur on mixed grounds. I.O.M.: 22–27 m., on coarse sand and broken shell, one.

Tryphosites longipes (Bate & Westwood): on muddy ground in moderate depths. I.O.M.: 68 m., muddy sand, few.

Orchomenella nana (Kröyer): commonly found on sand or mixed grounds in fairly shallow water. There are a number of records of its occurrence in dead crabs and it seems to be attracted by crustacean flesh. I.O.M.: 23–33 m., particularly on coarse sand and broken shell, fairly common.

AMPELISCIDAE

Ampelisca brevicornis (Costa): burrowing in muddy sand, usually in shallower water than the other species of the genus. I.O.M.: intertidal, in muddy sand at L.W.S., P.StM. Outer Harbour, few. Offshore: 2–18 m., fine sand, few.

A. macrocephala Lilljeborg: burrowing in mud in moderate depths. I.O.M.: 75 m., very muddy sand, one.

A. spinipes Boeck: burrowing in mixed grounds in moderate depths. Many of the records are from gravels and coarse grounds. Apparently never occurs in fine sand without mud. I.O.M.: 18–82 m., particularly in muddy sand, fairly common.

A. diadema (Costa): burrowing in muddy and mixed grounds mainly in water of moderate depth. In common with the other species of the genus it is a suspension feeder (Hunt, 1925). I.O.M.: 18–59 m., muddy sand and muddy sand with broken shell, fairly common.

A. typica (Bate): burrowing in coarse deposits usually in fairly shallow water. I.O.M.: 11–61 m., particularly in gravel and mixed grounds, few.

A. tenuicornis Lilljeborg: habitat similar to that of *A. diadema*. I.O.M.: 11–82 m., usually in muddy sand or muddy sand with broken shell, fairly common.

ARGISSIDAE

Argissa hamatipes (Norman): probably burrowing in fine or coarse deposits containing mud. I.O.M.: 15–38 m., on mixed grounds, rare.

HAUSTORIIDAE

Bathyporeia guilliamsoniana (Bate): burrowing in fine or muddy sand at L.W.S. and in shallow water. I.O.M.: intertidal, in muddy sands at L.W.S., Chapel Bay and P.StM. Outer Harbour, few. Offshore: 2–18 m., in fine sand, common.

B. elegans Watkin: burrowing in fine sand at L.W.S. and in shallow water. Its bathymetrical range is roughly the same as that of *B. guilliamsoniana* but it probably prefers cleaner sand and is usually more abundant than the latter species. I.O.M.: intertidal, in sand at L.W.S., P.E. Bay and P.StM. Outer Harbour, few. Offshore: 2–15 m., fine sand, abundant.

B. pelagica Bate: strictly intertidal, burrowing in fine sand below H.W.N. Its optimum zone is from M.T.L. to L.W.N. I.O.M.: in fine sand from H.W.N. downwards, P.E. Bay and Brewery Beach, common.

B. gracilis G. O. Sars: does not occur between tide-marks. May inhabit coarser sand than the other species in the genus and is confined to deeper water. I.O.M.: 11–35 m., mainly in coarse sand, few.

Haustorius arenarius (Slabber): usually intertidal, burrowing in sand. Its optimum zone is probably about the level of H.W.N. I.O.M.: in fine sand from H.W.N. to L.W.N., common.

Urothoë brevicornis Bate & Westwood: burrowing in fine sand from M.T.L. downwards and in shallow water. I.O.M.: not found below low-water mark. In fine sand from M.T.L. to L.W.S., P.E. Bay, few.

U. elegans Bate: usually in deeper water than *U. brevicornis* and may inhabit more muddy ground. I.O.M.: intertidal, in muddy sand at E.L.W.S., P.StM. Outer Harbour and Chapel Bay, common. Offshore: 9–60 m., particularly in sand, common.

U. marina Bate: burrows in coarser deposits than the other species of *Urothoë*, usually in coarse sand and fine gravels in moderate depths. I.O.M.: 18–37 m., particularly in coarse sand and broken shells, few.

PHOXOCEPHALIDAE

Paraphoxus oculatus G. O. Sars: probably mainly in fairly deep water, burrowing in mud or muddy sand. I.O.M.: 60 m., muddy sand, few.

Metaphoxus fultoni (T. Scott): characteristic of fine gravels, usually in rather shallow water. The animal is very small and flattened laterally and is adapted for

burrowing in deposits consisting mainly of rather large particles. I.O.M.: 11–60 m., particularly in gravel, common.

Harpinia antennaria Meinert: burrowing in muddy sand, usually in fairly shallow water but may extend down to moderate depths. It is often found in company with other species of the genus, particularly *H. pectinata*. I.O.M.: intertidal, muddy sand at E.L.W.S., P.StM. Outer Harbour, fairly common. Offshore: 11–82 m., particularly in muddy sand, few.

H. pectinata G. O. Sars: burrowing in muddy sand or mud. Has not been recorded from between tide-marks. I.O.M.: 46–88 m., in muddy sand, few. In the Irish Sea it usually occurs in deeper water than *H. antennaria* but the two species overlap.

H. crenulata Boeck: burrows in mud, usually in rather deep water. I.O.M.: 59–88 m., muddy sand and mud, fairly common.

AMPHILOCHIDAE

Amphilochus manudens Bate: probably lives mainly among hydroids in moderate depths. I.O.M.: 11–37 m., particularly on hydroids and mixed grounds, fairly common.

A. spence-batei (Stebbing): perhaps usually on muddy sand but the species has been too seldom recorded for a reliable estimate to be formed. The other British records are from Torbay and Plymouth. I.O.M.: 15–18 m., on gravel and sand, rare.

Amphilochoides serratipes (Norman): on mixed ground with mud at moderate depths. I.O.M.: 27–33 m., on muddy sand with broken shell, few. The species was obtained in six hauls, all on the same type of ground.

Peltocoxa brevirostris (T. & A. Scott): on mixed grounds in moderate depths. The animal is very small, usually about 1 mm. in length, and may live interstitially. I.O.M.: 27–33 m., on mixed grounds, fairly common.

Gitana sarsi Boeck: usually in shallow water on algae and hydroids and in gravel. Occasionally occurs at low-water mark. I.O.M.: intertidal, in *Laminaria*, P.StM. Ledges, few. Offshore: 7–82 m., particularly on gravel and mixed grounds, common.

LEUCOTHOIDAE

Leucothoë spinicarpa (Abildgaard): commensal in sponges and the branchial sacs of ascidians, usually in fairly deep water but at low-water mark in suitable localities. I.O.M.: intertidal, in sponges, Poyllvaaish, few. Offshore: 60 m., in sponges, few.

L. lilljeborgi Boeck: on muddy sand or mud in which it probably burrows, as suggested by Crawford (1937*b*). I.O.M.: 11–88 m., usually on muddy ground, few.

STENOTHOIDAE

Metopa bruzelii Goës: usually associated with hydroids in fairly shallow water. I.O.M.: 18–33 m., particularly on coarse sand with broken shell, fairly common.

M. pusilla G. O. Sars: habitat similar to that of *M. bruzelii*. I.O.M.: 33–60 m., particularly on coarse sand with broken shell, few.

M. borealis G. O. Sars: habitat probably similar to that of *M. bruzelii* but it may extend into deeper water. The three species may occur together. I.O.M.: 18–82 m., particularly on mud, few.

Stenothoë monoculoides (Montagu): occurs mainly above low-water mark but may extend into shallow water. Inhabits algae and is particularly common among *Corallina* in pools from M.T.L. to L.W.N., and in *Laminaria* holdfasts at L.W.S. Occasionally found on sponges. I.O.M.: occurs throughout the area and is abundant in *Corallina* pools and in *Laminaria* holdfasts. Intertidal: P.StM. Ledges and Outer Harbour, Poyllvaaish, below Biol. Stat., Chapel Bay, Castletown Bay. Offshore: 4–18 m., on algae, few.

S. marina (Bate): usually on hydroids in fairly shallow water and occasionally on algae. I.O.M.: 11–55 m., particularly on hydroids, few.

S. antennulariae Della Valle = *S. crassicornis* Walker: probably found only on hydroids in moderately deep water, sometimes in company with *S. marina*. I.O.M.: 31–60 m., on hydroids, few.

CRESSIDAE

Cressa dubia (Bate): usually on hydroids in moderately deep water. It is fairly abundant but is probably often missed owing to its small size. I.O.M.: 22–82 m., on hydroids and mixed ground, common.

COLOMASTIGIDAE

Colomastix pusilla Grube: always associated with sponges down to moderate depths. I.O.M.: 60 m., in sponges, few.

LAPHYSTIIDAE

Laphystius sturionis Kröyer: parasitic on fish. I.O.M.: on a cod caught on a long line, many.

ACANTHONOTOZOMATIDAE

Panoploea minuta (G. O. Sars): among algae in shallow water and may extend into moderate depths on hydroids. I.O.M.: 7–64 m., on hydroids and algae, fairly common.

P. eblanae (Bate): probably on hydroids in moderately deep water. Also reported in company with *Rhizostoma*. I.O.M.: 27–60 m., on hydroids, few.

Iphimedia obesa Rathke: habitat similar to that of *Panoploea minuta* but usually occurs in rather deeper water. I.O.M.: 11–82 m., on hydroids and algae, few.

LILLJEBORGIIDAE

Lilljeborgia brevicornis Bruzelius: has a wide bathymetrical range. It is difficult to form an estimate of its habitat but it possibly occurs mainly on hydroids. I.O.M.: 60 m., on hydroids, few.

L. kinahani (Bate): on fine gravels and mixed grounds in fairly shallow water. I.O.M.: 11–55 m., on gravel and mixed grounds, common.

OEDICEROTIDAE

Perioculodes longimanus (Bate & Westwood): burrowing in fine or muddy sand at L.W.S. and in shallow water. Occasionally extends into moderate depths. I.O.M.: intertidal, in muddy sand at extreme L.W.S., P.StM. Outer Harbour and Chapel Bay, fairly common. Offshore: 4–82 m., fine and muddy sand, common.

Pontocrates arenarius (Bate): burrows in fine sand preferably without mud from M.T.L. to a depth of a few metres. Its main centre of distribution is about L.W.S. I.O.M.: intertidal, in fine sand from M.T.L. downwards, P.E. Bay and Chapel Bay, fairly common. Offshore: 2–11 m., fine sand, few.

P. norvegicus Boeck: burrows in fine or muddy sand from M.T.L. to shallow water. I.O.M.: more abundant in shallow water than between tide-marks. Extends into rather deeper water than *P. arenarius* and will burrow in muddy sand, whereas *P. arenarius* prefers clean sand. Intertidal: in fine and muddy sand, from M.T.L. downwards, P.E. Bay, P.StM. Outer Harbour, and Chapel Bay, few. Offshore: 2–18 m., fine sand, common.

Synchelidium haplocheles Grube: in fairly shallow water but usually at a greater depth than the species of *Pontocrates*. Burrows in a wide range of deposits from fine

sand to gravel. I.O.M.: 4–35 m., in fine sand, gravel and mixed grounds, fairly common.

Monoculodes carinatus Bate: usually in fairly shallow water and probably burrows mainly in rather coarse deposits. I.O.M.: 11–33 m., mixed grounds, few.

M. subnudus Norman: habitat similar to that of *M. carinatus* but may occur in deeper water. I.O.M.: 7–33 m., mixed grounds, few.

Westwoodilla caecula (Bate): in mud or muddy sand usually in fairly deep water. I.O.M.: 82 m., mud, fairly common.

TIRONIDAE

Syrrhoites fimbriatus (Stebbing & Robertson): probably mainly on mixed grounds in moderately deep water. This species has only once been found outside the Irish Sea and the Firth of Clyde, but as this record was from north of Spitsbergen it must have a much wider geographical range than would appear from the few published finds. It has probably been missed largely because of its minute size. I.O.M.: 11–55 m., mixed grounds, few.

CALLIOPIIDAE

Calliopius crenulatus Chevreux & Fage: mainly on algae near low-water mark. I.O.M.: on sand at extreme L.W.S., P.E. Bay, few.

Apherusa cirrus (Bate): near or just below low-water mark on algae. I.O.M.: occurs particularly on *Halidrys siliquosa* with which it tones so well that it is difficult to distinguish. At L.W.S., Chapel Bay, Castletown Bay, Niarbyl, common.

A. bispinosa (Bate): usually on algae in shallow water and between tide-marks in pools and sheltered situations, but it may extend into fairly deep water where it probably lives on hydroids. I.O.M.: intertidal, in *Laminaria* holdfasts, on *Halidrys* and red algae at low-water mark, P.StM. Ledges and Outer Harbour, Chapel Bay, Castletown Bay, Poyllvaaish, few. Offshore: 2–59 m., mainly on algae and fine gravel, common.

A. jurinei (Milne-Edwards): among algae between tide-marks, only occasionally occurring offshore. Found in *Corallina* and *Enteromorpha* pools from H.W.N. downwards. Particularly common in the holdfasts of *Laminaria digitata*. I.O.M.: occurs throughout the area but is more abundant on exposed situations on rock. In sheltered places it is largely replaced by *Apherusa bispinosa*. Exhibits a large number of colour varieties. Intertidal: P.StM. Ledges and Inner Harbour, Poyllvaaish, Niarbyl, below Biol. Stat., Spaldrick, Chapel Bay, Castletown Bay. Offshore: 9–31 m., on algae, few.

PLEUSTIDAE

Parapleustes monocuspis (G. O. Sars): on hydroids in moderate depths. I.O.M.: 33–37 m., on hydroids, few. This species has not previously been recorded from the Irish Sea, but Walker (1895) stated that he had collected specimens on the north coast of Wales corresponding to this form, which he regarded as the young of *P. bicuspis*. He had constantly collected the two forms together and observed that only the largest specimens had a dorsal tooth on the first pleon segment, and this varied in length in proportion to the size of the specimen. It seems clear, however, from the descriptions of Sars (1895), that the two species are really distinct.

Stenopleustes nodifer (G. O. Sars): on mixed grounds and hydroids in fairly deep water. I.O.M.: 29–82 m., on mixed grounds and hydroids, few.

PARAMPHITHOIDAE

Epimeria cornigera (J. C. Fabricius): on coarse ground and particularly on corals in rather deep water. I.O.M.: 55–84 m., mud and coarse sand with broken shell, few.

ATYLIDAE

Nototropis swammerdami (Milne-Edwards): among algae on sandy bottoms in very shallow water and occasionally above low-water mark on sandy shores. I.O.M.: intertidal, on sand and among weed at L.W.S. P.E. Bay, Chapel Bay, Castletown Bay, fairly common. Offshore: 2–15 m., among weed on sand, common.

N. vedlomensis (Bate & Westwood): on mixed grounds and gravels, usually in deeper water than *N. swammerdami*. Conceals itself in the deposit in the manner described by Crawford (1937*b*) for *Maera othonis*. I.O.M.: 7–60 m., on gravel and mixed grounds, common.

MELPHIDIPPIDAE

Melphidippella macra (Norman): habitat similar to that of *Nototropis vedlomensis*. The two species often occur in the same hauls. I.O.M.: 9–82 m., mainly on mixed grounds, common.

EUSIRIDAE

Eusirus longipes Boeck: usually on mixed or muddy grounds in moderate depths. I.O.M.: 11–60 m., mainly on mixed grounds, fairly common.

GAMMARIDAE

Gammarellus homari (J. C. Fabricius): among algae in shallow water and at low-water mark. I.O.M.: intertidal, among *Laminaria* at low-water mark and on floating buoy, P.StM. Ledges, Niarbyl, P.E. Breakwater, common. Offshore: 1–18 m., on weed, few. The specimens from P.StM. included a number of adults of the form *homari* while those from P.E. Bay were all of the form *angulosus*. I am unable to detect any morphological difference between the two forms other than the length of the projections of the dorsal carina, and therefore treat them as belonging to one species. At the same time the collections included ovigerous females about 10 mm. in length of both forms.

Cheirocratus sundevalli (Rathke): mainly on mixed grounds and gravels in moderate depths. Also occurs occasionally on loose weed. I.O.M.: 9–37 m., in gravel and mixed grounds, fairly common.

C. intermedius G. O. Sars, probably on muddy grounds in moderate depths. I.O.M.: 66–84 m., in muddy sand, few.

C. assimilis Lilljeborg: habitat similar to that of *C. sundevalli*, but usually less abundant. I.O.M.: 27 m., mixed grounds, few.

Megaluropus agilis Hoek: burrowing in fine sand in shallow water. Also occurs on loose weed. I.O.M.: 2–15 m., in fine sand and on weed, common.

Melita pellucida G. O. Sars: among decaying vegetation in slightly brackish water of salinity up to about 3 $^{o}/_{oo}$. I.O.M.: upper part of Silverburn estuary, few.

M. palmata (Montagu): under stones and on algae mainly from M.T.L. to L.W.N. Can tolerate reduction in salinity down to about 13 $^{o}/_{oo}$ and penetrates some distance into estuaries. I.O.M.: under stones from M.T.L. to L.W.S., P.StM. Ledges and Inner Harbour, Chapel Bay, Spaldrick, Brewery Beach, lower part of Silverburn, Niarbyl, fairly common.

M. hergensis Reid: habitat similar to that of *M. palmata* but probably does not penetrate estuaries and occurs from above L.W.S. to shallow water. I.O.M.: intertidal, under stones on sand at L.W.S., Chapel Bay, common. Offshore: 2–22 m., on weed on sand, few.

M. obtusata (Montagu): on sublittoral algae and occasionally at low-water mark, and also on *Asterias rubens* and *Luidia ciliaris* in deeper water. The specimens from echinoderms are coloured white, while those from algae are brown or dark coloured.

I.O.M.: intertidal, on algae at L.W.S., P.E. Bay, Chapel Bay, P.StM. Inner Harbour, few. Offshore: 11–33 m., mainly on *Asterias* and *Luidia*, common.

Ceradocus semiserratus (Bate): in fine gravels and mixed grounds usually in fairly shallow water. I.O.M.: 11–55 m., on gravel and coarse sand with broken shell, common.

Maera othonis (Milne-Edwards): usually on coarse deposits mixed with fine or muddy sand in moderate depths. Occasionally at L.W.S. It shelters under pieces of shell and pebbles. I.O.M.: the most widely distributed amphipod on the offshore grounds of the area. It occurs on most types of bottom but is most abundant on mixed grounds and gravel. Intertidal: 7–82 m., gravel, mixed grounds, and hydroids, common.

Maerella tenuimana (Bate): appears to occur only in coarse sand deposits in moderate depths and to be absent from ground containing mud. Probably burrows in the substratum. I.O.M.: 22–35 m., in coarse sand and coarse sand with broken shell, few.

Gammarus locusta (L.): typically a marine species occurring on sandy ground beneath stones and among algae usually at low-water mark and in shallow water. Can tolerate some reduction in salinity and normally penetrates for a short distance up estuaries. In certain parts of its range *G. locusta* may live in salinities down to as low as 5–6 $^{o}/_{oo}$. (See Spooner, 1947, and Segerstråle, 1947.) I.O.M.: intertidal, under stones on sand at low water, P.E. Bay, P.StM. Inner Harbour, Chapel Bay, Poyllvaaish, Castletown Bay, abundant. Offshore: 0–18 m., weed on sand, particularly abundant near water's edge. In the area it is only found in fully marine conditions. Young individuals may occur higher up the shore than the adults.

G. zaddachi Sexton subsp. *zaddachi* Spooner 1947: inhabits the upper end of estuaries (roughly about the top third of the estuarine zone) and brackish water of low salinities. In some areas can maintain itself under pure fresh-water conditions (Spooner, 1947). I.O.M.: occurs only in the Silverburn from the overlap zone with *G. z. salinus* to the upper limit of salt-water penetration, where the water is entirely fresh except for short periods at H.W.S.

G. zaddachi Sexton subsp. *salinus* Spooner 1947: inhabits the middle reaches of estuaries where the salinity fluctuation is greatest; mingling with *G. locusta* at the seaward end, and with *G. z. zaddachi* towards the river end. Cannot tolerate fresh water (Spooner, 1947). I.O.M.: occurs in all streams which run into the sea over sandy shores, from L.W.N. to about M.T.L. It is absent from streams which run their entire course over rocks and through boulders. Also occurs in the Silverburn in weed and under stones in the stream from the harbour mouth to the limit of high salinity at high tide where it overlaps with *G. z. salinus*.

G. duebeni Lilljeborg: in brackish and fresh water on the upper part of the shore, in estuaries, and in small streams. Under stones, in rock pools, and among algae and fresh-water vegetation. Occurs in water of salinity up to about 21 $^{o}/_{oo}$ but usually lower than this value, and is very resistant to unfavourable conditions (Segerstråle, 1946). I.O.M.: occurs from H.W.N. to H.W.S. in streams which flow over rock and shingle into the sea, and in brackish pools above high-water mark. It is also found in some small fresh-water streams up to a considerable height above sea-level. These streams are reduced to mere trickles in times of drought. In other larger streams it was not found above high-water mark. Found with *G. pulex* only in the Silverburn. Presumably the animals occurring on the shore belong to Reid's type α, while those found in streams are of type β (Reid, 1939). Localities: Spaldrick, P.E. Bay, Brewery Beach, common.

G. pulex (L.): among vegetation and under stones in fresh-water streams and lakes. Cannot tolerate increased salinity. It is apparently commoner in hard than in soft water. I.O.M.: occurs only in the Silverburn above the tidal zone.

Marinogammarus marinus (Leach): strictly intertidal, optimum between H.W.N. and M.T.L. Under stones, in fucoids, on gravel, mixed grounds, and soft mud, but absent or scarce on cleaner sands or shingle. Penetrates estuaries for some distance, and can tolerate salinities down to about 10‰. I.O.M.: occurs on all rocky shores and is sometimes very abundant. Only found below M.T.L. where there is fresh-water influence. Occurs in streams where it is subject to considerable salinity changes and spends the greater part of each day in water which is entirely fresh. Localities: P.StM. Ledges and Outer Harbour, Spaldrick, Niarbyl, Poyllvaaish, Brewery Beach, Castletown Bay, lower part of Silverburn estuary.

M. obtusatus (Dahl): essentially intertidal, optimum about M.T.L. to L.W.N. Under stones and seaweed. Associated with less muddy and more stony grounds, and capable of existing on comparatively clean pebbles. Penetrates only a short distance into estuaries. Probably cannot tolerate salinities below about 33·5‰ except for short periods. I.O.M.: occurs on all rocky shores. Found sparingly in fresh-water streams running over the beach. Intertidal: P.StM. Ledges and Outer Harbour, Spaldrick, Niarbyl, Poyllvaaish, Brewery Beach, Castletown Bay, abundant. Offshore: 9–5 m. in weed on gravel, one.

M. finmarchicus (Dahl): habitat similar to that of *M. obtusatus* but more local and never so abundant. Possibly a tendency to inhabit situations which remain wet at low tide. I.O.M.: Spaldrick, below Biol. Stat., Bradda Head, locally common.

M. stoerensis Reid: strictly intertidal between H.W.N. and L.W.N. where fresh-water streams or seepage flow over the tidal zone. Under flat stones. Penetrating only a short distance into estuaries. Apparently this species can only exist where there are large periodical changes in salinity. I.O.M.: under stones in fresh-water streams, Spaldrick, Brewery Beach, lower part of Silverburn, common.

M. pirloti Sexton & Spooner: between H.W.N. and L.W.N. on stony beaches where there is fresh-water influence. I.O.M.: occurs in streams running over reefs or pebbles and emptying into the sea over sand. Its optimum zone is from H.W.N. to M.T.L. Occasional specimens may apparently occur away from fresh-water influence, as one individual was obtained from *Fucus serratus* at L.W.S., in company with *Gammarus locusta*. Brewery Beach, abundant.

Pectenogammarus planicrurus Reid: the records are too few to formulate a general idea of its habitat, but apparently it is usually intertidal and occurs under boulders (Reid, 1944). I.O.M.: under stones between H.W.N. and L.W.N., Spaldrick, few.

DEXAMINIDAE

Dexamine spinosa (Montagu): among algae at low-water mark and in shallow water. I.O.M.: intertidal, among algae at L.W.S., Chapel Bay and Castletown Bay, few. Offshore: 4–22 m., among weed on sand and gravel, common.

D. thea Boeck: among algae. A more pronounced littoral form than *D. spinosa* but also found in shallow water. I.O.M.: occurs in sheltered situations particularly among *Laminaria* at L.W.S. Intertidal: P.StM. Outer Harbour, below Biol. Stat., Chapel Bay, Castletown Bay, locally common. Offshore: 7–18 m., weed on sand and gravel, few.

Tritaeta gibbosa (Bate): among algae and associated with sponges usually at low-water mark but sometimes extending into fairly deep water. I.O.M.: intertidal, in sponges and *Laminaria* holdfasts at low-water mark, P.StM. Ledges, below Biol. Stat., Chapel Bay, Poyllvaaish, Niarbyl, fairly common. Offshore: 15–60 m., among algae and on sponges, few.

Guernea coalita (Norman): on coarse or mixed grounds in fairly shallow water where it probably lives interstitially. I.O.M.: 11–33 m., in coarse sand and mixed grounds, fairly common.

TALITRIDAE

Talitrus saltator (Montagu): burrowing into loose sand and beneath rotting weed on sandy beaches at H.W.S. In the winter months they are found some distance below the surface and possibly hibernate in the manner described for *Talorchestia megalophthalma* by Edwards & Irving (1943). I.O.M.: in loose sand at H.W.S., P.E. Bay, abundant.

Orchestia mediterranea A. Costa: usually under stones at about H.W.N. Occupies a zone distinctly below that of *O. gammarella*, though the two species overlap. Usually found with *Marinogammarus marinus*. I.O.M.: under stones at H.W.N., Niarbyl, Poyllvaaish, abundant.

O. gammarella (Pallas): usually on rocky shores at and above H.W.S. under stones and among rotting algae. In damp places it may extend considerably above sea-level. I.O.M.: abundant on all shingle beaches beneath rotting weed at H.W.S., P.StM. Outer Harbour, Spaldrick, Poyllvaaish, Niarbyl, Castletown Bay.

Talorchestia deshayesei (Audouin): under rotting weed and stones on sand at H.W.S. I.O.M.: very local and was not collected with *Talitrus*, although the habitats of the two species are very similar. Castletown Bay, Derby Haven.

Hyale prevosti (Milne-Edwards) = *H. nilssoni* (Rathke): among algae, usually between H.W.N. and L.W.N. Can tolerate fresh water for some time and penetrates estuaries. I.O.M.: occurs on all rocky shores. It lives over most of the tidal zone, being common in *Pelvetia* at H.W.N. and among algae in pools down to L.W.N. It is found more rarely in *Laminaria* holdfasts and red algae at L.W.S. and occasionally offshore in a few metres depth. Intertidal: P.StM. Ledges, Spaldrick, below Biol. Stat., Niarbyl, Brewery Beach, Castletown Bay, lower part of Silverburn. Offshore: 9–15 m., one.

H. pontica (Rathke): among algae near L.W.S., usually in rather exposed situations. I.O.M.: occurs only among algae from L.W.S. and on a floating buoy. Was not found offshore. In one collection it occurred in company with *H. prevosti* at L.W.S. but was considerably more abundant. P.E. Breakwater, fairly common.

AORIDAE

Aora typica Kröyer: on algae and hydroids in fairly shallow water and occasionally at L.W.S. in sheltered situations. I.O.M.: intertidal in *Laminaria* holdfasts, P.StM. Outer Harbour, below Biol. Stat., Castletown Bay, few. Offshore: 11–33 m., on algae and hydroids, common.

Microdeutopus damnoniensis (Bate): among algae at low-tide mark and in very shallow water. I.O.M.: on muddy sands at L.W.S., P.StM. Inner Harbour, and Chapel Bay, few.

Lembos websteri Bate: at L.W.S. and in shallow water on algae, particularly in the holdfasts of *Laminaria digitata*. I.O.M.: intertidal, abundant everywhere in *Laminaria* holdfasts, P.StM. Ledges and Outer Harbour, below Biol. Stat., Chapel Bay, Poyllvaaish, Castletown Bay, Niarbyl. Offshore: 7–22 m., on algae, few.

L. longipes (Lilljeborg): burrowing in coarse to muddy sand in moderate depths. I.O.M.: 29–82 m., in coarse and muddy sand, fairly common.

PHOTIDAE

Microprotopus maculatus Norman: always associated with a sandy bottom in shallow water. May sometimes burrow into the deposit but its usual habitat is among loose algae. I.O.M.: 2–18 m., in weed on sand, common.

Photis longicaudata (Bate & Westwood): probably burrows in fine to muddy sand or mixed grounds in moderate depths. I.O.M.: 27–82 m., on mixed grounds, few.

Eurystheus melanops (G. O. Sars): probably on mixed grounds in moderately deep water. I.O.M.: 11–33 m., mixed grounds, few. This species has not previously been

recorded with certainty from the British Isles as there has been some confusion with *E. erythrophthalmus*. It is much scarcer than the next species.

E. erythrophthalmus (Lilljeborg) = *E. maculatus* (Johnston): usually on hydroids in moderate depths and on algae in shallow water. Between tide-marks it seems to be almost confined to the holdfasts of *Laminaria digitata*, of which it is a typical and sometimes abundant inhabitant. I.O.M.: intertidal, in *Laminaria* at low-water mark, P.StM. Ledges, below Biol. Stat., Poyllvaaish, Castletown Bay, Niarbyl, common. Offshore: 2–82 m., mainly on hydroids and algae, common.

E. palmatus (Stebbing & Robertson): on hydroids and muddy or mixed grounds in fairly deep water. I.O.M.: 29–60 m., on hydroids and mixed grounds, few.

Podoceropsis nitida Stimpson: in moderately deep water on hydroids or commensal in the shells inhabited by hermit crabs. I.O.M.: 11–60 m., in shells of *Buccinum* inhabited by *Eupagurus bernhardus* and on hydroids, common.

Megamphopus cornutus Norman: in mixed grounds usually in fairly shallow water. I.O.M.: 7–33 m., on mixed grounds, fairly common.

Protomedeia fasciata Kröyer: in muddy sand at moderate depths. I.O.M.: 73 m., muddy sand, few. Not previously obtained so far south.

Leptocheirus hirsutimanus Bate: probably in rather coarse or mixed grounds in moderate depths. I.O.M.: 33 m., mixed grounds, few.

L. pectinatus Norman: in fine gravel or mixed grounds, usually in fairly shallow water. I.O.M.: 9–36 m., in gravel and mixed grounds, common.

Isaeidae

Isaea montagui Milne-Edwards: only found on the mouthparts of *Maia squinado*. I.O.M.: on *Maia*, Langness Point, few.

Amphithoidae

Amphithoë rubricata (Montagu): on the shore or in shallow water on algae or under stones. Occurs in pools and among algae from M.T.L. downwards, and is particularly abundant in the holdfasts of *Laminaria digitata*. I.O.M.: intertidal, under stones and in *Laminaria* holdfasts, P.StM. Ledges and Outer Harbour, below Biol. Stat., Chapel Bay, Poyllvaaish, Castletown Bay, common. Offshore: 7–18 m., on algae, few.

Pleonexes gammaroides Bate: habitat similar to that of *Amphithoë rubricata* but favours more exposed positions. I.O.M.: intertidal, among algae at L.W.S., P.StM. Ledges, Chapel Bay, P.E. Breakwater, few. Offshore: 7–15 m., on algae, one.

Sunamphithoë pelagica (Milne-Edwards): usually on algae in shallow water. I.O.M.: intertidal, on *Fucus* at L.W.S., P.StM. Inner Harbour, one. Offshore: 4–9 m., on algae, one.

Biancolina cuniculus (Stebbing): probably among algae in very shallow water. I.O.M.: on red algae at extreme L.W.S., Chapel Bay, one. Not previously recorded farther north than Devon. Apparently very rare.

Jassidae

Jassa falcata (Montagu) incl. *J. dentex* (Czerniavski), Chevreux & Fage (1925, p. 348): among algae at low-water mark and in very shallow water. Particularly abundant and grows to a large size among the algae on floating buoys. The younger forms are sometimes found in sponges. On the authority of Mrs E. W. Sexton (*in litt.*) I have included the form described by Chevreux & Fage as *J. dentex* (Czerniavski) in the species *J. falcata* (Montagu). Both forms of the male are common in the area and sometimes occur together. This was noted by Walker (1911). I.O.M.: intertidal, in *Laminaria* holdfasts and occasionally on sponges at L.W.S., P.StM. Ledges and Outer Harbour,

below Biol. Stat., P.E. Breakwater and buoy, Poyllvaaish, Castletown Bay, Niarbyl, common. Offshore: 2–15 m., on algae, few.

J. pusilla (G. O. Sars): on hydroids and among sponges on *Inachus dorsettensis* in moderately deep to deep water. Apparently never in very shallow water. I.O.M.: 27–33 m., on *Inachus*, common.

J. ocia (Bate): among algae and particularly in sponges from M.T.L. to L.W.S. I.O.M.: in sponges and *Laminaria* holdfasts on the shore from M.T.L. downwards, P.StM. Ledges, below Biol. Stat., Poyllvaaish, Castletown Bay, fairly common.

Parajassa pelagica (Leach): habitat similar to that of *Jassa falcata* but generally scarcer. I.O.M.: among algae at L.W.S., P.StM. Ledges, P.E. Breakwater and buoy, Poyllvaaish, Niarbyl, few.

Microjassa cumbrensis (Stebbing & Robertson): mainly on mixed grounds in moderately deep water. It may live interstitially or on hydroids. I.O.M.: 11–64 m., on mixed grounds and hydroids, fairly common.

COROPHIIDAE

Ericthonius brasiliensis (Dana): on algae at L.W.S. in sheltered situations and extending into deeper water on hydroids. I.O.M.: intertidal, in *Laminaria* holdfasts, P.StM. Inner and Outer Harbours, Poyllvaaish, few. Offshore: 1–82 m., on algae and hydroids, fairly common.

Unciola planipes Norman: probably burrowing in coarse sand or mixed grounds, usually in fairly deep water. I.O.M.: 35 m., in coarse sand, few.

Siphonoecetes dellavallei Stebbing: on fine sand in shallow water living in tubes constructed of sand grains and fine gravel usually attached to small pieces of shell. I.O.M.: 2–18 m., on fine sand, common.

Corophium volutator (Pallas): burrowing in muddy sand in sheltered places between tide-marks on the shore, but usually in estuaries. It is found in varying salinity conditions from about 1 to 34 $^{0}/_{00}$. The controlling factor in its distribution is probably the presence or absence of a suitable soil which depends largely on the amount of shelter from winds and currents (Hart, 1930; Beanland, 1940). I.O.M.: in muddy sand from H.W.N. to M.T.L., P.StM. Inner Harbour and Silverburn Estuary, abundant. The species seems to be absent or very scarce in P.StM. Inner Harbour during the early part of the year. At least it could not be found there at the end of March 1946, but was abundant a month later, and the population then included many large ovigerous females. It is possible that in this locality it is a summer visitor (see Beanland, 1940).

C. affine Bruzelius: burrowing in muddy sand, usually in moderately deep water. I.O.M.: 59 m., muddy sand, fairly common.

C. crassicorne Bruzelius: burrowing in muddy sand in shallow water and at L.W.S. in sheltered situations. I.O.M.: in muddy sand at E.L.W.S., P.StM. Outer Harbour, few.

C. bonelli (Milne-Edwards): in tubes on algae and hydroids at L.W.S. and in fairly shallow water. I.O.M.: intertidal, in *Laminaria* holdfasts and on sponges, P.StM. Outer Harbour, below Biol. Stat., Chapel Bay, Poyllvaaish, Niarbyl, few. Offshore: 11–27 m., on algae and hydroids, few.

CHELURIDAE

Chelura terebrans Philippi: bores in wooden piles, etc., and in submerged driftwood in shallow water. I.O.M.: in submerged wood, 18 m., abundant.

PODOCERIDAE

Dulichia porrecta (Bate): in moderately deep water. The type of ground that it usually inhabits is difficult to estimate from the records. It may be often associated with *Pecten maximus* on mixed grounds. I.O.M.: 29 m., on muddy sand with broken shell, one.

Caprellidea

CAPRELLIDAE

Phtisica marina Slabber: usually on algae or hydroids from L.W.S. to fairly deep water. I.O.M.: intertidal, in mud in deep clefts at L.W.S., P.StM. Ledges, few. Offshore: 2–82 m., on algae, hydroids, and mixed grounds, common.

Pseudoprotella phasma (Montagu): on algae and hydroids at L.W.M. and in fairly shallow water, sometimes descending to greater depths. I.O.M.: 7–33 m., on algae and hydroids, common. It is particularly common on *Obelia* growing on the fronds of *Laminaria*.

Podalirius typicus Kröyer = *Pariambus typicus* (Kröyer): usually in fairly shallow water on fine sand or in the ambulacral grooves of *Asterias* or *Solaster*. I.O.M.: intertidal, muddy sand at L.W.S., P.StM. Outer Harbour, one. Offshore: 4–29 m., mainly on fine sand, fairly common.

Parvipalpus capillaceus (Chevreux): habitat impossible to estimate. I.O.M.: 29 m., muddy sand with broken shell, one. Apparently a very rare species, being known previously only from the west coast of France and Valencia Harbour, Eire.

Caprella acanthifera Leach: from L.W.N. to shallow water offshore, mainly on algae and sponges, seldom on hydroids. I.O.M.: intertidal, in *Laminaria* holdfasts and on sponges under stones from L.W.N. downwards, P.StM. Ledges and Outer Harbour, Chapel Bay, Castletown Bay, Niarbyl, few. Abundant at Poyllvaaish. Offshore: 4–29 m., on algae, common.

C. acutifrons Latreille: on floating wreckage, buoys, and breakwater. In deep water on sponges and hydroids. Sometimes associated with tunicates (Harrison, 1944). I.O.M.: on red algae at L.W.S., P.E. Breakwater, common.

C. linearis (L.): on hydroids and floating wreckage, buoys, etc. Usually below low-water mark but intertidally in suitable localities. I.O.M.: intertidal, on red algae at L.W.S., P.E. Breakwater, few. Offshore: 11–37 m., on hydroids and algae, locally common.

C. fretensis Stebbing: probably on algae and hydroids in fairly shallow water. I.O.M.: intertidal, on red algae at L.W.S., P.E. Breakwater, one. Offshore: 11–18 m., on algae, one. Previously known only from the Channel and farther south.

Hyperiidea

HYPERIIDAE

Hyperia galba (Montagu): at or near the surface, usually commensal with Scyphomedusae. I.O.M.: commensal with *Rhizostoma*, common.

Themisto abyssorum (Boeck) = *Parathemisto oblivia* (Kröyer): pelagic usually in deep water, sometimes near surface. I.O.M.: 82 m., in young fish trawl, one.

DISCUSSION

Economic Importance

The most striking fact that emerges from intensive collecting of amphipods is that of their general abundance. On the shore species of *Marinogammarus* or *Bathyporeia*, on rocky or sandy facies respectively, are often present in large numbers and sometimes extremely plentiful. Near low-water mark a variety of species are very common among algae. It is, however, in moderate depths that the group reaches its maximum importance. In shallow water it is no uncommon occurrence to obtain several hundred individuals in a short haul.

Table I. The Distribution of the Offshore Species

(The figures opposite each species give the total number captured on each type of ground or habitat)

Type of ground or habitat ...	Algae	Fine sand (and algae)	Sand and gravel with algae	Stony gravel	Coarse sand	Coarse sand and broken shell	Hydroids	Muddy sand and broken shell	Muddy sand	Mud	Sponges
Number of hauls	10	12	11	4	3	12	11	17	6	3	2
Lysianassidae:											
Acidostoma obesum	—	—	—	—	—	—	—	—	1	—	—
Euonyx chelatus	—	—	—	—	—	—	1	—	—	—	—
Nannonyx goësi	—	—	—	—	—	—	1	—	—	—	—
Lysianassa plumosa	—	—	3	—	—	11	3	16	1	—	—
Perrierella audouiniana	—	—	—	—	—	—	2	—	—	—	23
Orchomene humilis	2	—	—	—	—	—	8	—	—	—	—
Socarnes erythrophthalmus	—	13	296	95	—	—	—	—	—	—	—
Hippomedon denticulatus	—	1	—	—	1	—	—	—	—	—	—
Scopelocheirus crenatus	—	—	—	—	—	1	1	3	6	1	—
Tryphosa sarsi	—	3	—	—	—	—	—	—	—	—	—
Tmetonyx similis	—	—	—	—	—	1	—	—	—	—	—
Orchomenella nana	4	—	—	—	—	51	—	6	—	—	—
Ampeliscidae:											
Ampelisca brevicornis	—	9	2	—	—	—	—	—	—	—	—
A. spinipes	—	—	2	1	—	6	1	18	21	—	—
A. diadema	—	—	2	—	—	7	1	31	18	—	—
A. typica	—	—	8	2	—	5	—	3	1	—	—
A. tenuicornis	—	—	—	1	—	7	—	31	30	1	—
Argissidae:											
Argissa hamatipes	—	—	1	—	—	—	—	1	—	—	—
Haustoriidae:											
Bathyporeia guilliamsoniana	—	51	5	—	—	—	—	—	—	—	—
B. elegans	—	336	52	—	—	—	—	—	—	—	—
B. gracilis	—	1	—	2	7	2	—	1	—	—	—
Urothoë elegans	—	11	60	—	—	4	—	5	4	—	—
U. marina	—	—	—	—	1	12	1	1	—	—	—
Phoxocephalidae:											
Paraphoxus oculatus	—	—	—	—	—	—	—	—	2	—	—
Metaphoxus fultoni	—	2	48	40	—	19	—	12	1	—	—
Harpinia antennaria	—	16	—	—	—	1	—	4	3	—	—
H. pectinata	—	—	—	—	—	3	—	—	19	1	—
H. crenulata	—	—	—	—	—	—	—	—	25	6	—
Amphilochidae:											
Amphilochus manudens	1	—	3	—	—	14	14	16	—	—	—
A. spence-batei	—	—	2	—	—	—	—	—	—	—	—
Amphilochoides serratipes	—	—	—	—	—	—	—	9	—	—	—
Peltocoxa brevirostris	—	—	—	—	—	15	1	17	—	—	—
Gitana sarsi	1	—	86	6	—	5	6	19	—	—	—
Leucothoidae:											
Leucothoë spinicarpa	—	—	—	—	—	—	3	—	—	—	4
L. lilljeborgi	—	—	2	—	—	—	—	2	1	1	—
Stenothoidae:											
Metopa bruzelii	—	—	—	—	—	17	2	7	—	—	—
M. pusilla	—	—	—	—	—	4	1	1	—	—	—
M. borealis	—	—	—	—	—	2	1	2	—	4	—
Stenothoë monoculoides	3	—	3	—	—	—	—	—	—	—	—
S. marina	3	3	3	—	—	—	10	—	1	—	—
S. antennulariae	—	—	—	—	—	4	18	1	—	—	—

Table I (*cont.*)

Type of ground or habitat...	Algae	Fine sand (and algae)	Sand and gravel with algae	Stony gravel	Coarse sand	Coarse sand and broken shell	Hydroids	Muddy sand and broken shell	Muddy sand	Mud	Sponges
Number of hauls	10	12	11	4	3	12	11	17	6	3	2
Cressidae:											
Cressa dubia	1	—	—	—	—	66	90	61	—	—	—
Colomastigidae:											
Colomastix pusilla	—	—	—	—	—	—	—	—	—	—	5
Acanthonotozomatidae:											
Panoploea minuta	5	—	7	—	—	2	25	1	—	—	—
P. eblanae	—	—	—	—	—	—	3	2	—	—	—
Iphimedia obesa	1	—	—	—	—	1	1	—	—	1	—
Lilljeborgiidae:											
Lilljeborgia brevicornis	—	—	—	—	—	—	4	—	—	—	—
L. kinahani	—	1	39	4	—	48	9	7	—	—	—
Oedicerotidae:											
Perioculodes longimanus	1	40	22	—	—	—	—	—	9	7	—
Pontocrates arenarius	—	26	—	—	—	—	—	—	—	—	—
P. norvegicus	2	103	14	—	—	—	—	—	—	—	—
Synchelidium haplocheles	—	10	28	3	1	16	2	25	—	—	—
Monoculodes carinatus	—	—	4	—	—	4	—	6	—	—	—
M. subnudus	—	1	2	—	—	1	—	7	—	—	—
Westwoodilla caecula	—	—	—	—	—	—	—	—	—	53	—
Tironidae:											
Syrrhoites fimbriatus	—	—	5	—	—	5	—	1	—	—	—
Calliopiidae:											
Apherusa bispinosa	31	30	263	2	—	—	2	4	3	—	—
A. jurinei	—	3	2	—	—	—	—	1	—	—	—
Pleustidae:											
Parapleustes monocuspis	—	—	—	—	—	—	10	—	—	—	—
Stenopleustes nodifer	—	—	—	—	—	11	4	7	—	—	—
Paramphithoidae:											
Epimeria cornigera	—	—	—	—	—	2	—	—	—	1	—
Atylidae:											
Nototropis swammerdami	56	4	—	—	—	—	—	—	—	—	—
N. vedlomensis	—	2	9	12	—	19	—	30	1	—	—
Melphidippidae:											
Melphidippella macra	—	1	3	—	—	16	3	36	3	—	—
Eusiridae:											
Eusirus longipes	—	—	1	—	—	16	4	6	1	—	—
Gammaridae:											
Gammarellus homari	—	1	1	—	—	—	—	—	—	—	—
Cheirocratus sundevalli	—	—	13	1	—	10	—	11	1	—	—
C. assimilis	—	—	—	—	—	5	—	2	—	—	—
Megaluropus agilis	—	94	12	—	—	—	—	—	—	—	—
Melita hergensis	1	2	2	—	—	—	—	—	—	—	—
M. obtusata	1	—	2	—	—	—	—	1	—	—	—
Ceradocus semiserratus	2	—	200	24	—	13	1	2	—	—	—
Maera othonis	1	2	13	3	—	58	17	38	2	—	—
Maerella tenuimana	—	—	—	—	4	4	—	—	—	—	—
Gammarus locusta	1	8	8	—	—	—	—	—	—	—	—
Marinogammarus obtusatus	—	—	1	—	—	—	—	—	—	—	—

Table I (*cont.*)

Type of ground or habitat ...	Algae	Fine sand (and algae)	Sand and gravel with algae	Stony gravel	Coarse sand	Coarse sand and broken shell	Hydroids	Muddy sand and broken shell	Muddy sand	Mud	Sponges
Number of hauls	10	12	11	4	3	12	11	17	6	3	2
Dexaminidae:											
Dexamine spinosa	52	2	53	—	—	—	—	—	—	—	—
D. thea	—	1	3	—	—	—	—	—	—	—	—
Tritaeta gibbosa	—	—	1	—	—	—	—	—	—	—	1
Guernea coalita	—	—	6	—	3	7	—	9	—	—	—
Talitridae:											
Hyale prevosti	1	—	—	—	—	—	—	—	—	—	—
Aoridae:											
Aora typica	39	—	13	—	—	1	30	1	—	—	—
Lembos websteri	22	3	2	—	—	—	—	—	—	—	—
L. longipes	—	—	—	—	17	4	—	—	16	—	—
Photidae:											
Microprotopus maculatus	70	60	21	—	—	—	—	—	—	—	—
Photis longicaudata	—	—	—	—	—	8	2	11	—	—	—
Eurystheus melanops	—	—	1	—	—	—	—	7	—	—	—
E. erythrophthalmus	14	2	3	—	—	15	85	15	1	—	—
E. palmatus	—	—	—	—	—	1	6	8	3	—	—
Podoceropsis nitida	—	—	—	—	—	1	15	—	—	—	—
Megamphopus cornutus	1	—	22	4	—	27	3	41	1	—	—
Leptocheirus hirsutimanus	—	—	—	—	—	1	—	1	—	—	—
L. pectinatus	1	—	109	79	—	3	—	19	2	—	—
Amphithoidae:											
Amphithoë rubricata	—	—	2	—	—	—	—	—	—	—	—
Pleonexes gammaroides	—	—	1	—	—	—	—	—	—	—	—
Sunamphithoë pelagica	—	1	—	—	—	—	—	—	—	—	—
Jassidae:											
Jassa falcata	4	5	8	—	—	—	—	—	—	—	—
J. pusilla	—	—	—	—	—	1	3	6	—	—	—
Microjassa cumbrensis	1	—	7	—	—	13	8	21	—	—	—
Corophiidae:											
Ericthonius brasiliensis	15	—	15	—	—	—	13	1	—	—	—
Unciola planipes	—	—	—	—	2	—	—	—	—	—	—
Siphonoecetes dellavallei	4	63	35	—	—	—	—	—	—	—	—
Corophium affine	—	—	—	—	—	—	—	—	53	1	—
C. bonelli	2	—	—	—	—	—	—	2	—	—	—
Podoceridae:											
Dulichia porrecta	—	—	—	—	—	—	—	1	—	—	—
Caprellidae:											
Phtisica marina	28	4	114	3	—	10	69	11	16	—	—
Pseudoprotella phasma	40	—	12	—	—	—	2	—	—	—	—
Podalirius typicus	1	35	9	—	—	—	—	1	—	—	—
Parvipalpus capillaceus	—	—	—	—	—	—	—	1	—	—	—
Caprella acanthifera	41	9	9	2	—	—	—	1	—	—	—
C. linearis	2	2	—	—	—	—	61	—	—	—	—
C. fretensis	2	—	—	—	—	—	—	—	—	—	—

The number of species obtained is often great, and in haul no. 70, taken with a tow-net attached to the otter trawl, there were over 325 individuals belonging to thirty-three separate species; while on several occasions a haul of the small fine-meshed dredge, which was towed only a few yards, brought up over 100 individuals belonging to more than twenty species. As a rule two or three species are predominant and make up the greater part of the catch, but the majority are widely distributed on the kind of bottom that they favour. I have found the Amphipoda to be a dominant group, in numbers if not in bulk, on all types of offshore ground that I have investigated. It is therefore possible that they are of even greater economic importance than has been realized. They have been shown to be an important source of food for fish, especially young fish, and they are probably largely eaten by other animals upon which fish feed (see Hunt, 1925, and Steven, 1930).

Habitats of Offshore Species

I have compiled lists of the species which I consider to be significant for various habitats. These lists are obtained by finding the average number of individuals per haul of each species on the different grounds. They are not of equal value for each habitat, as the number of hauls taken varied from only three on a mud bottom to seventeen on a bottom of muddy sand with broken shell. The hauls also varied in distance covered and were taken with a variety of gear. The lists do not, therefore, provide any information as to the absolute density of population but they are an attempt at classification into habitat groups with an observed numerical basis, however imperfect.

In assessing the significance of any species on a particular ground I have included it where it has an average value per haul of 0·8 or more. This is an arbitrary figure which may be considered low, but the area covered by any one haul is usually small and when the species may be present in an average of eight hauls out of ten the total population on the ground is probably large. The average occurrence in hauls is not necessarily related to the average number of individuals per haul as the latter is sometimes brought up by large numbers in one or two hauls only, but on the whole the spreadover is fairly even and I have not thought it practical to discriminate between these two values. Habitat (1) consisted of weed, often lying loose on the bottom, mainly brought up in the otter trawl. Habitat (2) was to some extent mixed, as a certain amount of loose weed was present. Habitat (3) included parts of (1), (2) and (4). Habitat (7) consisted of hydroids and polyzoans usually attached to shells and stones on mixed grounds. Where the habitat is a combination of two or more of the others listed, that to which each species should properly be referred is indicated by the number set in heavier type following the name of the species. After the name of the species are also indicated (in brackets) the other habitats for which it was significant.

Species which are probably significant for Various Habitats

(1) Weed, including loose weed, on fine sand. Depth 4–22 m.

Apherusa bispinosa
Nototropis swammerdami
Dexamine spinosa
Aora typica (7)
Lembos websteri
Microprotopus maculatus (2)
Eurystheus erythrophthalmus (6, 7, 8)
Ericthonius brasiliensis (7)
Phtisica marina (6, 7, 9)
Pseudoprotella phasma
Caprella acanthifera

(2) Fine sand (with some loose weed). Depth 2–12 m.

Socarnes erythrophthalmus (4)
Bathyporeia guilliamsoniana
B. elegans
Urothoë elegans (9)
Stenothoë marina (1, 7)
Perioculodes longimanus (9, 10)
Pontocrates arenarius
Pontocrates norvegicus
Synchelidium haplocheles (6, 8)
Apherusa bispinosa (1)
Megaluropus agilis
Microprotopus maculatus (1)
Siphonoecetes dellavallei
Podalirius typicus

(3) Sand and fine gravel (with some weed). Depth 11–18 m.

Socarnes erythrophthalmus (2, 4)
Bathyporeia elegans (2)
Urothoë elegans (2)
Metaphoxus fultoni (4)
Gitana sarsi (4)
Lilljeborgia kinahani (4)
Perioculodes longimanus (2)
Pontocrates norvegicus (2)
Synchelidium haplocheles (2)
Apherusa bispinosa (1)
Nototropis vedlomensis (4)
Cheirocratus sundevalli (6)
Megaluropus agilis (2)
Ceradocus semiserratus (4)
Maera othonis (6, 7, 8)
Dexamine spinosa (1)
Aora typica (1)
Microprotopus maculatus (1)
Megamphopus cornutus (4)
Leptocheirus pectinatus (4)
Ericthonius brasiliensis (1)
Siphonoecetes dellavallei (2)
Phtisica marina (1)
Pseudoprotella phasma (1)
Podalirius typicus (2)
Caprella acanthifera (1)

(4) Stony gravel. Depth 18–35 m.

Socarnes erythrophthalmus (2)
Metaphoxus fultoni (6)
Gitana sarsi (8)
Lilljeborgia kinahani (6, 7)
Nototropis vedlomensis (6, 8)
Ceradocus semiserratus (6)
Megamphopus cornutus (6, 8)
Leptocheirus pectinatus (8)

(5) Coarse sand. Depth 18–35 m.

Bathyporeia gracilis
Maerella tenuimana
Guernea coalita
Lembos longipes (9)

(6) Coarse sand with broken shell. Depth 18–55 m.

Lysianassa plumosa (8)
Orchomenella nana
Urothoë marina
Metaphoxus fultoni (4)
Amphilochus manudens (7, 8)
Peltocoxa brevirostris (8)
Metopa bruzelii
Cressa dubia (7, 8)
Lilljeborgia kinahani (4, 7)
Synchelidium haplocheles (2, 8)

Stenopleustes nodifer
Nototropis vedlomensis (4, 8)
Melphidippella macra (8)
Eusirus longipes
Cheirocratus sundevalli (3)
Maera othonis (3, 7, 8)
Eurystheus erythrophthalmus (1, 7)
Megamphopus cornutus (4, 8)
Microjassa cumbrensis (8)
Phtisica marina (1, 7, 8)

(7) Hydroids and polyzoans, mainly on mixed grounds. Depth 20–82 m.

Amphilochus manudens (6, 8)
Stenothoë marina
S. antennulariae
Cressa dubia (6, 8)
Panoploea minuta
Lilljeborgia kinahani (4, 6)
Parapleustes monocuspis
Maera othonis (3, 6, 8)
Aora typica (1)
Eurystheus erythrophthalmus (1, 6)
Ericthonius brasiliensis (1)
Phtisica marina (1, 6, 8)
Caprella linearis

(8) Muddy sand with broken shell. Depth 27–38 m.

Lysianassa plumosa (6)
Ampelisca spinipes (9)
A. diadema (9)
A. tenuicornis (9)
Amphilochus manudens (6, 7)
Peltocoxa brevirostris (6)
Gitana sarsi (4)
Cressa dubia (6, 7)
Synchelidium haplocheles (2, 6)
Nototropis vedlomensis (4, 6)
Melphidippella macra (6)
Maera othonis (3, 6, 7)
Eurystheus erythrophthalmus (1, 6, 7)
Megamphopus cornutus (4, 6)
Leptocheirus pectinatus (4)
Microjassa cumbrensis (6)

(9) Muddy sand. Depth 31–82 m.

Scopelocheirus crenatus
Ampelisca spinipes (8)
A. diadema (8)
A. tenuicornis (8)
Urothoë elegans (2)
Harpinia pectinata
Harpinia crenulata (10)
Perioculodes longimanus (2, 9)
Lembos longipes (6)
Corophium affine
Phtisica marina (1, 6, 8)

(10) Mud. Depth 82–88 m.

Harpinia crenulata (9)
Metopa borealis
Perioculodes longimanus (2, 9)
Westwoodilla caecula

(11) Sponges, growing on a bed of *Modiolus*. Depth 60 m.

Perrierella audouiniana
Leucothoë spinicarpa
Colomastix pusilla
Tritaeta gibbosa

(12) Specialized habitats

Laphystius sturionis, parasitic on fish
Melita obtusata, on *Asterias* and *Luidia*
Podoceropsis nitida, in hermit crab shells
Jassa pusilla, in sponges on *Inachus*
Chelura terebrans, in submerged wood

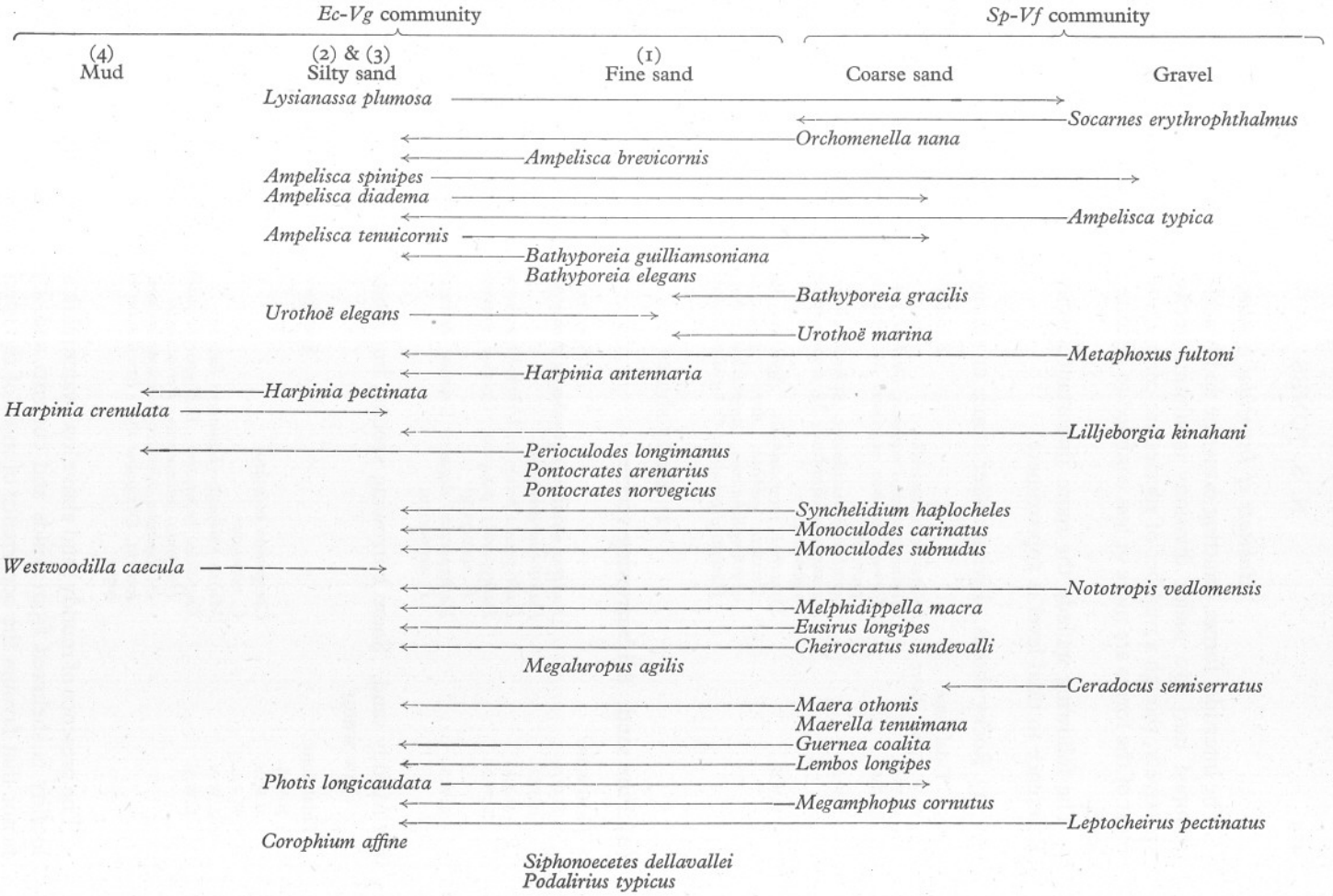
The habitats shown include all those that have been found offshore in the area. It will be seen that many species are common to two or more habitats or grounds, while some are confined to one, but where a species occurs on several grounds these have as a rule some common feature. Thus stony gravel and mixed grounds may be grouped together as containing coarse deposits. Some species which occur on algae also occur on hydroids. On the whole, in fact, the Amphipoda are quite selective with regard to the type of ground on which they live.

Certain closely related species occupy very different habitats in the same area. *Leucothoë spinicarpa* is associated, probably as a commensal, with sponges and ascidians, while *L. lilljeborgi* burrows in silty ground; *Nototropis swammerdami* lives on sublittoral algae while *N. vedlomensis* is found in mixed grounds and gravels; *Lembos websteri* occurs on algae mainly at low water while *L. longipes* probably burrows in various grades of sand in at least some metres depth. Differences in habitat of this description are not always so clear but in other genera a trend from one grade of deposit to another can be seen over a series of species as in the example of *Ampelisca* and *Harpinia* set out below. A similar trend may be observed among the closely related genera of the Oedicerotidae.

From the information so far obtained as to the distribution of the Amphipoda on various grounds some idea may be gained of the place of a number of species in the general schema of animal communities in the sea. In this matter I shall refer to the system used by Ford (1923). He divided the infaunal communities on the sea-bottom near Plymouth into two main series, the *Echinocardium cordatum-Venus gallina* (*Ec-Vg*) community characteristic of finer soils, and the *Spatangus purpureus-Venus fasciata* (*Sp-Vf*) community characteristic of coarser grades. The *Ec-Vg* series was divided into four subgroups, (1) being characteristic of clean sand, (2) and (3) of silty sand, and (4) of black mud. The communities in the neighbourhood of Port Erin fall within the limits of this system.

In the table below (Table II) certain species of Amphipoda from the area are referred to this system. As there is no rigid boundary between one community and another it is usually only possible to indicate a preference for one grade of deposit. An arrow-head opposite a specific name shows a tendency for that species to be found in mixed communities corresponding to a mixture of coarse and fine grades of deposit, or to occur in more than one grade. No species by itself could be used to diagnose the community in which it occurred, but a number of species together would provide a fairly sound indication of the other animals to be expected on the same ground.

TABLE II. HABITAT DISTRIBUTION OF OFFSHORE AMPHIPODA



Habitats of Intertidal Species

The intertidal forms, omitting estuarine and brackish-water species, may be grouped into two major divisions inhabiting rocky and sandy shores respectively, though a number of species are common to both. In each division most of the forms are more or less confined to definite zones in relation to tidal level.

The following include the more important species occurring in the area. Reference to tidal level is approximate.

(1) Rocky shores. Living under stones or among algae and in pools

Tidal zone	Species
H.W.S. and above	*Orchestia gammarella*
H.W.N.	*Marinogammarus marinus, Orchestia mediterranea, Hyale prevosti*
M.T.L.	*Marinogammarus marinus, M. obtusatus, M. finmarchicus, Hyale prevosti*
L.W.N	*Stenothoë monoculoides, Apherusa jurinei, Melita palmata, Marinogammarus obtusatus, M. finmarchicus, Hyale prevosti, Amphithoë rubricata*
L.W.S.	*Stenothoë monoculoides, Apherusa jurinei, Gammarellus homari, Tritaeta gibbosa, Aora typica, Lembos websteri, Eurystheus erythrophthalmus, Amphithoë rubricata, Jassa falcata, J. ocia, Parajassa pelagica, Corophium bonelli, Caprella acanthifera*

(2) Sandy shores

(*a*) Fine sand. All burrowing species

Tidal zone	Species
H.W.S.	*Talitrus saltator, Talorchestia deshayesei*
H.W.N.	*Haustorius arenarius*
M.T.L.	*Bathyporeia pelagica, Urothoë brevicornis, Pontocrates arenarius*
L.W.N.	*Bathyporeia pelagica, Urothoë brevicornis, Pontocrates arenarius, P. norvegicus*
L.W.S.	*Bathyporeia elegans, Urothoë brevicornis, Pontocrates arenarius, P. norvegicus*

(*b*) Muddy sand. Some burrowing species, but many found among algae at low water

Tidal zone	Species
H.W.S.	—
H.W.N.	*Corophium volutator*
M.T.L.	*C. volutator*
L.W.N.	*Melita palmata, Gammarus locusta*
L.W.S.	*Ampelisca brevicornis, Bathyporeia guilliamsoniana, Urothoë elegans, Harpinia antennaria, Perioculodes longimanus, Apherusa cirrus, A. bispinosa, Nototropis swammerdami, Melita hergensis, Gammarus locusta, Dexamine thea, Ericthonius brasiliensis, Corophium crassicorne*

The presence of muddy sand above low-water mark always indicates that the locality is sheltered from winds and currents and it is probably the shelter factor that favours the occurrence of most of the species in list (2*b*).

List (2*a*) was mainly compiled from collections made on the fine sand of Port Erin Bay. It may be compared with the data given for Kames Bay, Millport, by Watkin (1941), which may be set out as follows:

Tidal zone	Species
H.W.S.	—
H.W.N.	*Bathyporeia pilosa, Haustorius arenarius*
M.T.L.	*Bathyporeia pelagica, Urothoë brevicornis, Pontocrates norvegicus*
L.W.N.	*Bathyporeia pelagica, B. elegans, Urothoë brevicornis, Pontocrates arenarius, P. norvegicus*
L.W.S.	*Bathyporeia guilliamsoniana, B. elegans, Urothoë brevicornis, Pontocrates arenarius, P. norvegicus*

As Watkin was not concerned with the Talitridae it is evident that on the whole the two beaches show a similar amphipod fauna and zonation. The most striking difference is that *Bathyporeia pilosa* is present abundantly at Kames Bay but is absent from Port Erin Bay. This may be due to the absence of suitable ground about H.W.N. at Port Erin. The species probably occurs on the sandy shores at the north end of the Isle of Man, since it has been recorded from Millport and from the west coast of Wales. The density of population is generally lower at Port Erin than at Millport.

Colman (1940) investigated the faunas of intertidal seaweeds including *Laminaria digitata* holdfasts. It is interesting to compare the results for Plymouth and the Isle of Man for the latter habitat.

AMPHIPODA FROM *LAMINARIA* HOLDFASTS

Plymouth	Isle of Man
Stenothoë monoculoides	*Stenothoë monoculoides*
Apherusa jurinei	*Apherusa jurinei*
Tritaeta gibbosa	*Tritaeta gibbosa*
Lembos websteri	*Lembos websteri*
Eurystheus erythrophthalmus	*Eurystheus erythrophthalmus*
Amphithoë rubricata	*Amphithoë rubricata*
Jassa falcata	*Jassa falcata*
Caprella acanthifera	*Caprella acanthifera*
Leucothoë incisa	*Nannonyx goësi*
Elasmopus rapax	*Orchomene humilis*
Microdeutopus damnoniensis	*Gitana sarsi*
M. chelifer	*Leucothoë spinicarpa*
Microjassa cumbrensis	*Lilljeborgia brevicornis*
Podocerus variegatus	*Apherusa bispinosa*
Caprella linearis	*Dexamine thea*
	Hyale prevosti
	Aora typica
	Pleonexes gammaroides
	Jassa ocia
	Parajassa pelagica
	Ericthonius brasiliensis
	Corophium bonelli

Allowing for the fact that the investigations at Port Erin were more extensive and that a number of species were collected once only the two lists are very similar. Eight out of fifteen species recorded at Plymouth occur in the same habitat at Port Erin, and of the remaining seven *Leucothoë incisa*, *Elasmopus rapax*, *Microdeutopus chelifer* and *Podocerus variegatus* have not been recorded from the Isle of Man. It is evident that this habitat is an important one, as the population of the holdfasts is often very large. According to Colman there may be as many as 175 amphipods per 100 g. of damp weed. I have several times obtained 300 or more individuals from six medium-sized holdfasts of a total weight of about 300 g.

Goodhart & Harrison (1940) gave a list of six amphipods found on the shore above L.W.S. at Oldany Harbour, Sutherland. These were *Ampelisca spinimana*, *Harpinia antennaria*, *Leucothoë spinicarpa*, *Coremapus versiculatus*, *Phtisica marina* and *Caprella acanthifera*. With two exceptions they had not been recorded elsewhere between tide-marks. It was suggested as a possible explanation of this distribution that these species occurred above low-water mark only on coasts washed by the North Atlantic Drift.

At Port Erin four of these species occur and they have all been found above low-water mark, *Caprella acanthifera* sometimes abundantly. *Ampelisca spinimana* and *Coremapus versiculatus* have not been recorded from the area. The water near the coasts of the Isle of Man is considerably less saline than unaltered Atlantic water. There is a gradual diminution in surface salinity from St George's Channel towards the Lancashire coast, indicating a drift from south to north through the Irish Sea. The mean value near Port Erin is 34·1‰ (Proudman, 1946). There is probably no current from the North Channel reaching the Isle of Man. In view of these facts I do not think that the hypothesis of Goodhart and Harrison can be maintained.

It is unlikely that temperature or the time of occurrence of spring tides are controlling factors, since spring tides occur during the hottest part of the day in the north of Scotland (Reid, 1935).

It is more likely that the intertidal distribution of some amphipods is regulated by the amount of shelter available. All the records of the four species mentioned above in the Isle of Man were from locations affording a good deal of shelter from heavy seas, and which consequently contained a large amount of silt, and it is probably this last factor that the amphipods find favourable. In this connexion it is interesting to note that in Port St Mary Outer Harbour, which is almost completely protected from wave action, the sand at L.W.S. is rather muddy and contains among other species *Urothoë elegans*, *Harpinia antennaria*, *Perioculodes longimanus* and *Podalirius typicus*, none of which is frequently found above low-water mark.

It is possible that the scarcity of intertidal records from other districts is merely due to the comparative lack of intensive collecting.

Brackish-Water and Estuarine Habitats

It will be convenient to describe three locations in the area in which the distribution of the amphipods is affected by variations in salinity.

Spaldrick

This location is situated at the north side of Port Erin Bay. It consists of a rocky beach with a small fresh-water stream emptying on to the beach over a fall just above H.W.S., and running into the sea through stones and boulders covered with *Fucus* and *Ascophyllum*. In the summer the stream is often reduced to a mere trickle. The animals living in the stream are therefore subjected to considerable variations in salinity, especially during spring tides, from almost fresh water at low tide when the stream is in flood to almost full salinity at high tide when the stream is low. In winter the salinity under the fall reaches about 17 $^{0}/_{00}$ at H.W.S. The whole tidal zone will be subjected to these extremes for varying periods, the inhabitants of the topmost zone living in fresh water for most of the time and those of the lowest zone in sea water except for short periods at L.W.S. The length of the beach from high- to low-water mark is about 70 yards.

Brewery Beach

This location is situated just east of Gansey. A fresh-water stream enters the shore through a tunnel just above H.W.S. and runs over rock with scattered stones to below H.W.N., the remainder of its course over the shore being over fine sand with a number of flat stones down to L.W.N. The amphipods occur under the stones. The stream is rather larger than that at Spaldrick. The distance from high- to low-water mark is about 120 yards. Fig. 1 shows the relative distributions of the amphipod species found in these two streams.

Salinity conditions are approximately the same at both locations.

It will be seen that the zonation of species common to both streams is generally similar. At Spaldrick the presence of *Marinogammarus obtusatus* and *M. finmarchicus* may be regarded as accidental since both species are much more abundant outside the limits of the stream. They are evidently able to tolerate almost fresh water for some hours at a time. *Hyale prevosti* and *Melita palmata* are also more abundant away from the stream at both locations. *Marinogammarus marinus* is abundant both in and away from the streams, but extends to a lower level on the shore where there is fresh-water influence. The remaining species are confined to the area of the streams. At both places the brackish-water type of *Gammarus duebeni* occurs from about H.W.S. to H.W.N. At Spaldrick this species also occurs for some distance up the stream to a height of at least about 100 ft. above sea-level. Since the stream enters the shore over a fall there is a distinct break in the distribution of *G. duebeni*, and presumably the animals found above the fall belong to the fresh-water type.

The species is apparently absent above the shore at Brewery Beach since a number of collections in the stream failed to produce any. I am unable to give any reason for its absence from the upper reaches.

Marinogammarus pirloti occupies much the same zone as *M. marinus* at Brewery Beach but is absent from Spaldrick, and *Gammarus zaddachi salinus* occurs from about M.T.L. to L.W.N. at Brewery Beach but not at Spaldrick. Since the salinity conditions are much the same in both streams some other factor must control their distribution and the most obvious one is the nature of

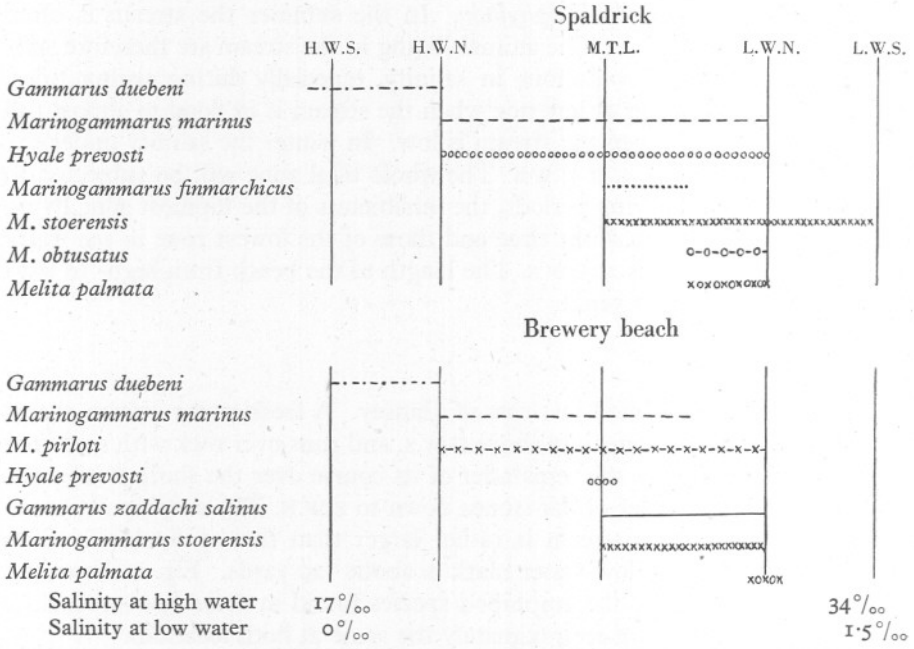


Fig. 1. The relative distributions of various amphipods in the streams at Spaldrick and Brewery Beach. The vertical lines indicate the tidal level. Limits for each species are only approximate.

the ground over which the streams flow. At Brewery Beach the stream flows over sand from just below H.W.N. to low-water mark, while at Spaldrick the whole course is over rocks and through boulders. It is possible that *Marinogammarus pirloti* and *Gammarus zaddachi* can establish themselves only where fine deposits are present.

The Silverburn

This is the only stream in the area of this survey with a distinct estuary. It is a small river which enters the sea through Castletown Harbour. At L.W.S. the whole of the estuary is emptied of sea water. The length of the tidal zone is about $\frac{1}{2}$ mile but the upper 400 yards of this zone is tidal only when high tide at

Liverpool reaches 28 ft. or more above datum, that is during H.W.S. This effect is due to the stream passing over a weir above which neap tides do not reach. The amphipod fauna lives among algae and fresh-water vegetation and under stones on the bottom of the stream and on the banks. Those animals which live in the stream below the weir will be subjected to considerable salinity changes twice daily, the range depending on the distance from the

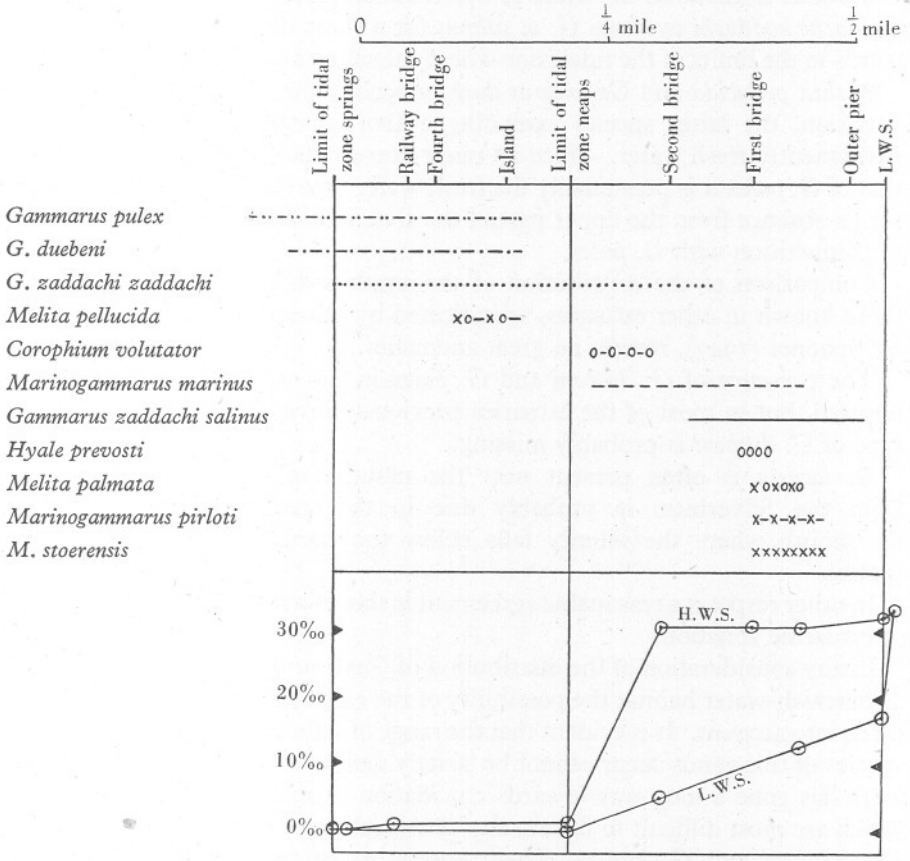


Fig. 2. The relative distribution of the amphipods in the Silverburn. The salinity diagram is based on samples taken near the bottom with a reversing water-bottle.

harbour mouth. Individuals living on the banks above the stream will not experience the lower limits of the salinity range. Above the weir the animals will remain in fresh water for long periods but will at intervals experience a slight rise in salinity. The distribution of the amphipods in the Silverburn is indicated in Fig. 2.

The species which inhabit that part of the estuary which is subjected to a wide range in salinity, i.e. the region below the upper limit of neap tides,

occupy zones comparable to those which they inhabit on the shore. *Marinogammarus marinus*, *M. stoerensis*, *M. pirloti*, *Hyale prevosti* and *Melita palmata* are confined to this region. Their distribution may be compared with that shown in Fig. 1.

Gammarus zaddachi salinus is confined to the region in which the salinity at high water rises to 33‰ at the bottom, and in this respect experiences similar conditions to those on the shore at Spaldrick and Brewery Beach. *Gammarus zaddachi zaddachi* overlaps *G. z. salinus* for a short distance but extends upwards to the limits of the tidal zone where the salinity does not rise above 1‰.

Melita pellucida and *Gammarus duebeni* occupy the region of small salinity variation, the latter species extending a short distance into the region of permanently fresh water. Since its range corresponds with the lower part of that of *G. pulex* it is presumably the fresh-water type (Reid, 1939). The reason for its absence from the upper part of the river is unknown but it may be due to competition with *G. pulex*.

Comparison of the distribution of the amphipods in the Silverburn with those known in other estuaries, as indicated by Bassindale (1942) and revised by Spooner (1947), reveals no great anomalies.

The presence of *G. duebeni* and *G. pulex* in the same zone appears to be unusual, but in most of the estuaries previously investigated the fresh-water type of *G. duebeni* is probably missing.

G. locusta is often present near the mouths of estuaries. Its absence from the Silverburn is probably due to the steep salinity gradient at the mouth where the salinity falls below the normal lower limit for the species.

In other respects a reasonable agreement is shown with previous descriptions of estuarine zonation.

In any consideration of the distribution of *Gammarus* species in an estuarine or brackish-water habitat the possibility of the existence of local races must be taken into account. It is evident that the range of salinities in which the various species of this genus occur cannot be strictly defined at present, though recent work has gone a long way towards elucidation of this problem. The species which are most difficult to fit into any classification of habitats are *G. duebeni*, *G. zaddachi* and *G. locusta*. There appear to be two types of *G. duebeni* adapted respectively to brackish and fresh water, but they cannot be separated by any morphological criterion (Reid, 1939). *G. zaddachi*, on the other hand, may be grouped according to external characters into three subspecies (Spooner, 1947, and Segerstråle, 1947). *G. locusta* is a marine species and is usually intolerant of salinities below about 28‰ but in some locations, as in the Baltic (Segerstråle, 1946), it lives in greatly reduced salinities.

It seems probable that the genus *Gammarus* is in a state of active evolution. There is evidently more than one form of each of the three species mentioned and even where no alteration in external characters has taken place there

appear to be physiological races. Huxley (1942, p. 279) suggested that the explanation of the occurrence of *G. duebeni* in the fresh waters of Ireland lies in its adoption of a peculiar habitat in atypical conditions at the margin of its range, an occurrence that has been observed in other animals. Salinity is the most obvious factor controlling the evolution of races in the genus and it is not unlikely that in regions of very gradual salinity change forms have evolved which are adapted to different conditions from those in which the parent species is found. These forms may then be able to colonize new areas and, in the absence of interspecific competition, to establish themselves. The presence of *G. duebeni* in the fresh waters of Ireland and its virtual absence from those of England may be due to the fact that *G. pulex* is a more successful competitor but has never become established in Ireland.

The manner in which an amphipod adapted to brackish water extends its range is unknown. There is no possibility of transport of the eggs since they are always carried by the female until hatched. Some species can tolerate high salinities for long periods and can even be bred in sea water in the laboratory (Sexton, 1928), and it is possible that they may occasionally be carried from one estuary to another by way of the sea; however, there are few authentic records of the capture of brackish-water species under fully marine conditions. The most probable method of transport from one brackish-water situation to another is on the bodies of birds as suggested by Segerstråle (1946).

Summary

A survey was made during 1946 covering the Amphipoda of the south end of the Isle of Man from an ecological aspect. The object was to revise the records of the area and to obtain additional information about the habitats of the species found.

The area in which the survey took place was within a radius of 10 miles from Port Erin.

Various sorts of gear were used for collection offshore and it is concluded that the fine-meshed dredge is the most useful instrument for collecting amphipods.

A systematic treatment of the species found includes a summary of records regarding habitat for each species.

It is suggested that the Amphipoda may be of greater economic importance than has previously been realized.

A discussion of offshore, intertidal, and brackish-water habitats includes lists of the more important species obtained grouped according to type of ground and zonation with regard to tidal level and salinity. The importance of offshore species is assessed on a basis of the average number of individuals collected per haul.

Comparison of the zonation of littoral and estuarine species with that known from other areas reveals a general agreement.

The existence of more than one form of several species of the genus *Gammarus* is discussed and it is suggested that they are in an active state of evolution.

I am indebted to Prof. J. H. Orton for encouragement and discussion, and to Mr G. M. Spooner for allowing me to read a proof of his recent paper.

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APPENDIX I

Additions to the Fauna of the Isle of Man

The following species are here recorded for the first time from the area:

Acidostoma obesum (Bate)
Nannonyx spinimanus Walker
Tryphosa sarsi Bonnier
Tryphosites longipes (Bate & Westwood)
Ampelisca diadema (Costa)
Bathyporeia gracilis G. O. Sars
Urothoë elegans Bate
Harpinia pectinata G. O. Sars
Amphilochus spence-batei Stebbing
Metopa borealis G. O. Sars
M. pusilla G. O. Sars
Laphystius sturionis Kröyer
Monoculodes subnudus Norman
Calliopius crenulatus Chevreux & Fage
Parapleustes monocuspis (G. O. Sars)
Epimeria cornigera (J. C. Fabricius)
Cheirocratus intermedius G. O. Sars
Melita hergensis Reid
Gammarus pulex (L.)
Marinogammarus finmarchicus (Dahl)
M. stoerensis (Reid)
Pectenogammarus planicrurus Reid
Microdeutopus damnoniensis Bate
Eurystheus melanops (G. O. Sars)
Protomedeia fasciata Kröyer
Isaea montagui Milne-Edwards
Sunamphithoë pelagica (Milne-Edwards)
Biancolina cuniculus Stebbing
Unciola planipes Norman
Corophium crassicorne Bruzelius
C. affine Bruzelius
Parvipalpus capillaceus (Chevreux)
Caprella acutifrons Latreille
C. fretensis Stebbing
Themisto abyssorum (Boeck)

APPENDIX II

Other Records and Corrections

The following species have been recorded from the area previously but were not collected during the course of this survey:

Normanion quadrimanus (Bate & Westwood)
Harpinia laevis G. O. Sars
Peltocoxa marioni Catta
Parapleustes assimilis (G. O. Sars)
Calliopius laeviusculus Kröyer
Ericthonius difformis Milne-Edwards
Siphonoecetes colleti Boeck

The following species have been recorded incorrectly from the area (Marine Fauna of the Isle of Man, 1937, and additions in *Annual Reports*, Marine Biological Station, Port Erin):

Amphilochoides odontonyx Boeck. Walker's original record was named in accordance with Sars (1895), and should have been referred to *A. serratipes* (Norman).

Apherusa ovalipes Norman & Scott. Examination of the specimens in the collection referred to this species shows that they are all *A. bispinosa* (Bate).

Gammarus campylops Leach. These were probably immature specimens of *G. locusta* and possibly of *G. zaddachi*. *G. campylops* is now a doubtful species (Sexton & Spooner, 1940).

Leptocheirus pilosus Zaddach. Walker's original record refers to *L. pectinatus* Norman.

Jassa dentex (Czerniavski). The forms referred to this species should now be included under *J. falcata* (Montagu).

APPENDIX III

Record of Hauls taken Offshore

Serial no.	Date	Position	Type of bottom or contents of haul	No. of species	No. of individuals	Depth (m.)	Gear used
1	29. iii. 46	¾ mile W. of Bradda Head	Coarse sand	1	1	27	Naturalist's dredge
2	8. iv. 46	¾ mile N. of Kitterland	Algae on sand and broken shell	6	13	22	N.D.
4	10. iv. 46	½ mile N. of Fleshwick	Stony gravel	7	119	15	Small fine-meshed dredge
5	12. iv. 46	1 mile N. of Kitterland	Coarse sand and broken shell	5	36	27	N.D.
6	12. iv. 46	Inside Port Erin Bay	Fine sand with algae	8	22	6–9	Small F.-M.D.
7	16. iv. 46	¾ mile N.W. of Bay Fine	Hydroids on broken shell	8	71	27	N.D.
8	17. iv. 46	1 mile N. of Kitterland	Coarse sand and broken shell	7	61	27	N.D.
9	24. iv. 46	Mouth of Port Erin Bay	Fine sand with algae	7	24	9–13	D-net
10	24. iv. 46	Inside Port Erin Bay	Fine sand with weed	12	36	4–9	D-net
11	25. iv. 46	½ mile W.S.W. of Bradda	Coarse sand and broken shell	7	14	29	N.D.
12	26. iv. 46	1 mile W.N.W. of Bradda	Muddy sand and broken shell	6	9	29	D-net
13	26. iv. 46	Inside Port Erin Bay	Algae on sand	10	34	4–9	D-net
14	1. v. 46	1½ miles W.N.W. of Bradda	Muddy sand and broken shell	8	14	33	N.D.
16	3. v. 46	3 miles W.N.W. of Charran	Muddy sand and broken shell	6	21	33	Small F.-M.D.
17	3. v. 46	Mouth of Port Erin Bay	Fine sand with algae	10	27	9–13	D-net
18	8. v. 46	2 miles W.N.W. of Charran	Muddy sand and broken shell	18	46	31	Small F.-M.D.
19	10. v. 46	½ mile N.W. of Fleshwick	Fine sand with weed	5	5	11–15	Small F.-M.D.
20	10. v. 46	Off Raglan Pier, Port Erin Bay	Fine sand with weed	12	158	2–4	Small F.-M.D.
21	13. v. 46	50 yards off Bay Fine	Sand and stony gravel	15	170	11–15	Small F.-M.D.
22	13. v. 46	N. side of Port Erin Bay	Fine gravel and sand	14	68	18–11	Small F.-M.D.
23	15. v. 46	2 miles W.N.W. of Bradda	Muddy sand and broken shell	15	40	33	Small F.-M.D.

24	17. v. 46	50 yards off Bradda	Stony gravel	4	7	22–27	Small F.-M.D.
26	20. v. 46	N. side of Port Erin Bay	Fine gravel and sand	29	114	7–15	Small F.-M.D.
27	20. v. 46	Inside Port Erin Breakwater	Fine sand with weed	28	156	11	Small F.-M.D.
28	24. v. 46	6 miles W.N.W. of Fleshwick	Muddy sand	6	20	46	Small F.-M.D.
29	14. vi. 46	400 yards off Bay Fine	Sand and fine stony gravel	15	22	18	Small F.-M.D.
30	14. vi. 46	Off Raglan Pier	Fine sand with weed	5	162	2–4	Small F.-M.D.
31	15. vi. 46	Bay Fine to Port Erin Breakwater	Algae on sand and gravel	5	6	18–11	Otter trawl
32	19. vi. 46	¾ mile W.N.W. of Bradda	Muddy sand and broken shell	25	81	29	Small F.-M.D.
33	19. vi. 46	N. side of Port Erin Bay	Sand and fine gravel	18	76	15–18	Small F.-M.D.
34	21. vi. 46	2½ miles W. of Bradda	Muddy sand and broken shell	24	46	33	Small F.-M.D.
35	21. vi. 46	1½ miles W. by N. of Bradda	Muddy sand and broken shell	9	15	31	Small F.-M.D.
36	24. vi. 46	2 miles N.W. of Kitterland	Coarse sand and broken shell	18	60	31	Small F.-M.D.
37	24. vi. 46	2½ miles N. by W. of Kitterland	Muddy sand and broken shell	10	24	33	Small F.-M.D.
38	26. vi. 46	2½ miles W.N.W. of Kitterland	Muddy sand and broken shell	9	12	31	Small F.-M.D.
39	26. vi. 46	2 miles W.N.W. of Charran	Muddy sand and broken shell	13	30	33	Small F.-M.D.
40	1. vii. 46	½ mile N.N.W. of Calf Stack	Coarse sand and broken shell	20	128	33	Small F.-M.D.
41	1. vii. 46	Bay Fine to Breakwater	Algae on sand and gravel	5	15	18–11	Otter trawl
42	3. vii. 46	3½ miles W. of Bradda	Coarse sand	6	33	35	Small F.-M.D.
43	8. vii. 46	3 miles N. by W. of Calf Stack	Hydroids on coarse sand and shells	6	10	33	Small F.-M.D.
44	8. vii. 46	2 miles W. by S. of Bradda	Coarse sand and broken shell	8	11	31	Small F.-M.D.
45	10. vii. 46	Bradda Head to Breakwater	Algae on fine sand	6	42	18–11	Otter trawl
46	11. vii. 46	Bay Fine to Breakwater	Algae on sand and gravel	8	29	18–11	Otter trawl
48	12. vii. 46	½ mile N. of Fleshwick	Fine stony gravel	14	132	11–15	Small F.-M.D.

APPENDIX III (*cont.*)

Serial no.	Date	Position	Type of bottom or contents of haul	No. of species	No. of individuals	Depth (m.)	Gear used
49	16. vii. 46	½ mile N. of Bay Fine	Sand and fine gravel	7	18	15–18	Small F.-M.D.
50	16. vii. 46	Mouth of Port Erin Bay	Fine sand with weed	10	22	11–15	Small F.-M.D.
51	24. vii. 46	2 miles S. of Kitterland	Hydroids on shell gravel	6	116	18–22	Otter trawl
53	26. vii. 46	¾ mile W. of Bradda	*Nemertesia* on shell	4	8	29	On long line
54	1. viii. 46	5 miles N.W. by W. of Bradda	Muddy sand	13	46	59	Large F.-M.D.
55	1. viii. 46	5 miles N.W. by W. of Bradda	Hydroids on muddy sand	7	56	55	Scallop dredge
56	8. viii. 46	6 miles W. of Port Erin	Muddy sand	14	30	61	Large F.-M.D.
57	8. viii. 46	6 miles W. of Port Erin	Hydroids on muddy sand	9	38	64	S.D.
58	16. viii. 46	1½ miles W.N.W. of Bradda	Muddy sand and broken shell	18	28	31	Large F.-M.D.
59	16. viii. 46	Bradda Head to Breakwater	Algae on sand	13	32	18–11	Otter trawl
60	16. viii. 46	Bay Fine to Breakwater	Algae on sand and gravel	12	71	18–11	Otter trawl
61	20. viii. 46	3 miles S.E. of Kitterland	Hydroids on stones	14	123	37	S.D.
62	20. viii. 46	3 miles S.E. of Kitterland	Hydroids on stones and gravel	9	26	37	Large F.-M.D.
63	21. viii. 46	2½ miles W. of Port Erin	Coarse sand and broken shell	11	21	33	Large F.-M.D.
64	26. viii. 46	8 miles W.N.W. of Port Erin	Mud	5	11	82	Young fish trawl
65	26. viii. 46	8 miles W. ½ N. of Bradda	Muddy sand	10	24	82	Large F.-M.D.
66	26. viii. 46	8 miles W. by N. of Bradda	Hydroids on muddy sand	11	46	82	Otter trawl
67	2. ix. 46	5 miles S. of Spanish Head	Sponges on *Modiolus*	4	20	60	N.D.
68	2. ix. 46	5 miles S. of Spanish Head	Hydroids on *Modiolus*	13	61	60	N.D.
69	4. ix. 46	Bay Fine to Breakwater	Sand and fine stony gravel	19	108	18–11	Bottom tow-net
70	11. ix. 46	Bay Fine to Breakwater	Sand and gravel	33	325	15–11	Bottom tow-net
71	20. ix. 46	Mouth of Port Erin Bay	Fine sand with weed	14	85	7–15	Bottom tow-net
72	20. ix. 46	Mouth of Port Erin Bay	Algae on sand	16	214	7–15	Otter trawl
73	24. ix. 46	1 mile N. of Bay Fine	Coarse sand and broken shell	20	57	26	Large F.-M.D.
74	25. ix. 46	400 yards off Bay Fine	Sand and fine gravel	25	178	18	Large F.-M.D.

75	30. ix. 46	4½ miles S.W. by W. of Calf Stack	Coarse sand and broken shell	13	48	55	Large F.-M.D.
76	1. x. 46	1½ miles W. of Charran	Muddy sand	8	25	31	Large F.-M.D.
77	3. x. 46	8⅓ miles N.W. by N. of Bradda	Mud	3	8	88	Large F.-M.D.
78	7. x. 46	3 miles W.N.W. of Dalby	Muddy sand and broken shell	10	30	38	Large F.-M.D.
79	7. x. 46	½ mile W. of Cronk-ny-Arrey-Laa	Fine stony gravel	9	23	18	Large F.-M.D.
80	10. x. 46	50 yards off S.W. of Calf	Coarse sand	2	2	18	Large F.-M.D.
81	10. x. 46	1½ miles N. of Calf Stack	Hydroids on sand and shells	24	192	33	N.D.
82	14. x. 46	2½ miles W.N.W. of Charran	Muddy sand and broken shell	15	29	33	Large F.-M.D.
83	22. x. 46	400 yards N. of Bay Fine	Submerged wood	4	108	18	Otter trawl
84	23. x. 46	½ mile W. of Bradda	Muddy sand and broken shell	25	97	27	Large F.-M.D.
85	29. x. 46	Breakwater to Castle Rock	Sand and fine gravel	27	536	11–15	Bottom tow-net
87	29. x. 46	Inside Port Erin Bay	Fine sand	10	204	2–4	Large F.-M.D.
88	15. xi. 46	½ mile N. of Bay Fine	Algae on sand and broken shell	6	30	22	S.D.
89	16. xi. 46	Inside Port Erin Bay	On dead fish	1	50	4	Lobster pot
90	29. xi. 46	2½ miles W. by S. of Bradda	Coarse sand and broken shell	17	37	33	Large F.-M.D.
91	18. iv. 47	1½ miles N. of Kitterland	Coarse sand and broken shell	20	120	31	Large F.-M.D.
92	5. v. 47	2½ miles N.W. of Bradda	Muddy sand and broken shell	16	55	33	Large F.-M.D.
93	7. v. 47	2 miles W.N.W. of Bradda	Muddy sand and broken shell	14	46	33	Large F.-M.D.
94	9. v. 47	1 mile N.W. of Bay Fine	Coarse sand and broken shell	16	55	29	Large F.-M.D.
95	12. v. 47	Inside Port Erin Bay	Fine sand	6	48	8	Large F.-M.D.
96	12. v. 47	Off Castle Rock	Sand and fine gravel	12	78	8	Large F.-M.D.
97	13. v. 47	5½ miles N.W. by W. of Bradda	Muddy sand	8	101	59	Large F.-M.D.
98	16. x. 47	9 miles W.N.W. of Bradda	Mud	7	60	84	Young fish trawl

A NEW SPECIES OF CILIATE, *TRICHODINA BRANCHICOLA*, FROM SOME FISHES AT PLYMOUTH

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From the Plymouth Laboratory

(Text-figs. 1, 2)

While examining fishes at Plymouth for parasites I found the gills of eight species of Teleosts infected with a new urceolarid peritrich ciliate which I describe here as *Trichodina branchicola* n.sp. I also found the same species on rocklings (*Ciliata mustela* and *Gaidopsaurus tricirratus*) at Roscoff in March 1947.

All the fishes were caught at Plymouth and examined fresh, studies being made on living as well as fixed material. The living ciliates were stained with neutral red and methylene blue (1 : 5000 in sea water). The fixatives used were Bouin-Duboscq's fluid, Zenker's fluid (with formalin) and 2% aqueous osmium tetroxide. The slides were stained in iron-alum-haematoxylin with eosine, or in Delafield's haematoxylin with or without eosine.

The host species and the incidence of infection are shown in Table I.

TABLE I

Host species	No. examined	No. infected
Ciliata mustela (L.)	26	21
Gaidopsaurus tricirratus (Bloch)	8	6
Cottus bubalis Euphrasen	4	4
Spinachia spinachia (L.)	4	3
Blennius pholis L.	2	2
B. gattorugine Bloch	1	1
Trigla lucerna L.	1	1
Pleuronectes platessa L.	1	1

The intensity of infection was generally high in the first five and low in the last three hosts, but no exact quantitative studies of the parasitic population were made. *Ciliata mustela*, which is normally heavily parasitized, had a low grade of infection by *Trichodina branchicola* when caught from the brackish water of the Laira estuary near Plymouth. Four specimens of this fish taken from the estuary after a heavy rainfall had an appreciably low infection.

The following experiment supports the view that reduction in salinity of the water was the operative factor in reducing the parasitic population. The infected gills of *Ciliata mustela* were placed in diluted sea water (75 c.c. sea water and 25 c.c. distilled water) and it was found that after 3 hr. the ciliates were dead. The experiments were conducted in a bath of circulating sea water to ensure the requisite low temperature. Casual observations have repeatedly shown that *Trichodina branchicola* n.sp. is far more sensitive to changes of

temperature and salinity than its hosts. This evidence tends to show that this species of *Trichodina* is a stenohaline organism and will not be found on fresh-water fishes.

After removal from its fish host *T. branchicola* does not survive in sea water for more than 2–3 hr.: it may live much longer on the dead host provided that it is kept at a low enough temperature. I have observed the ciliates still moving 32 hr. after the infected gills were removed from *Ciliata mustela* and had been placed in a refrigerator, although the sea water covering them had a thin film of ice. Richardson (1938) has done some experiments to show the viability of *Cyclochaeta domerguei* Wallengren, under different temperatures and salinity. Fresh-water fishes when infected with *Trichodina* spp. in the hatcheries are cured of the infection by a bath in saline water, or by putting them 'for 1 hour in a solution composed of 1 part of formalin (40%) to 4000 parts water' (Davis, 1947).

In *Ciliata mustela* and *Gaidopsaurus tricirratus* the only other gill parasite was a species of *Gyrodactylus*. This monogenean occurred as a moderate infection irrespective of the presence of *Trichodina branchicola* and there is no reason to think that they are incompatible.

In one *Spinachia spinachia* examined on 9 July 1946 the gills were infected with a species of *Amoeba* (6–11 μ in diameter) and hardly a dozen *Trichodina branchicola* were present. None was found to be infected by the *Amoeba*. The high infection of *Amoeba* was associated with hyper-secretion of mucus. *Trichodina branchicola*, normally a very actively moving animal, was hampered in its movements by the presence of the viscous mucus on the gills, and this may account for the unusually low infection. Chatton (1910) has described *Amoeba mucicola* infecting *Trichodina labrorum* Chatton 1910, from the gills of *Symphodus tinca*, but does not mention any pathological effect on the ciliate.

Morphology

The shape of the organism is surprisingly variable. The oral or anterior end is strongly arched and the saucer shape of the posterior or aboral end is maintained by the 'skeletal complex', which in life is applied to the gill surface of the host (Figs. 1*b*; 2*a*, *b*). The general shape may be hemispherical or subspherical. When viewed from above the organism is round (30–53 μ in diameter) (Fig. 1*a*, *b*). Its height (antero-posterior) is 22–36 μ.

In the genus *Trichodina* there are two rings of cilia, an anterior oral and a posterior aboral ring—the latter is the chief organ of locomotion. In between these two rings of cilia is the velum—a fold of protoplasmic pellicle, capable of great extensions and contractions (Fig. 2*a*, *b*). It attains its greatest size when the animal is crawling on the gills or moving freely in the water (as seen on the slide under the microscope), and almost disappears when the animal is motionless or nearing death.

On the posterior surface, internal to the aboral ring of cilia, lies the 'skeletal complex' composed of the outer striated and the inner denticulate rings (Figs. 1*b*; 2*b*, *c*).

The skeletal complex and the centre of the ring are covered by a thin protoplasmic pellicle (Fig. 2*b*). The denticulate ring (10–19 μ in diameter) consists of 20–26 denticles. The following table shows the percentage of individuals with different numbers of denticles and the average diameter of the ring. These measurements are from 142 individuals from a single *Ciliata mustela*.

No. of denticles	...	20	21	22	23	24	25	26
Percentage of organisms	...	5	26·4	29·5	17·2	12·7	5	4·3
Average diameter of the ring (μ)	...	12·8	13	13·5	14·8	15·2	15·6	16

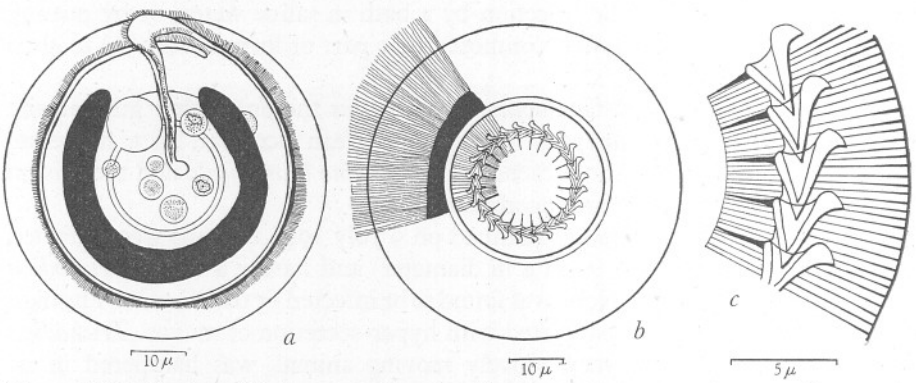

Fig. 1. *Trichodina branchicola*: *a*, anterior view showing oral groove and contractile vacuole and macronucleus; *b*, posterior view showing the skeletal complex and aboral cilia; *c*, portion of skeletal complex (further magnified).

The highest percentage of individuals is with 22 denticles; individuals with 21 and 23 denticles coming second and third. The size of the ring increases with the number of the denticles. Each denticle (Fig. 1*c*) is cone-shaped, 1·5–2·0 μ thick and 3·5 μ long, with a broad curved hook (2·3 μ long) on the outer side. The concave side of the hook is slightly thicker than the convex side. Each hook is attached to the cone at an angle like an oar. On the inner side of the cone there is a ray (3·2 μ long) pointing centrally and slightly anteriorly. It is thicker at the base, tapering towards the centre. In young *Trichodina branchicola* which has just undergone fission the number of denticles is halved, being 10–13 only. The outer hook is a little in front of the inner ray, as in *T. truttae* Mueller, and not exactly opposite, as in *T. pediculus* Ehrb. as described by Mueller (1937). The denticulate ring stains beautifully red with eosine and this helps very much in counting the number of denticles under the oil immersion lens.

External and somewhat anterior to the denticulate ring is a striated ring band 19–33 μ in diameter (Figs. 1*b*, *c*; 2*c*). There are six striae to each denticle. Each stria extends inwards as far as the inner ray. Each also is

connected with the ciliary girdle by a strand of material which takes up the eosine stain less readily than the stria. Mueller (1938) supposed these structures to be myonemes. The outer borders of the striae fuse to form the edges of the adhesive disk. Each stria is thicker at the outer ends and tapers towards the centre. The ciliate secures itself to the gill surface with the adhesive disk. The skeletal complex is capable of movement as a whole in the horizontal plane but whether it can move in the antero-posterior direction as well is not clear.

The aboral cilia, 15–20 μ long, are fused at their base to form a thin membranelle, their distal ends being free (Figs. 1*b*; 2*c*). In *T. pediculus* Ehrb., *T. steinii* (Clar. & Lach.) and *T. renicola* (Mueller) the cilia are fused to form a series of membranelles. In the present species there is one continuous membranelle like that in *T. spheroidesi* Padnos & Nigrelli. The cilia do not fuse to form any membranelle in *T. halli* Padnos & Nigrelli and *T. urinicola* Fulton.

The oral groove runs round the anterior surface of the organism and makes little more than one anti-clockwise turn and then descends into the vestibulum which is connected with the gullet at its posterior end (Figs. 1*a*; 2*b*). The vestibulum descends obliquely and makes an angle of nearly 45° with the vertical axis of the organism. The base of the groove is slightly thickened and from its edges two parallel rows of cilia arise which are fused at their bases to form two parallel membranelles. The outer cilia are slightly longer than the inner. The inner membranelle ends at the entrance of the vestibulum. In the anterior three-quarters of the vestibulum the cilia are curved at their free ends and fused at their bases. The wave of movement starts at the anterior end and passes towards the gullet. In the last quarter of the vestibulum the cilia are longer and beat quickly towards the gullet. The undulatory motion of the cilia of the oral groove appears to form four waves in the whole circumference. The gullet is devoid of any cilia. The food vacuoles arise near the fundus of the gullet and then move peripherally in spirals. When stained *intra vitam* with neutral red (1 : 5000 in distilled water) the food vacuoles take the stain in 5 min. The young vacuoles stain yellowish and later on become pinkish. This shows a change in the pH content of the food vacuoles from basic to acidic, i.e. at a rough estimate initially at pH 8 or more to pH 7·5 or thereabout. The food vacuoles are mostly in the anterior half of the body. The food of the ciliate consists of desquamated epithelial cells and erythrocytes of the host fish. Diller (1928) states that *Trichodina* on the gills of tadpoles feed only on bacteria.

The contractile vacuole is 6·0–11·5 μ in diameter and situated near the vestibule (Fig. 1*a*). It pulsates every 30–35 sec. In *T. urinicola*, Fulton (1923) records that the pulsation is very sluggish, while in *T. pediculus* Ehrb., Mueller (1937) records the pulsation at the interval of 10–12 sec. The opening of the vacuole is not clear in the living or fixed specimens, but in sections it can be seen as a very thin short duct opening in the anterior part of the vestibulum. When the organism is stained *intra vitam* the vacuole does not take any stain.

No accessory vacuoles are present in any species of *Trichodina* such as have been reported for *Cyclochaeta domerguei* (Wallengren) by MacLennan (1939) and in various vorticellids by Fauré-Fremiet (1925).

The macronucleus is situated in the posterior half of the body, is horseshoe-shaped, and lies parallel to the plane of the 'skeletal complex' (Figs. 1*a*; 2*a*). It is 6·0–10·5 μ thick in the centre and somewhat thinner at the ends. The two ends of the nucleus are separated by the vestibulum and the contractile vacuole. The micronucleus is situated in a pocket in the macronucleus on its outer edge and can be seen only in the sections. During vegetative life the macronucleus is uniformly granular.

The body of the ciliate is covered by a thin protoplasmic pellicle. Within this is the dense granular layer of the protoplasm. The endoplasm is less granular and has many food vacuoles and the contractile vacuole.

Locomotion

The organism moves by the help of the posterior cilia. Each cilium moves with a gyratory motion while the resultant movement of the whole ring appears as a wave motion. The animal rotates in a clockwise or anti-clockwise direction and moves very quickly with the posterior end forwards when swimming freely in water. There does not seem to be any relation between gyratory and rotatory motion. During movement the body is very much constricted antero-posteriorly and the velum increases in size. When the animal is 'browsing' on the gill with its adhesive disk in contact with the gill surface, it does not move very quickly.

Pathogenic Effect

When *Trichodina* settles on the gills and makes itself secure by the application of the adhesive disk then considerable local irritation must be caused, and this seems to bring about the desquamation of the epithelial cells of the gills. No very marked pathogenic effect was observed on the gills of the fishes examined during the course of the present studies, though there is no doubt that considerable harm could be done to the host if the ciliates were present in large numbers. At times, in fresh-water fish hatcheries, some species of *Trichodina* cause epidemic mortality.

Reproduction

As a rule in the family Urceolaridae reproduction is by binary fission, though conjugation and endomixis also take place. Some stages of fission have been observed in *T. branchicola*. Preparatory to the fission the macronucleus becomes round and then oblong and stains deeply with haematoxylin. The shape of the organism changes and it too becomes oblong and later on dumb-bell-shaped. The micronucleus divides into two and can be seen lying on the outside of the macronucleus (Fig. 2*d*). There is a constriction in the adhesive disk, and then in the denticulate ring. The macronucleus and the whole body then divide into two daughter individuals.

The young individual has about half the adult number of the denticles in the denticulate ring. The old vestibulum persists in one of the daughter individuals while a new one seems to develop in the other. Peshkowsky (1923) states that the oral cilia, gullet and contractile vacuole are absorbed during division in *T. mitra* and *T. steinii*. Padnos & Nigrelli (1942) report that these organs definitely remain in *T. spheroidesi* during the fission, as occurs in the present species. The fate of the contractile vacuole could not be followed, though according to Padnos & Nigrelli it cleaves at the same time as the macronucleus in *T. spheroidesi*. Diller (1928) states that the contractile vacuole probably persists in one of the daughter individuals in *Trichodina* sp. on the gills of tadpoles.

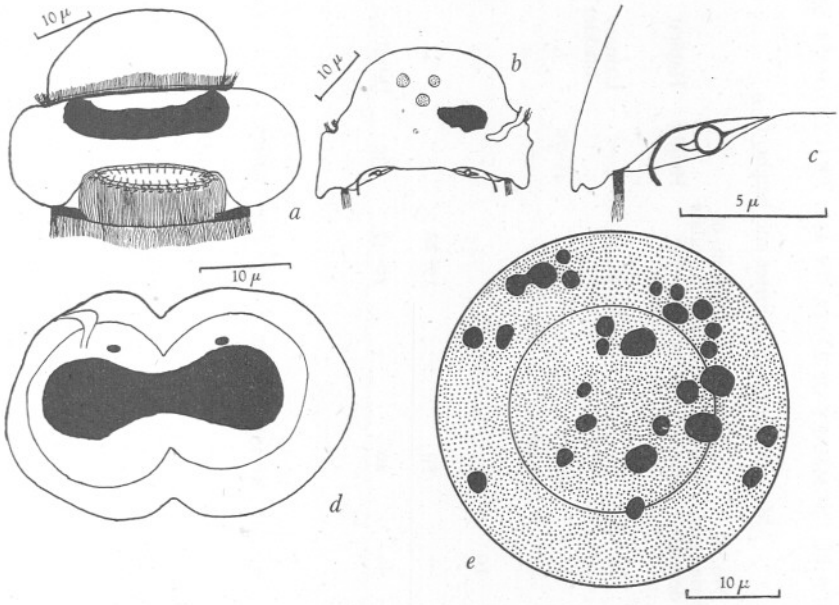

Fig. 2. *Trichodina branchicola*: *a*, side view; *b*, vertical section showing food vacuoles and oral groove; *c*, portion of the same showing skeletal complex in section; *d*, individual undergoing fission, showing macro- and micro-nuclei and a part of the oral groove; *e*, macronucleus broken up into small fragments.

The young individual has the shape of the adult. The 'skeletal complex' arises *de novo* in the adult exterior to the smaller skeletal complex inherited from the parent which is gradually absorbed. Various stages in the development of the new denticulate ring are seen and they confirm fully the findings of Fauré-Fremiet & Thareaux (1944, pl. 1, figs. 1–5). At first the central cone is formed, then the outer hook and lastly the inner ray. At first the denticle is thin but gradually it becomes thick. The young individuals, with one ring only, measure 25–33 μ in diameter, the diameter of the denticulate ring being 6·5–10 μ. The number of denticles is 10–13. The macronucleus is round or

TABLE II. SUMMARY OF DATA ON ALL *TRICHODINA* SPECIES

Measurements (of diameter and height) in μ

Species	No. of denticles	No. of striae per denticle	Total diam.	Diam. of striated ring	Diam. of denticulate ring	Height	Habitat	Host	Habitat of host
ENDOPARASITES:									
T. urinicola Fulton, 1923	26–36	—	—	—	34–35	—	Urinary bladder	*Triton cristatus*, *Bufo* sp.	Fresh water
T. okajimae Ibara, 1931	34–38	—	40–60	20–40	—	45–70	,,	*Hynobius tokyoensis*	,,
T. [*V.*] *renicola* (Mueller, 1931) n.comb.	56	—	70–96	—	—	—	,,	*Esox niger*	,,
T. [*V.*] *nephritica* (Mueller, 1938) n.comb.	36–40	10	—	62–75	—	—	,,	*Esox masquinongy*	,,
T. vesicularum Fauré-Fremiet, 1943	21–33	—	50	—	19–22	—	,,	*Triton cristatus*, *T. palmatus*	,,
T. fariai Da Cunha & Pinto, 1928	24–28	—	40	—	20–42	32	Intestine	*Spheroides testudineus*	Marine
ECTOPARASITES:									
T. labrorum Chatton, 1910	21	—	30–34	—	—	18–22	Gills	*Symphodus tinca* and *S. melops*	,,
T. chelidonichthys Fantham, 1930	30	—	30–45	19–32	—	19–27	,,	*Chelidonichthys cupensis*	,,
T. mugilis Fantham, 1930	32	—	33–34	23–28	—	14–20	,,	*Mugil capito*	,,
T. blennii Fantham, 1930	24–32	5	40–45	24–27	—	20–32	Gills and pericardium	*Blennius cornutus*	,,
T. clini Fantham, 1930	24	—	37	20	—	20	,,	*Clinus anguillaris*	,,
T. halli Padnos & Nigrelli, 1942	26–34	—	45–86	41–81	30–54	—	Gills and skin	*Spheroides maculatus*	,,
T. spheroidesi Padnos & Nigrelli, 1942	21–31	—	17–54	18–32	14–22	12–42	,,	,,	,,
T. branchicola n.sp.	20–26	6–8	30–54	19–33	10–20	22–36	Gills	See p. 440	,,

T. pediculus Ehrenberg, 1838	16–26	—	60	—	—	—	Body surface and gills	Various fresh-water fishes, tadpoles and salamanders; *Hydra fusca, H. viridis*	Fresh water
T. steinii Clarapede & Lachmann, 1858	21–26	—	40	—	—	40	Body surface	*Polycelis nigra, Dugesia lugubris, Polycelis cornuata*	,,
T. [*C.*] *spongillae* Jackson, 1875, n.comb.	37	—	*c.* 61–60	—	—	—	Inside the body	*Spongilla fluviatilis*	,,
T. [*C.*] *domerguei* (Wallengren, 1897), n.comb.	18–25	—	23–56	—	—	$\frac{1}{5}$th of diameter	Gills	On various fishes	,,
T. truttae Mueller, 1937	28–31	20	120–140	110–125	75–85	—	,,	*Salmo clarkii*	,,
T. myakkae Mueller, 1937	17–24	4–5	—	21–25	11–12	—	,,	*Aplites salmoides, Ictiobus bubalus, Carpoides carpio, Salvalinus fontinalis*	,,
T. [*C.*] *guberleti* MacLennan, 1939, n.comb.	28–32	—	50–100	—	—	25	Gills and skin	*Richardsonius balteatus, Apocope oscula carringtoni*	,,
T. tenuidens Fauré-Fremiet, 1943	27–37	—	—	—	—	41–55	,,	*Gasterosteus aculeatus*	,,
T. discoidea Davis, 1947	18–30	6–8	—	35–50	19–29	—	Gills	*Lepomis macrochirus, Pomoxis sparoides, Ambloplites rupestris, Ictalurus punctatus*	,,
T. platyformis Davis, 1947	26–35	10	—	56–75	31–50	—	,,	*Margariscus margarita, Rhinichthys atronasus*	,,
T. vallata Davis, 1947	18–21	10	—	38–48	25–30	—	,,	*Ictalurus punctatus*	,,
T. fultoni Davis, 1947	25–30	12–14	100	75–90	50–58	—	,,	*Huro salmoides, Micropterus dolomieu, Lepomis macrochirus, Ambloplites rupestris, Salmo irideus*	,,
T. symmetrica Davis, 1947	21–28	5	—	24–35	13–22	—	,,	*Ictalurus punctatus, Margariscus margarita, Rhinichthys atronasus*	,,
T. californica Davis, 1947	25–32	8–10	—	38–50	25–33	—	,,	*Oncorhynchus tschawytscha*	,,
T. tumefaciens Davis, 1947	19–26	7	—	29–30	18–23	—	,,	*Cottus bairdii*	,,
T. bulbosa Davis, 1947	19–24	5–6	—	22–26	10–12	—	,,	*Margariscus margarita*	,,
T. brusiformis Davis, 1947	24–27	5	—	25–35	14–18	—	,,	*Ambloplites rupestris*	,,

ellipsoidal at first but gradually becomes horseshoe-shaped. No stages of conjugation were observed. I have found one individual in which the nucleus is broken up into small spherules (Fig. 2*e*) and is very much like the fig. 14, pl. II of Padnos & Nigrelli (1942) and figs. 29–36, pl. 3 of Diller (1928). In the absence of other stages it is very difficult to interpret correctly the nucleus phase of this individual.

The rate of fission during the summer increases, for it is only during the months of March to August that various individuals undergoing fission were observed. No quantitative population count was done to find the percentage of the dividing forms. Only one young individual was observed during the period September 1946–March 1947, while several have been observed from April 1947 to August 1947.

Discussion on Taxonomy

Fauré-Fremiet (1943) has recently given a very good systematic review of the family Urceolaridae. He retains only two genera, *Trichodina* Ehrb. and *Urceolaria* Stein., in this family; and he relegates the genus *Cyclochaeta* Jackson as a sub-genus of *Trichodina*, and *Leiotrocha* Fabre-Domergue as a sub-genus of *Urceolaria*.

The members of the genus *Trichodina* are generally found as ectoparasites on the gills and skin of fishes and tadpoles, and as endoparasites in the urinary bladder of fishes and Urodela and rarely in Anura. Mueller (1938) has created a new genus *Vauchomia* for endoparasitic urceolarids. The only difference between *Vauchomia* and *Trichodina*, according to Mueller, is that the former has a system of 'myonemes' and the oral groove makes more than two turns. The shape of the denticulate ring is similar in both these genera. The 'myonemes' have, however, since been described in additional species of ectoparasitic *Trichodina*. The distinction, therefore, does not strictly hold, and the name *Vauchomia* is relegated to a synonym of *Trichodina*.

In distinguishing the different species of the genus *Trichodina* (which now includes *Cyclochaeta* and *Vauchomia* as well) the size, shape and the number of the denticles are of great value. Fauré-Fremiet (1943, p. 163) and Davis (1947, p. 7) have also emphasized this point. Biometrical studies on the variation in the number of denticles in a population of *Trichodina* are of great help in finding out whether that particular population contains one or two species of *Trichodina*. Mueller (1937) pointed out that Wallengren (1897) was probably dealing with more than one species of *Trichodina* which he described as *T. pediculus*. Recently Fauré-Fremiet (1943), on the basis of his biometrical studies, has pointed out that there are really two species of *Trichodina* on the skin of *Gasterosteus aculeatus*. The one with 20–26 denticles is *T.* [*Cyclochaeta*] *domerguei* (Wallengren), and the other with 30–34 denticles he named *T. tenuidens*. The variation in the number of denticles is the same in *T.* [*Cyclochaeta*] *domerguei* and *T. branchicola* n.sp., but the size of the denticles and the shape of the body differ in the two species.

At present there are 31 species of *Trichodina* out of which 16 are freshwater, 8 marine, 6 endoparasites in the urinary bladder, and one in the intestine of *Spheroides testudineus*. In Table II the measurements, habitats and hosts of all the species of *Trichodina* are given.

I take this opportunity to thank the Director and the Staff of the Plymouth Laboratory for their kind interest and facilities offered to me. My hearty thanks are due to Miss N. G. Sproston, under whose guidance this work was done, for her helpful criticisms and encouragement, and to Miss T. Skilton for her help in translating the French papers.

Summary

The morphology and reproduction of *Trichodina branchicola* n.sp. are described. Its total diameter is 30–54 μ, and its height is 20–36 μ; the diameter of the striated ring is 19–33 μ, and the diameter of the denticulate ring 10–20 μ. There are 20–26 denticles in the ring, with 6–8 striations to each denticle. The denticles measure 1·5–2 μ in thickness, 3·5 μ in length, each with a broad outer hook 2·3 μ long and an inner ray 3·2 μ long. The aboral cilia are fused at their base to form a complete membranelle.

This organism is found on the gills of the following marine fishes at Plymouth: *Ciliata mustela*, *Gaidopsaurus tricirratus*, *Cottus bubalis*, *Spinachia spinachia*, *Blennius pholis*, *B. guttorugine*, *Trigla lucerna* and *Pleuronectes platessa*.

The genus *Vauchomia* Mueller 1938 is regarded as a synonym of *Trichodina*.

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METHODS OF IDENTIFICATION OF THE LARVAE OF *BALANUS BALANOIDES* (L.), *B. CRENATUS* BRUG. AND *VERRUCA STROEMIA* O. F. MÜLLER

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(Text-figs. 1–6)

CONTENTS

INTRODUCTION

A review of the literature on barnacle larvae suggests that the information available falls roughly into two categories. A number of accounts are now available which give detailed descriptions of the full sequence of larval stages for a particular species. Willemoes-Suhm (1876) described the larval stages of *Lepas fascicularis*; Groom (1894) those of *Balanus perforatus*; Herz (1933) the larval stages of *B. crenatus*; Kühnert (1935) the larval stages of *Alcippe lampas*; Bassindale (1936) those of *Balanus balanoides*, *Chthamalus stellatus* and *Verruca stroemia*; Yasugi & Ishida (1937) the free-swimming stages of *Balanus amphitrite albicostatus*; and Batham (1946) the larval stages of *Pollicipes spinosus*. As well as these fuller accounts, a number of references are available which describe and figure some of the larval stages; these range from the more comprehensive accounts of Hoek (1909) and Nilsson-Cantell (1921), through shorter accounts in papers dealing more specifically with other topics (e.g. Runnström, 1925, 1926; Lochhead, 1936), to brief descriptions of unidentified material (e.g. Stubbings, 1940).

Barnacle larvae, both nauplii and cyprids, are prominent components of the zooplankton in early spring and none of the sources of information just mentioned provides a means of rapid identification which would enable species and stages to be recognized when working through a plankton haul. Because of this difficulty, many workers have had to be content with recording the

presence of Cirripede larvae without attempting further identification (e.g. Lebour, 1917; Marshall, 1925), others have made tentative identifications based on the identity of the commonest barnacles on the neighbouring shore (e.g. Moore, 1935), or on a knowledge of the spawning seasons of shore barnacles of the area studied (e.g. Fish, 1925).

In the course of biological work carried out in connexion with research on the fouling problem, studies have been made on the barnacle larvae occurring in the plankton at Millport. It has proved possible to discover characteristics which are sufficiently clear to be recognized at a glance under a low-power binocular microscope, and which therefore enable the species and, to a considerable extent, the stage of barnacle larvae to be identified *in situ* in a dish of plankton.

The larvae of *Balanus balanoides*, *B. crenatus* and *Verruca stroemia* form the chief constituents of the Cirripede larvae in the plankton at Millport, and in this paper attention will be confined to methods for recognizing the larval stages of these three species. Other Cirripede larvae occur in much smaller numbers, in particular the nauplius of a species of *Sacculina* (probably *S. carcini*), the nauplius of a species of *Peltogaster* and nauplii and cyprids which may be those of *Balanus porcatus*. All these can be distinguished from the larval stages of the three species first mentioned, but knowledge of their appearance is too fragmentary to warrant their inclusion in the scheme set out below.

That the larval stages of only three species should be common in plankton hauls taken at Millport imposes, it is recognized, a disadvantage on the identification scheme set out later. Though these larval stages are fairly readily distinguishable *inter se*, some of them may resemble the larval stages of other species of barnacles, not present on the Clyde, so closely as to make this scheme difficult to use elsewhere. Inspection of the published figures of other species of barnacles does not suggest any obvious difficulties in this respect, but reasonable certainty that the characteristics suggested below are truly distinctive must await further work on the larvae of other species.

The Characteristics of the Larval Stages

Balanus balanoides, *B. crenatus* and *Verruca stroemia* each pass through six naupliar stages before reaching the cyprid stage. Herz (1933) has described eight naupliar stages for *Balanus crenatus*, but Bassindale (1936), after reviewing the evidence put forward by Herz, concludes that 'it seems possible to regard the number of stages as not being perfectly established'. The present author has reared *B. crenatus* in the laboratory and has not been able to discover more than six naupliar stages so that it would appear that *B. crenatus* at Millport agrees with *B. balanoides* in possessing the same number of naupliar stages.

Outline diagrams of the six naupliar stages of *B. balanoides* are shown in Fig. 1; these will be used to illustrate the characteristic features of each of these

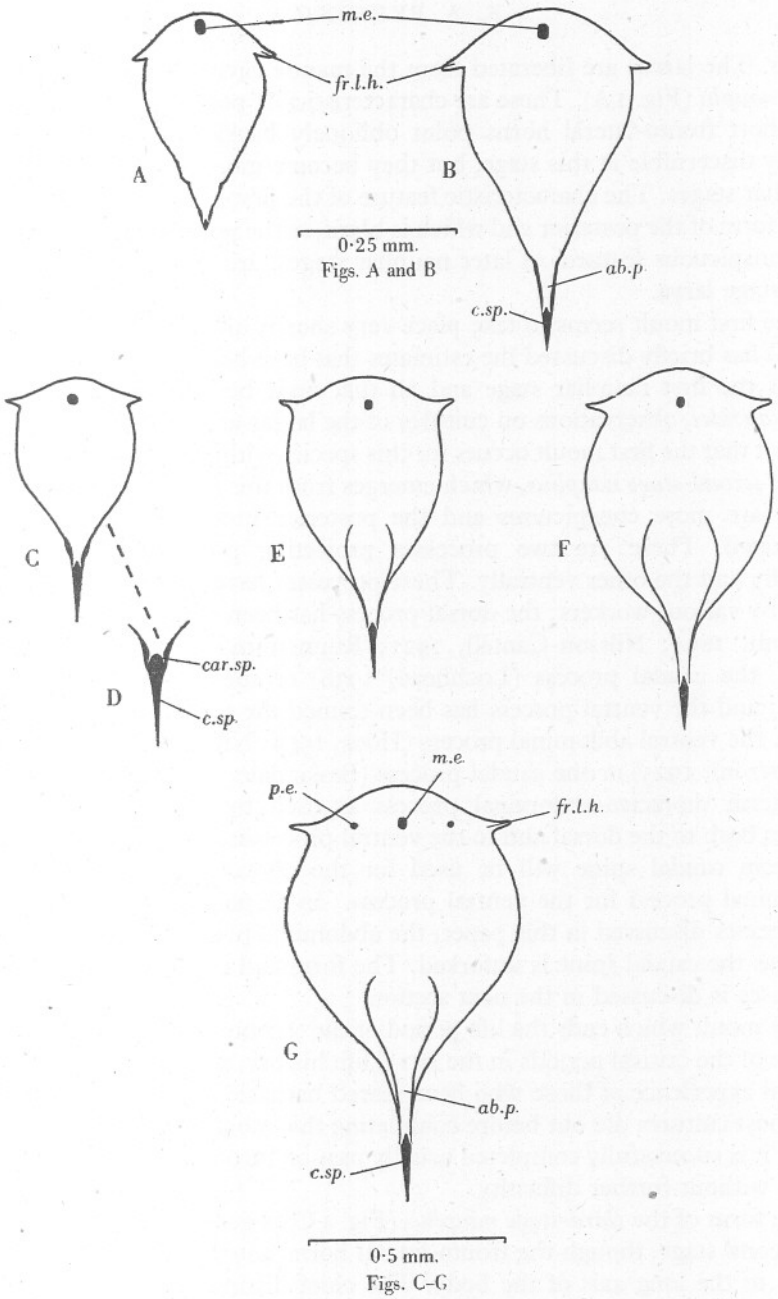

Fig. 1. Outline diagrams of the six naupliar stages of *Balanus balanoides* in ventral view. A, first-stage nauplius; B, second stage; C, third stage; D, dorsal view of hinder edge of carapace of third-stage nauplius, showing carapace spines; E, fourth stage; F, fifth stage; G, sixth-stage nauplius. *ab.p.*, abdominal process; *c.sp.*, caudal spine; *car.sp.*, carapace spine; *fr.l.h.*, fronto-lateral horn; *m.e.*, median eye; *p.e.*, paired eye.

stages. The larvae are liberated from the mantle cavity of the parent as *first-stage nauplii* (Fig. 1 A). These are characteristically pear-shaped in outline and the short fronto-lateral horns point obliquely backwards. These horns are clearly discernible at this stage, but they become more prominent in the later naupliar stages. The characteristic feature of the first-stage nauplius, however, is the form of the posterior end which is blunt, as the posterior processes, which are conspicuous features of later naupliar stages, are barely developed in the first-stage larva.

The first moult seems to take place very shortly after hatching. Bassindale (1936) has briefly discussed the estimates that have been made of the length of life of the first naupliar stage and all that need be added here is that, for *B. balanoides*, observations on cultures of the larvae and plankton studies both suggest that the first moult occurs for this species within 24 hr. after liberation. In the *second-stage nauplius*, which emerges from this moult, the fronto-lateral horns are more conspicuous and the posterior processes are much better developed. There are two processes projecting posteriorly, one situated dorsally and the other ventrally. These processes have been named in various ways by various workers; the dorsal process has been termed the caudal spine (Groom, 1894; Nilsson-Cantell, 1921; Runnström, 1925; and Bassindale, 1936), the caudal process (Lochhead, 1936) or the carapace spine (Hoek, 1909); and the ventral process has been termed the spinous process (Groom, 1894), the ventral abdominal process (Hoek, 1909; Nilsson-Cantell, 1921; and Runnström, 1925) or the caudal process (Bassindale, 1936; Lochhead, 1936). The term thoracico-abdominal process as used by Herz (1933) evidently applies both to the dorsal and to the ventral processes. In the present account the term caudal spine will be used for the dorsal process, and the term abdominal process for the ventral process. In *B. balanoides*, as in the other two species discussed in this paper, the abdominal process is forked at its tip, whereas the caudal spine is unforked. The form and relationship of these two processes is discussed in the next section.

The moult which ends the life period of the second-stage nauplius seems to be one of the critical periods in the larval life history, since it appears to be the general experience of those who have reared barnacle larvae in the laboratory that most cultures die out before completing this moult, and those cultures in which it is successfully completed usually pass on through the remaining larval stages without further difficulty.

The form of the *third-stage nauplius* (Fig. 1 C) is generally similar to that of the second stage, though the fronto-lateral horns now project roughly at right angles to the long axis of the body. The chief distinguishing feature of this stage is the form of the carapace. In the earlier naupliar stages the carapace has no definite posterior margin, but is produced without interruption into the caudal spine. In the third-stage nauplius, however, the posterior border of the carapace is distinct and is further marked by the presence of two short, simple

spines each of which projects backwards a short distance on each side of the mid-dorsal line (Fig. 1D). These spines, which occur in the later naupliar stages both of *B. balanoides* and *B. crenatus*, are here termed carapace spines. In his diagrams of the outlines of the six naupliar stages of *B. balanoides* Bassindale (1936, fig. 2A, p. 59) does not figure these carapace spines, though he indicates the definite posterior margin of the carapace. They are present, however, in third-stage *B. balanoides* nauplii from the Clyde.

It is not at first easy to distinguish the *fourth- and fifth-stage nauplii* (Fig. 1E, F) from the third-stage larvae, as the main difference between these three stages is one of size. If they occur together in a plankton haul (as frequently happens) distinction is not difficult, as the fifth-stage nauplius is roughly half as long again as the third-stage larva, but if only one stage occurs or if the numbers present are small, it may be necessary to measure individual larvae to check their stage. Practice, however, soon enables any one of these three stages to be recognized even if the other stages are not available for comparison. The swollen base of the abdominal process (within which the rudiments of the post-mandibular appendages can be discerned) helps to distinguish the fourth- and fifth-stage nauplii from the third stage, since this swelling first becomes obvious in the fourth-stage nauplius and is prominent in the succeeding stage. A careful examination of the third-stage nauplius, however, shows that the base of the abdominal process is slightly swollen in this stage also, so that this character is not diagnostic.

Finally, the *sixth-stage nauplius* (Fig. 1G) can be recognized by the appearance of the paired eyes. The median eye, which has been present throughout the larval sequence, also persists in the sixth-stage nauplius. When the paired eyes are fully pigmented they are immediately obvious but it would seem that pigmentation proceeds slowly, probably during the life period of this naupliar stage, as some nauplii occur in which pigmentation is only just beginning and others in which the pigment is distinctly red in colour. When fully developed the pigment is black. This gradual development of pigmentation in the sixth-stage nauplius is found not only in *B. balanoides*, but also in the corresponding stage of *B. crenatus* and *Verruca stroemia*.

It is more convenient to reserve a description of the characteristic features of the *cyprid larva* for the next section, where it will be compared with the corresponding stage of the other two species under discussion.

Distinctions between the Larvae of *Balanus balanoides*, *B. crenatus* and *Verruca stroemia*

Second-stage nauplii. Comparisons of the naupliar stages of *Balanus balanoides*, *B. crenatus* and *Verruca stroemia* can most profitably be made, in the first instance, between the second-stage nauplii. This is the most convenient stage for this purpose for two reasons: second-stage nauplii are those most frequently encountered in tow-nettings, and at this stage the main specific characteristics

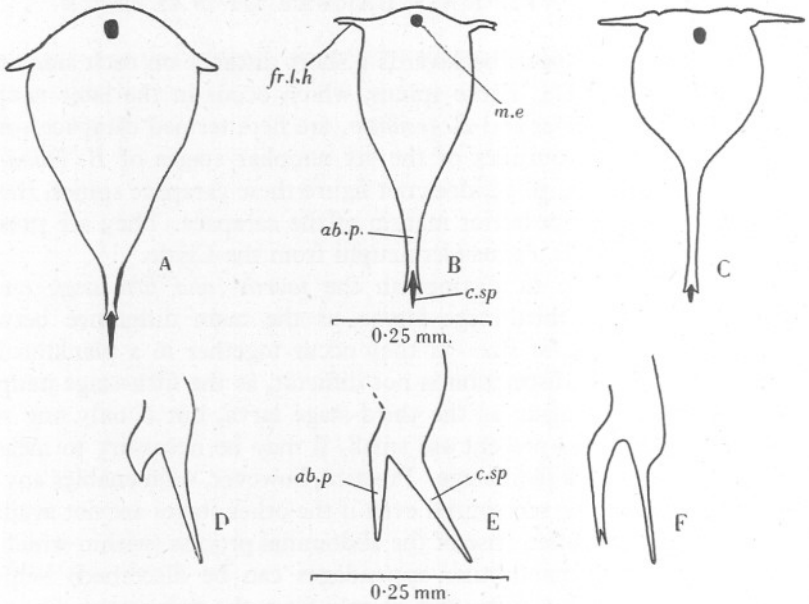

Fig. 2. Second-stage nauplii of (A) *Balanus balanoides*, (B) *B. crenatus*, and (C) *Verruca stroemia*, in ventral view; (D) *Balanus balanoides*, (E) *B. crenatus*, and (F) *Verruca stroemia*, lateral view of abdominal process and caudal spine. *ab.p.*, abdominal process; *c.sp.*, caudal spine; *fr.l.h.*, fronto-lateral horn; *m.e.*, median eye.

Table I. Characteristics of the Second-Stage Naupliar Larvae

Character	*Balanus balanoides*	*Balanus crenatus*	*Verruca stroemia*
General shape (in ventral view)	Pear-shaped	Triangular	Circular
Anterior margin	Curved	Curved, but radius of curvature greater than that of *B. balanoides*	Almost straight
Fronto-lateral horns	Short, pointing posteriorly	Moderately long, slightly curved, projecting roughly at right angles to main axis	Long and slender, projecting at right angles to main axis
Posterior processes	Caudal spine much longer than abdominal process	Caudal spine longer than abdominal process	Caudal spine either slightly longer than, or equal in length to, abdominal process
	Terminal fork of abdominal process with rami practically parallel	Terminal fork of abdominal process with rami making a distinct angle with each other	
	In lateral view, abdominal process tap-like in form	In lateral view, abdominal process slender, diverging markedly from caudal spine	In lateral view, abdominal process and caudal spine practically parallel, or with former inclined towards latter

are apparent so that a means of recognizing the identity of the second-stage nauplii provides a useful basis for distinguishing the specific identity of later stages.

Fig. 2A–C shows outline diagrams of these second-stage nauplii seen in ventral view, and Fig. 2D–F shows the characteristics and relationships of the caudal spine and the abdominal process, as seen in lateral view. With these diagrams as illustrations, the distinguishing features of these nauplii can be listed as in Table I.

Using these characters, it is possible to recognize this stage of each of the three species under discussion virtually at a glance and distinction is further aided by the existence of other differences, less capable of precise description, but equally valuable as guides to identification. For example, the larvae of *Balanus balanoides* give an impression of bulk (this applies to all the naupliar stages, not only to the second stage) which produces a clumsy appearance, whereas the larvae of *B. crenatus* and *Verruca stroemia* are delicate and more transparent in appearance.

Later nauplii. Once the second-stage nauplii can be distinguished, the recognition of the specific identity of the later naupliar stages is not difficult as the main characteristics of the second-stage nauplii persist through the later naupliar stages, but there are a few complications which may cause some difficulty and therefore deserve mention.

The short, tap-like abdominal process of the second-stage nauplius of *Balanus balanoides* does not wholly retain this form through the later larval stages, since it becomes more elongated relative to the caudal spine. In the third-stage nauplius the ratio of the length of the caudal spine to that of the abdominal process is 3:1, but in the sixth-stage nauplius it has become 1·7:1. As this ratio for the sixth-stage nauplius of *B. crenatus* is of the order of 1·3:1 and as the abdominal process of the latter stage does not diverge so sharply from the caudal spine as in its second-stage larva—possibly owing to the swelling of the base of the abdominal process (see Fig. 3C), an examination of the posterior region of the larvae in lateral view may not be very helpful. Distinction can be made, however, on the basis of general appearance, the form of the forked tip of the abdominal process and the dorsal outline. The later naupliar stages of *B. balanoides* fully retain the bulky appearance of the earlier stages and the tissues of formalin-preserved specimens often appear particularly dense, whereas the corresponding stages of *B. crenatus* retain their delicacy and transparency. The rami of the forked tip of the abdominal process of *B. balanoides* remain more parallel than those at the tip of the abdominal process of *B. crenatus* (Fig. 3B, D), and the dorsal edge of the carapace is much more rounded in *B. balanoides* than it is in *B. crenatus* (Fig. 3A, C).

The series of structural changes in the sequence of nauplii of *Verruca stroemia* is not quite the same as that of the nauplii of *Balanus balanoides* and *B. crenatus*. In the latter the third-stage nauplius can be distinguished from

the earlier stages by the definite posterior margin of the carapace which bears the carapace spines. In *Verruca stroemia* the carapace does not acquire a definite posterior margin until the fourth-stage nauplius and this margin lacks

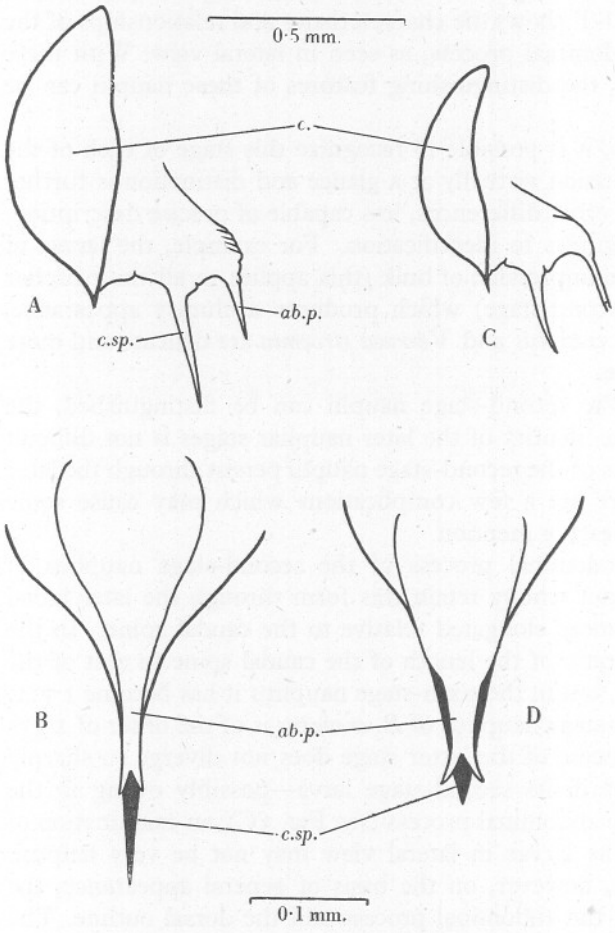

Fig. 3.

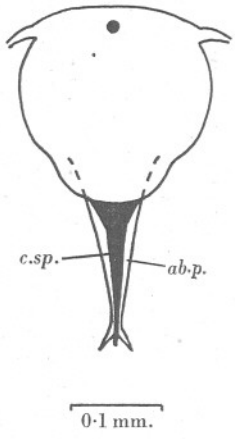

Fig. 4.

Fig. 3. Sixth-stage nauplii of (A, B) *Balanus balanoides*, and (C, D) *B. crenatus*. Above, lateral view of nauplius; below, posterior processes enlarged in ventral view. *ab.p.*, abdominal process; *c.*, carapace; *c.sp.*, caudal spine.

Fig. 4. Dorsal view of the fourth-stage nauplius of *Verruca stroemia*. The absence of carapace spines should be noted.

carapace spines, both in this and in the two succeeding naupliar stages (see Fig. 4). In *V. stroemia*, therefore, the fourth-stage nauplius is readily distinguished from the third stage, but the distinction between the second and third stages seems to be one of size only. Third-stage nauplii of *V. stroemia* do

not seem to occur at all commonly in plankton hauls made at Millport, though Bassindale (1936) found this stage to be that which occurred most commonly in his cultures of the nauplii of this species.

Details of the lengths of the naupliar stages of the three species are given in Table II. Those for *Balanus balanoides* and *Verruca stroemia* are compared with those given by Bassindale (1936), which are given in parentheses below the measurements recorded in the course of the present survey. The range of variation in the length of these larvae from one place to another is unknown but in view of the known differences in size between cyprids of *Balanus balanoides* from different latitudes (see p. 461), it would seem unwise to place too much reliance on the universal application of length measurements. In the present instance, these measurements are quoted to give an idea of the order of difference in length of the larval stages of the three species.

TABLE II. LENGTHS OF THE NAUPLIAR STAGES OF *B. BALANOIDES*, *B. CRENATUS* AND *V. STROEMIA*

	B. balanoides		*B. crenatus*		*V. stroemia*	
Stage	Full length (mm.)	Carapace length (mm.)	Full length (mm.)	Carapace length (mm.)	Full length (mm.)	Carapace length (mm.)
Nauplius I	0·34 (0·35)	—	0·28	—	— (0·27)	—
Nauplius II	0·54 (0·51)	—	0·44	—	0·40 (0·44)	—
Nauplius III	0·63 (0·62)	0·42 (0·37)	0·57	0·35	0·47 (0·50)	—
Nauplius IV	0·76 (0·69)	0·54 (0·41)	0·73	0·48	0·54 (0·58)	0·31 (0·34)
Nauplius V	0·92 (0·81)	0·63 (0·53)	0·84	0·59	0·62 (0·63)	0·39 (0·37)
Nauplius VI	1·05 (1·15)	0·73 (0·79)	0·91	0·63	0·73 (0·69)	0·47 (0·42)

Measurements in parentheses are taken from Bassindale (1936).

First-stage nauplii. Outline diagrams of the first-stage nauplii of these three species are shown in Fig. 5, from which it will be evident that each differs considerably in appearance from its succeeding stages; this difference is probably least for the first-stage nauplius of *B. balanoides*. The brevity of the posterior processes seems to be the common characteristic of these three first-stage nauplii.

The first-stage larva of *B. balanoides* (Fig. 5A) gives promise of the bulky appearance that has been mentioned as a general characteristic of the later naupliar stages; there is nothing that can be added here to the description given earlier (p. 454). The first-stage larvae of *B. crenatus* (Fig. 5B) and *Verruca stroemia* (Fig. 5C) are, in spite of their evident differences shown in these outline diagrams, curiously easy to confuse when examining a plankton haul.

Though the first-stage nauplius of *V. stroemia* has long, well-developed fronto-lateral horns, in formalin-preserved material these are often obscured by the first antennae, which lie over them (Fig. 5C)—it is interesting to find that this had evidently occurred in the specimen which Nilsson-Cantell (1921) used for his figure of the first-stage nauplius of this species. With the fronto-lateral horns obscured in this way, there is little to distinguish the first-stage nauplii of *V. stroemia* and the corresponding stage of *Balanus crenatus*, in ventral view, except size. This confusion can, however, readily be resolved, for if the first-stage larva of *Verruca stroemia* is turned forwards, by a needle placed under the posterior processes, on to its anterior edge, the fronto-lateral

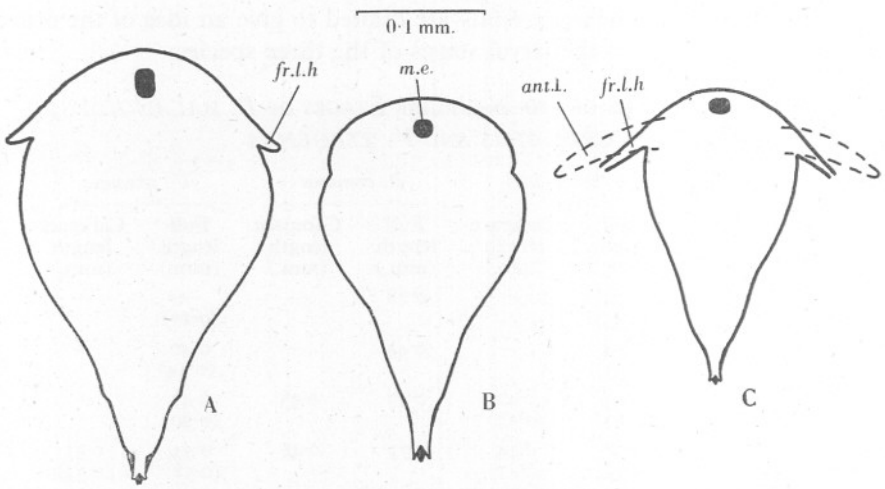

Fig. 5. First stage nauplii of (A) *Balanus balanoides*, (B) *B. crenatus*, and (C) *Verruca stroemia* in ventral view. *ant.*1, first antenna; *fr.l.h.*, fronto-lateral horn; *m.e.*, median eye.

horns are easily seen curving away from the body. Since the fronto-lateral horns of the first-stage nauplius of *Balanus crenatus* are closely bound to the sides of the carapace, this operation does not reveal their presence in this species.

Nilsson-Cantell (1921) has emphasized the ease with which liberation of larvae can take place in the laboratory and has stressed the fact that therefore larval stages may occur under aquarium conditions which are not normally liberated under more natural circumstances. It should therefore be added that all the first-stage larvae described in this section have been recovered from plankton hauls in considerable numbers and there is little doubt that these stages are members of the series of stages normally present in the sea.

Cyprids. Diagrams of the outline of the cyprid larvae of these three species, shown in Fig. 6, indicate that there are considerable differences in size between them, the cyprid of *B. balanoides* (Fig. 6A) being the largest, that of *Verruca stroemia* (Fig. 6C) the smallest.

Confusion is most likely to arise between the cyprid of *Balanus balanoides* and that of *B. crenatus*. The specimens chosen for Figs. 6A and 6B may be considered typical specimens of the two species of cyprids, but the limits of variation in length of the cyprids of the two species unfortunately are such that the biggest *B. crenatus* cyprids are as long as, or slightly longer than, the smallest cyprids of *B. balanoides*. Further, the cyprid of *B. balanoides* seems to vary in length with latitude; Runnström (1925) states that this larva is 1·2 mm. long, whereas Bassindale (1936) found specimens collected at Plymouth to be 0·94 mm. in length. Measurements of this cyprid from the plankton at Millport show that the length varied from 0·9 to 1·1 mm., though most lay within the range 0·98–1·06 mm. This suggests that the cyprid of *B. balanoides* attains a greater length in northern waters.

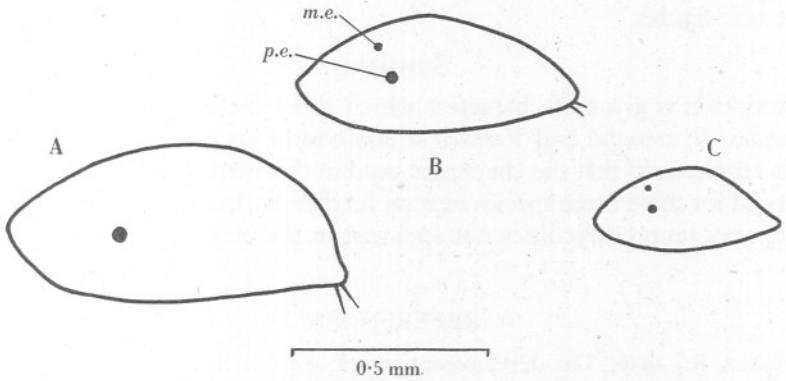

Fig. 6. Cyprid larvae of (A) *Balanus balanoides*, (B) *B. crenatus*, and (C) *Verruca stroemia*. *m.e.*, median eye; *p.e.*, paired eye.

Other characters can, however, be used to distinguish the cyprid of *B. balanoides* from that of *B. crenatus*. Typically, the former is brown in colour, whereas the latter is not coloured in this way. The degree of pigmentation of the cyprid of *B. balanoides* varies considerably, however, and some lightly pigmented specimens may not be easy to distinguish. In these the position of the eyes has proved a useful character. In the cyprid of *B. balanoides* the median eye lies at very much the same distance from the dorsal edge of the carapace, whereas in the cyprid of *B. crenatus* the paired eyes lie at a greater distance from the dorsal edge of the carapace than the median eye. Consequently, in lateral view, only one of the paired eyes can be seen in the cyprid of *B. balanoides* (Fig. 6A), whereas one of the paired eyes and the median eye can be seen in the cyprid of *B. crenatus* (Fig. 6B). The difficulties that may arise in distinguishing these two species of cyprids have been discussed at some length, as experience has shown that awkward specimens do occur, but it should be emphasized that they form only a small fraction of the whole and

that it is possible to distinguish *most B. balanoides* cyprids from *most B. crenatus* cyprids at a glance.

One of the paired eyes and the median eye can also be seen when the cyprid of *Verruca stroemia* is seen from one side, but there is little chance of confusing this cyprid with that of *Balanus crenatus* as the difference in size is pronounced. Moreover, the dorsal border of the carapace of the cyprid of *Verruca stroemia* is practically straight posteriorly, whereas that of the cyprid of *Balanus crenatus* is distinctly rounded.

The author is indebted to the Marine Corrosion Sub-Committee of the British Iron and Steel Research Association for permission to publish this work and to Prof. J. E. Harris for his interest in it. He also wishes to record his grateful thanks to Dr R. B. Pike for his skilled assistance in the preparation of the text-figures.

Summary

A description is given of characters which allow the larval stages of *Balanus balanoides*, *B. crenatus* and *Verruca stroemia* to be identified rapidly.

It is emphasized that the characters used in this method of identification are only valid for these three species *inter se*, further work on similar lines for other species may reveal difficulties not apparent in the present scheme.

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NOTES ON THE BIOLOGY OF CIRRIPEDES

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(Text-figs. 1–18)

CONTENTS

INTRODUCTION

In the course of some four years' work on the fouling problem opportunities have occurred for observations on the general biology of those barnacles whose larvae occur commonly in the plankton and settle in some numbers on surfaces continuously immersed below experimental rafts at Millport.

In his account of the Crustacea of the Clyde sea area, Scott (1901) recorded the presence of the following six free-living species of Cirripedes: *Balanus porcatus* Costa, *B. crenatus* Brug., *B. balanoides* (Linn.), *B. hameri* (Ascan.), *Verruca stroemia* (O. F. Müll.), and *Scalpellum vulgare* Leach.

Two parasitic forms, *Sacculina carcini* (Thomp.) and *Peltogaster paguri* (Rathke), were also recorded then. In the *Annual Reports of the Scottish*

Marine Biological Association for 1919 and 1920 the records of *Chthamalus stellatus* (Poli), *Lepas anatifera* L. and *L. pectinata* Spengler were added to this list, and more recently Moore & Kitching (1939) have estimated the abundance of *Chthamalus stellatus* on the shores of the Isle of Cumbrae.

Examination of tow-nettings shows that the larvae of *Balanus crenatus*, *B. balanoides* and *Verruca stroemia* are by far the most common Cirripede larvae in the plankton. The larvae of *Sacculina* occur in small numbers, chiefly from November to March; the larvae of *Peltogaster paguri* have been recorded practically the year round, and on occasions nauplii and cyprids which cannot be referred with certainty to their species have been present; it is possible that these are the larval stages of *Balanus porcatus*.

On submerged surfaces, the most common species have been *B. crenatus* and *B. balanoides*. *Verruca stroemia*, in spite of its abundance in the plankton, has not yet been recorded on a raft-exposed surface. Small numbers of *Balanus porcatus* have settled from time to time, but the remaining species listed as known in the Clyde have been represented only by a single specimen of *Lepas*, which settled on a raft-exposed surface during the summer of 1942. It should be added, however, that the surfaces available for settlement have not, in general, extended for more than about 4 ft. below the water surface, and this factor has probably limited the range of settling species. Further, the surfaces available have all been exposed vertically, a condition which may also have had its effect on settlement.

Under these circumstances, observations have chiefly been limited to *Balanus balanoides*, *B. crenatus* and *Verruca stroemia*; these are the three species principally discussed in this paper.

BALANUS BALANOIDES

Development of Larvae and their Occurrence in the Plankton

Balanus balanoides liberates its larvae at only one period of the year, during the early spring. Elmhirst (1923) states that fertilization of the ova occurs from August to November, and Moore (1935*a*) that fertilization takes place during November at Port Erin. Observations made in the course of the present study show that by the middle of January the ovigerous lamellae within the mantle cavity contain ova in an advanced stage of development. At this time these lamellae are pale yellow in colour and form firm, discrete masses within the mantle cavity. During the later stages of development of the ova, the colour of the ovigerous lamellae gradually darkens, passing from pale yellow, through yellowish brown to brown and dark brown, finally becoming, as the larvae are ready to hatch, virtually black. These colour changes, presumably due both to the absorption of yolk and to the appearance of dark-coloured structures within the developing larva, afford a means of tracing, roughly, the rate at which the later stages of larval development occur.

Fig. 1 shows the results of the examination of a series of small samples of *B. balanoides* (each sample roughly 100 individuals) during January to May 1946, in which the proportion of individuals containing ovigerous lamellae in the five colour stages mentioned in the preceding paragraph are expressed as a percentage of the whole sample. (In Stage 1 of Fig. 1 the ovigerous lamellae are pale yellow, in Stage 5 they are black in colour.) From this figure it is evident that development was well advanced when the observations were started, and that further development took place rapidly, a small percentage of individuals containing larvae ready to hatch first appearing towards the middle of February. The latter group reached its maximum in the population examined on 8 March and thereafter showed a general decline in importance,

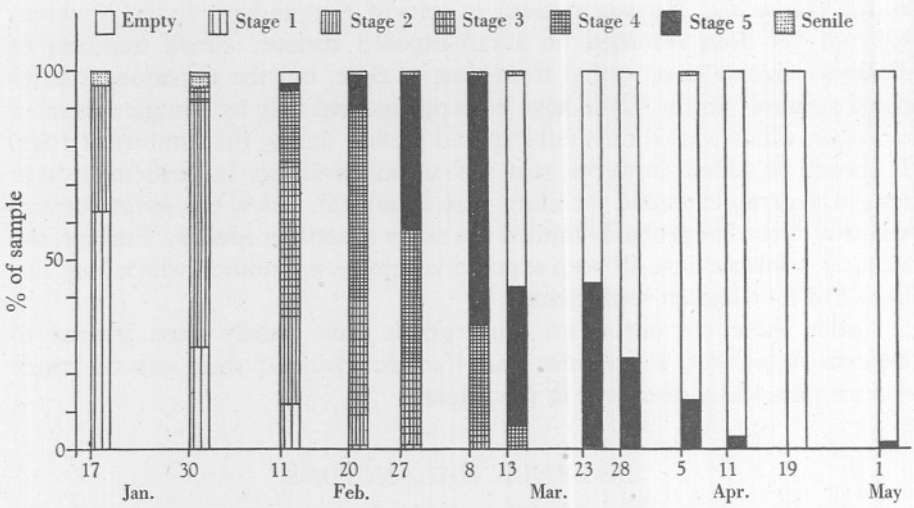

Fig. 1. *Balanus balanoides*. State of development of ova, 1946.

though 2% of the individuals examined on 1 May contained fully developed larvae. Spent individuals were first evident on 13 March and from then onwards progressively dominated the samples examined. These samples of *B. balanoides* were all collected below mid-tide level, that is, from a level below that termed B 1 by Moore (1935*b*).

Contemporaneous plankton hauls clearly indicate that the period at which most adults contain larvae ready to hatch corresponds with the appearance of large numbers of 1st and 2nd stage nauplii in the plankton, and suggest that in this species there is a short period when the majority of the adults liberate their larvae. In 1946 the numbers of these larvae present in the plankton were at a maximum on 7 March. Comparable liberations have occurred in the other years covered by these observations: in 1944 the heaviest swarms occurred on 16 March, in 1945 the maximum occurred on 14 March (though numbers

were small in comparison with other years), and in 1947 the greatest numbers were present on 11 March, though considerable numbers were present from 5 to 14 March.

Following the methods described in a previous paper (Pyefinch, 1948), it has been possible to trace the appearance and relative abundance of the later larval stages in the plankton, and the results for 1947 are given in Fig. 2. In this figure the average numbers of each stage present, over 3-day periods, have been expressed as a percentage of the total numbers of *B. balanoides* larvae present over each period. These and all other plankton hauls mentioned in this paper have been made from Keppel Pier, Millport; the conditions which allow such hauls to be made from this pier have been described in the *Annual Report of the Scottish Marine Biological Association* for 1922 (p. 7). The nets were fished for 1 hr. daily, starting 1 hr. after high water. Variations in tidal flow between a neap and a spring tide are, of course, considerable and this factor alone makes this method of tow-netting useless for any but the most approximate quantitative comparisons; it is for this reason that the data given in Fig. 2 are presented as percentages rather than as absolute numbers. The latter are too strongly influenced by variations in the speed of tidal flow.[1]

1st and 2nd stage nauplii (which may be considered together because the length of life of the 1st stage is very much shorter than that of the 2nd stage) were first present in small numbers on 20 February, 3rd and 4th stage nauplii first appeared on 4 March, 5th stage nauplii followed on 10 March, 6th stage nauplii on 13 March and the first cyprids were recorded on 22 March. This suggests that roughly 1 month elapses between hatching and the appearance of the cyprid. Moore (1935*a*), quoting Johnstone, Scott & Chadwick (1924), states that 2 months separate the naupliar and cyprid maxima in Port Erin Bay, but as the larval stages were not specifically identified, it is possible that this is an over-estimate, covering more than one species.

The histograms shown in Fig. 2 also suggest that at least three maxima of 3rd and 4th stage nauplii occurred, and indications of corresponding maxima are also present for the 5th and 6th naupliar stages. From the dates of occurrence of these maxima it is possible to make tentative suggestions of the life periods of the naupliar and cyprid stages; for the first two maxima shown in Fig. 2 these appear to be as in Table I.

As these estimates are based on 3-day averages, the shortest possible interval is 3 days. The differences in interval indicated in this table, therefore, with the exception of the interval between the 6th stage nauplius and the cyprid of the first 'brood', probably mean little and it would seem that the length of life of each larval stage is approximately the same, except that of the 1st naupliar stage which, as noted earlier, may be much shorter.

[1] As an indication of the numbers of larvae taken, the following are the average daily hauls, over the period shown in Fig. 2, for each larval stage: Nauplius I+II, 2430; Nauplius III, 1525; Nauplius IV, 1915; Nauplius V, 870; Nauplius VI, 710; Cyprid, 1750.

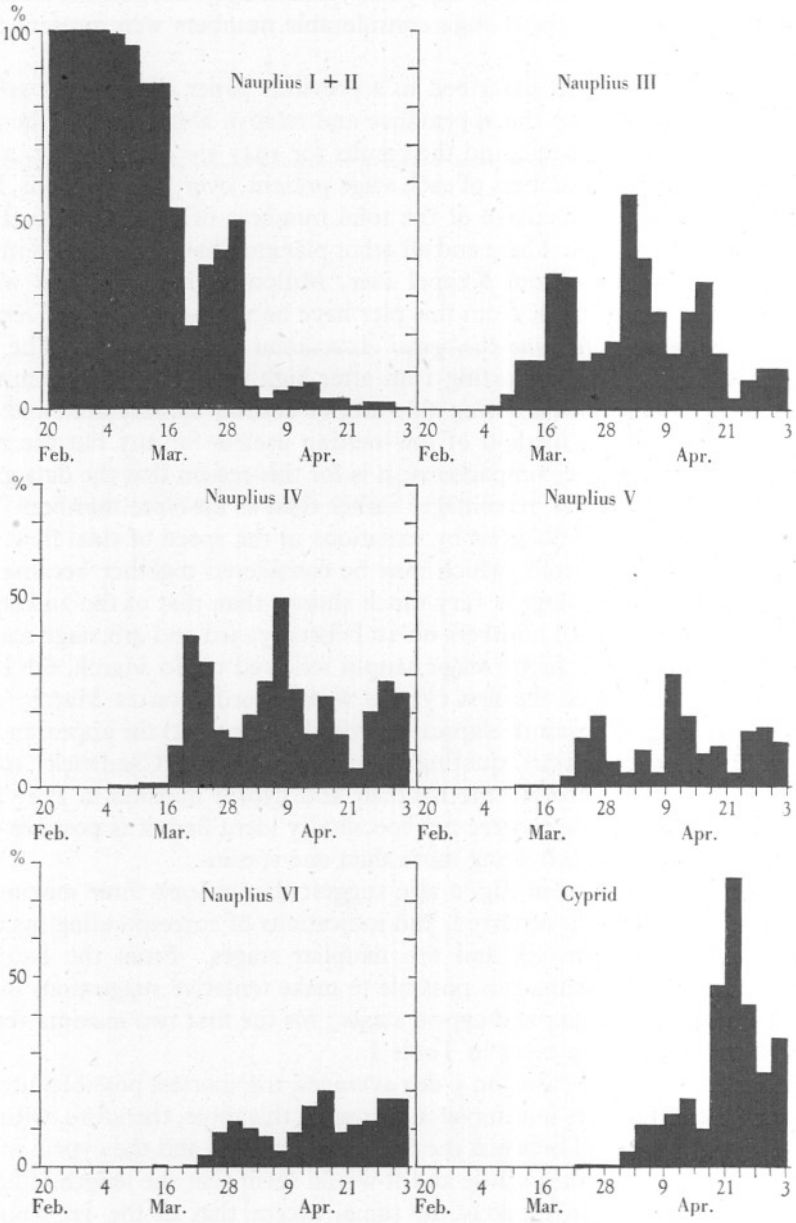

Fig. 2. Occurrence of the larval stages of *Balanus balanoides*, spring 1947. The numbers present, over 3-day periods, of each larval stage are expressed as a percentage of the total *B. balanoides* larvae present.

TABLE I. RATE OF DEVELOPMENT OF *BALANUS BALANOIDES*

Larval stage	Date beginning 3-day period of maximum	Interval (days)	Date beginning 3-day period of maximum	Interval (days)
Nauplius I and II	10. iii.	—	28. iii.	—
Nauplius III	16. iii.	6	31. iii.	3
Nauplius IV	19. iii.	3	6. iv.	3
Nauplius V	25. iii.	6	9. iv.	6
Nauplius VI	28. iii.	3	15. iv.	6
Cyprid	?12. iv.	?15	21. iv.	6

The interval between the 6th stage nauplius and the cyprid of the first 'brood' seems almost certainly too long and there are reasons for supposing that it has been caused by the action of other factors, which will be more fully discussed later (see p. 498). For the present it may be mentioned that it seems likely that the first 'brood' of nauplii was relatively unsuccessful, in the sense that they eventually produced only a small proportion of cyprid larvae; in this respect the second 'brood' was much more successful. The numbers of 1st and 2nd stage nauplii involved in the second maximum—that for the period beginning 28 March (Fig. 2)—were only about 35 % of those involved in the first maximum, yet over 45 % of these were recorded in the cyprid stage, whereas under 15 % of the first maximum were so represented. The number of cyprid larvae recorded from the later hatched nauplii was actually slightly greater than that produced from the earlier group.

The length of life of the cyprid stage is difficult to assess. Visscher (1928) states that the free-swimming period of the cyprid lasts from 3 days to 2 weeks or longer. Observations made on the cyprid of *B. balanoides*, taken from the plankton and stored in tanks in the laboratory, suggest that on the average a period of 5 days elapses before settlement begins. Such an estimate, based on artificial laboratory conditions, is not likely to be accurate and field observations suggest that the interval before settlement is much shorter as, when cyprid larvae are present in large numbers in the plankton, settlement on the shore is virtually coincident with the appearance of these swarms.

Visscher (*loc. cit.*) further states that the oil globules, which are present in some numbers in the tissues of the cyprid, particularly towards the anterior end, disappear towards the end of the free-swimming period. It has not been possible to confirm this observation, either for the cyprid of *B. balanoides* or for that of *B. crenatus*. McDougall (1943) states that he has confirmed Visscher's observation for *B. eburneus* but has not found that the oil globules of the cyprid of *Chthamalus fragilis* disappear. Nevertheless, it seems possible that physiological changes do take place during the free-swimming life of the cyprid, changes which must have reached a certain stage before settlement can occur; it is hoped to discuss this point more fully in a later paper.

These notes on the larval stages of *B. balanoides* have chiefly been based on observations made during 1947; the main outline of the story—liberation of

larvae during the first half of March, appearance of nauplii of the 3rd and later stages during the second half of that month and first appearance of cyprid larvae late in March or early in April—has been fully confirmed by the observations of the preceding 3 years. The differences between one year and another present some features of general interest, which are discussed more fully later in this paper.

Settlement and Metamorphosis

Settlement of the cyprid of *B. balanoides* occurs during April and, over the 4 years covered by this survey, seems to occur most heavily towards the beginning of that month.

Two methods of locomotion are commonly used by the cyprid larva: a swimming motion produced by rapid movements of the thoracic appendages, and a walking motion in which the tips of the antennae are used. It is also of interest to find that the normal response to a slight increase in speed of water movement is one of attachment to the surface; a weak current of water from a pipette can stimulate this clinging reaction and if the water is poured out of a dish or beaker containing *B. balanoides*, the majority of the larvae will remain clinging to the walls of the container. This response is not shown by the cyprid of *B. crenatus*; it is one which would seem of particular importance for an intertidal form. It is not known how far such temporary 'attachments' become permanent; in the laboratory they do not, but there the surfaces to which the cyprid clings are often widely different from those which it would encounter under natural conditions.

Some observations have been made on the cement by means of which secure attachment to the substratum is attained. It is clear that this takes place in two stages. Primarily, permanent attachment is secured by means of a large mass of cement, produced from the cement glands of the cyprid, which surrounds the bases of the antennae. After metamorphosis, this mass of cement lies approximately at the centre of the basis of the young barnacle and for some time constitutes the sole means of attachment to the substratum, so that the barnacle can be rotated about this central, pillar-like support. Later a thin layer of cement is secreted all over the basis, which then becomes attached to the substratum over the whole of its area.

The staining reactions of the cement, both of that secreted by the cyprid and of that secreted later by the young barnacle suggest, as Yonge (1938) has pointed out, that this secretion is identical with the cuticular layer of the integument. This would suggest that the setting of the cement might take place as a result of a similar series of reactions to those outlined by Pryor (1940). Some preliminary observations were made along these lines in the course of this work, but these need considerable amplification before it can be stated that the setting of the attachment cement is due to the 'tanning' of a muco-protein.

FACTORS INFLUENCING SETTLEMENT

Many workers have investigated the factors which seem to affect barnacle settlement, but it is difficult to find much unanimity of opinion as to the relative importance of the various factors concerned. The range of species covered in these investigations is, however, considerable and, as it is reasonable to suppose that any one factor will not necessarily play as important a part in influencing settlement of one species as it will with another, confusion may have arisen because of the range of species used as experimental material. The following notes apply only to the settlement of *B. balanoides* on structures continuously immersed; contrary to general experience, these studies have shown that this species can settle, metamorphose and grow on such surfaces, though settlement is probably less dense than on intertidal surfaces.

Nature of the Surface. In the course of its 'walking' motion over a surface, a cyprid can cover appreciable distances (Visscher, 1928, records that this type of motion may persist for an hour, during which a distance of the order of 12 mm. can be covered) and it is therefore possible for the larva to encounter a range of surface conditions, some of which may be more suitable for settlement than others. Primarily, settlement is usually confined to cracks, crevices and other irregularities of the surface, though, if the supply of settling stages is maintained, this distinction may later disappear. Pomerat & Weiss (1946) have recently described the extent to which settlement can occur on a wide range of substrata and have found wide variations in the populations of barnacles (*B. improvisus* and *B. amphitrite niveus*) which settled on these surfaces during an exposure period lasting 3 months. The results of exposures of a number of different types of surface during the settlement period for *B. balanoides* in 1944, when cyprid larvae were particularly abundant, are given in Table II.

TABLE II. DENSITY OF SETTLEMENT OF *B. BALANOIDES* ON DIFFERENT SURFACES

Coating applied	Nature of surface produced	Density of settlement No./sq.in.
Anti-corrosive paint (non-toxic)	Smooth; slight surface irregularities	105
Cementiferous protective composition	Granular	77
Rosin-paraffin wax	Waxy	90
Stearic acid	Fatty	68
Petroleum jelly-paraffin wax mixture	Greasy	1
Zinc ricinoleate	Surface completely covered with a heavy, jelly-like slime over period of settlement	1
Aluminium ricinoleate	Surface completely covered with a very heavy, jelly-like slime over period of settlement	1

Though settlement on the non-toxic paint surface proved heavier than that on the other types of surface exposed, settlement occurred to an appreciable extent on all surfaces except those covered with slime and that which had a greasy, unstable finish. The slime which developed on the metallic soap surfaces exposed was far heavier and more viscous than that which often develops on a painted surface, but observations made by Dr M. F. Spooner (personal communication) suggest that the latter type of slime (which is most commonly bacterial in origin) can be as effective in preventing settlement. During a very heavy settlement of the cyprid of *B. balanoides* at Caernarvon in April 1943, she noticed that some of the surfaces exposed had acquired a slime film, which in places was stripping away; settlement of cyprids was wholly confined to those areas thus denuded of slime.

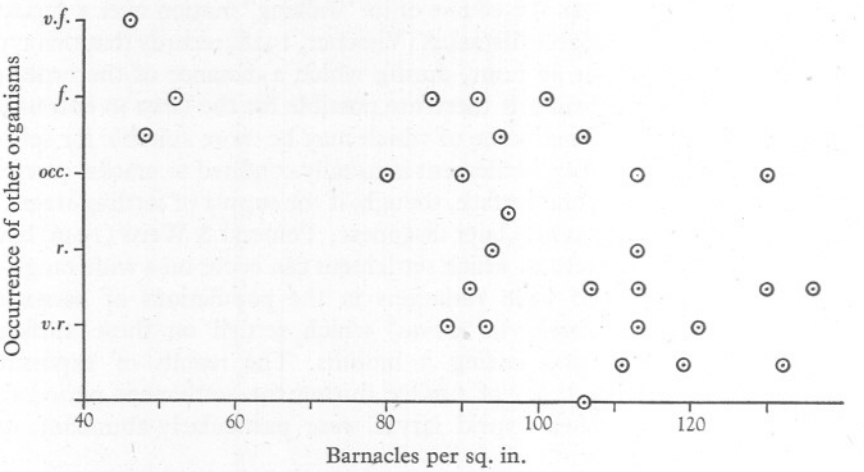

Fig. 3. Effect of the presence of other organisms on the density of settlement of *Balanus balanoides*. The former is classed in eight grades, ranging from 'none' to 'very frequent' (*v.f.*); alternate intermediate grades are denoted 'very rare' (*v.r.*), 'rare' (*r.*), 'occasional' (*occ.*), and 'frequent' (*f.*).

Presence of Other Organisms. Fig. 3 shows the relationship between the density of settlement of *B. balanoides* and the presence of other sedentary forms; in general, the presence of other organisms in any numbers would seem markedly to reduce the density of settlement of this species of barnacle. It should perhaps be added that the other organisms present on these surfaces were *Obelia*, *Ectocarpus* and *Enteromorpha*, since the type of organism present is clearly important; a surface covered with *Mytilus* or with other barnacles will not interfere with settlement to the same extent.

Light and Water Currents. A number of workers (Visscher, 1928; Visscher & Luce, 1928; Neu, 1933), have investigated the effect of light on the settlement of the cyprid larva of a number of barnacle species, and generally seem to

have concluded that this larva reacts negatively to light at the time of its attachment and that barnacle settlement takes place most readily on shaded surfaces. The colour of the substratum has also been found to be of importance, settlement of the cyprid taking place most readily on darker colours (Visscher & Luce, 1928). In their study of the effect of surface angle and of light on the attachment of barnacles, Pomerat & Reiner (1942) conclude that the photic factor is of primary importance in the attachment of *Balanus eburneus*. McDougall (1943), however, suggests that the effect of the quantity and quality of the incident light and the tone of the surface of submerged objects, though they influence the settlement of the cyprid, need further investigation before their action can be regarded as definite.

In the course of this work a series of exposures was made which illustrates the difficulty of drawing definite conclusions on the effect of light on settlement. In the spring of 1944 a number of mild steel panels, each 24 in. × 24 in., coated with a non-toxic paint, were exposed to investigate variations in density of settlement over a limited area. The upper edges of the panels lay some 18 in. below the surface and the fifteen panels exposed were distributed at random through the seven exposure bays available on the raft used for this experiment (see Fig. 4). Settlement of the cyprids of *B. balanoides* was heavy during April 1944, and at the end of this period the panels were withdrawn and the density of settlement estimated, for each panel, by counting the numbers of barnacles present with 2 in. × 2 in. areas chosen at random from each square foot of each face of each plate. Eight areas were thus counted for each panel. The mean density (as individuals/sq.in.) for each face of each plate is given in Fig. 4.

The exposure conditions of the panels situated at the end positions in each bay differed from those situated towards the centre of the bays in that the face towards the outside of the raft (i.e. those towards the nearest end of each bay) was shaded by the presence of a buoyancy drum lying immediately outside it. Inspection of the density of settlement on these faces shows that it was always higher than on the inner, better lit face. This suggests heavier settlement on the shaded surfaces; but an inspection of the density of settlement on the more centrally-situated panels shows similar—or even greater—differences between the two faces of most of them. The lighting of the two faces of these panels was certainly more equal than it was for panels exposed at the ends of the bays and therefore, if light were the most important factor concerned, a greater similarity in density of settlement should have occurred.

Walton Smith (1946) has recently drawn attention to the effect of water currents on the settlement of barnacles and his experiments have shown that permanent settlement of the cyprid can be prevented by currents of the order of 1 knot or less. This factor may clearly also play a part in controlling density of settlement. In the experiments under discussion, those panels at the ends of the bays are not only shaded on their outer faces, but these faces may possibly also, because of the buoyancy drums outside them, be in 'dead' water for

slightly longer periods in each tidal cycle. The limiting current speed for *B. balanoides* is not known, but it would seem possible to suppose that this was exceeded for slightly shorter periods per tidal cycle for the outer faces of these panels than for their inner faces and, given the presence of a considerable number of settling larvae, this might therefore lead to heavier settlement on the outer faces.

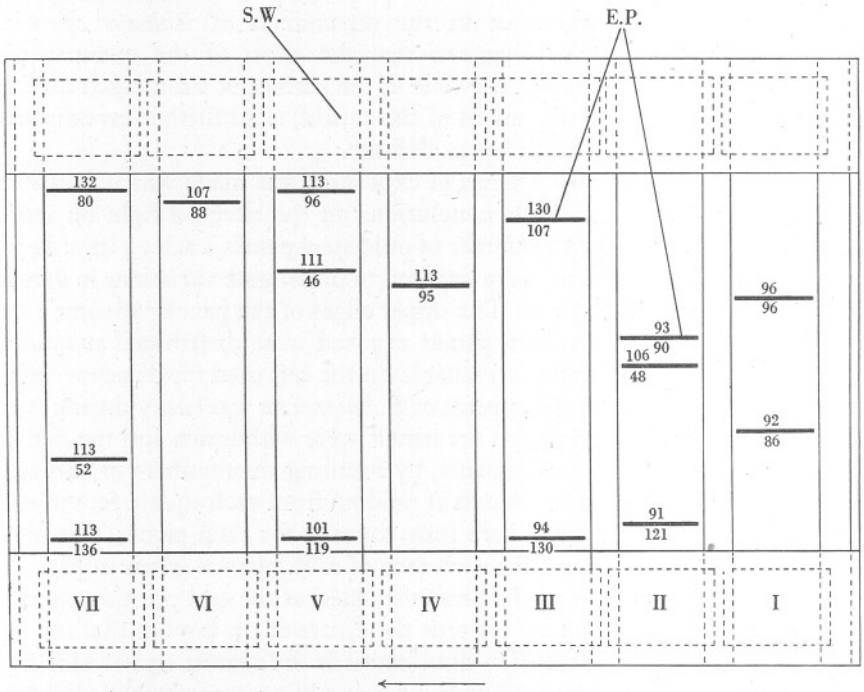

Fig. 4. Plan of raft, showing positions of exposures for observations on the density of settlement of *Balanus balanoides*. The roman numerals indicate the numbers of the exposure bays. The arabic figures, set against each side of the exposure panels (E.P.), represent density of barnacles, as explained in the text. S.W., side-walk with the buoyancy drums beneath. The arrow at the foot of the plan represents direction of the ebb tidal flow. The raft is moored with its ends facing NNE. (bay I) and SSW. (bay VII).

The possibility that current speeds play some part in influencing density of settlement also means that the occurrence of differences in density of settlement on the two faces of panels immersed towards the middle of these bays is less anomalous than if light were the sole controlling factor. This raft is moored so that its long axis lies approximately parallel with the tidal current but the panels suspended from it are not orientated accurately edge on to this current, hence it is possible that this, coupled with the effects of neighbouring panels, might lead to differences in the period of slack water per tidal cycle on the two faces of the panels, and so to differences in density of settlement. Other exposures suggest that marked differences in water flow may be present.

At the site where this exposure raft is moored, the ebb tide (indicated by an arrow on Fig. 4) is far stronger than the flow. If this suggestion of the effect of water currents is correct, therefore, those panels situated towards the middle of the end bay on the 'ebb end' of the raft should, because of the absence of interference from either buoyancy drums or other panels, be likely to show the most similar conditions on their two faces. It is therefore interesting to find that the population densities on the two faces of the panels immersed in this position (i.e. towards the centre of bay 1, see Fig. 4), are virtually identical.

It is clear that this hypothesis that current speeds play at least as important a part in affecting settlement as light can only be advanced tentatively, but laboratory evidence does not indicate that the cyprid of *B. balanoides* reacts negatively to light at the time of attachment. If these larvae are kept in a tank lit from one side, they cluster thickly on the lighted face. Settlement usually begins elsewhere in the tank, but later, when death and settlement have reduced the numbers of free-swimming larvae swarming over the lighted face, settlement takes place readily there, which suggests that lack of settlement over this face initially was due to the mechanical interference of large numbers of free-swimming forms. If the numbers of cyprids are reduced so that crowding on the lighted face is not excessive, settlement starts on that face.

Mention has been made earlier in this section of the effect of colour of substratum on settlement. This survey has provided little additional evidence, which can be regarded as at all conclusive, on this point. Though exposures of non-toxic paint surfaces of different colours, made in 1944, did not indicate any marked differences in density of settlement on the various colours employed, conditions of exposure were not critical enough to allow much importance to be attached to this result.

METAMORPHOSIS

Observations on shore settlements indicate that metamorphosis takes up to 48 hr. to be completed; these observations also emphasize the heavy mortality which occurs during this process, as roughly 50% of the original population observed died during metamorphosis.

DENSITY OF SETTLEMENT

In the course of other experiments, a number of observations have been made on the density of settlement at various stages. The most relevant observations are given in Table III.

TABLE III. POPULATION DENSITIES OF *B. BALANOIDES* AT DIFFERENT STAGES

Stage	Number/sq.in.	Date and place of record
Newly settled cyprids	480	Millport, April 1945
Young barnacles	106	Millport, April 1944
Young barnacles	76	Millport, April 1945
Barnacles, over 2 months old	65	Carnarvon, July 1943

These observations emphasize the heavy mortality which occurs after settlement, but it should be added that the difference between the newly settled cyprid and the young barnacle populations has possibly been over-emphasized, as the former count was made from an intertidal area and may thus include some larvae present on the surface due to the 'clinging' reaction mentioned earlier and which may not have been permanently attached. The density of the 2-month-old population is high compared with that of many intertidal areas, but all the individuals were extremely elongated and resembled the specimens of *B. balanoides* var. *elongatus* Gould. mentioned by Moore (1935*b*).

The Adult Barnacle

It is interesting to find that *B. balanoides*, so typically an intertidal form, can settle and metamorphose in some numbers under conditions of continuous immersion. Settlement under these conditions, moreover, is not confined to the region immediately below the water, as an exposure made in 1944 showed that settlement of *B. balanoides* could take place down to a depth of over 5 ft. below the water-line. Other records are available (Mr R. Elmhirst, personal communication) of the settlement of *B. balanoides* below low-water mark, e.g. it has been found, though not in large numbers, on *Laminaria*. No detailed observations have been made on the growth rate of individuals continuously immersed but it was evident to the eye that growth, at any rate over the period immediately following settlement, was more rapid in continuously immersed individuals than in forms which had settled on the shore. As opportunities for feeding are not limited for the individual below the water-line in the way they are for an intertidal specimen, this might be expected. It would be of interest to know more about the general biology of such individuals, since continuous immersion modifies a number of the factors of the normal habitat of this species. For example, such individuals are not subject to the high temperatures which can occur on the shore during the summer and therefore the curious intermittent growth, described by Hatton & Fischer-Piette (1932), might not occur.

BALANUS CRENATUS

Development of Larvae and their Occurrence in the Plankton

The breeding habits of *B. crenatus* differ markedly from those of *B. balanoides* for, whereas the latter liberates larvae only over a limited period of the year, *B. crenatus* liberates larvae at intervals from early spring onwards through the summer.

Fig. 5 shows the results of a series of examinations of small populations of this species, similar to those made for *B. balanoides*, over a period extending from mid-January to the beginning of May. Four arbitrary stages could be recognized in the development of the larvae, based upon the colour of the

ovigerous lamellae. In the 1st stage the ovigerous lamellae were white in colour and creamy in consistency; microscopic examination showed them to contain unsegmented ova. In the 2nd stage the white colour persisted, but each lamella now formed a discrete mass within the mantle cavity; the ova were beginning to segment. In the 3rd and 4th stages, the ovigerous lamellae became coloured; but at first they became much more transparent in appearance and pink or light brown in colour (stage 3), the colour later darkening to a deep purple (stage 4). In stage 3, the outlines of the larva are clearly visible within each vitelline membrane and the rudiments of the appendages could be detected; in ova from 4th stage ovigerous lamellae the larva was fully formed and the median eye spot visible.

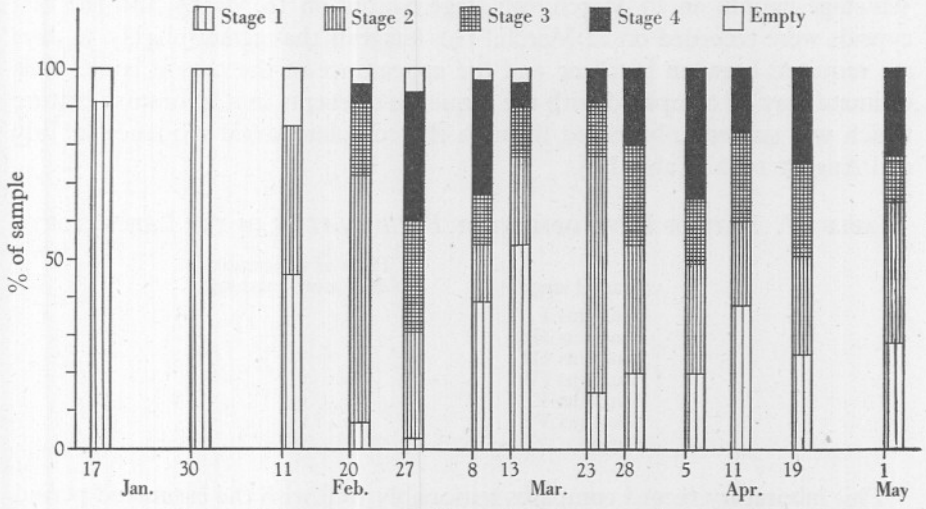

Fig. 5. *Balanus crenatus*. State of development of ova, 1946.

A comparison of Fig. 5 and Fig. 1 suggests that development of the ova started later in *B. crenatus* than in *B. balanoides* but, once started, progressed more rapidly as populations of both species showed a small proportion of individuals containing larvae ready to hatch at about the same time. From then onwards some proportion of the barnacles examined contained larvae ready to hatch. Examinations of samples of *B. crenatus* were continued for the rest of the year and individuals containing larvae ready to hatch were last recorded in a sample collected on 30 August. The maximum percentage of individuals containing larvae ready to hatch, however, fell steadily through the summer, in February 34% was the maximum recorded, in March 30%, in April 34%, May 20%, June 18%, July 8% and in August 6%. This suggests that over each period of liberation, the numbers of larvae liberated decreased and therefore the numbers of cyprids available for settlement should grow steadily less: fig. 8 confirms this.

Fig. 6 presents the analysis of counts of *B. crenatus* larvae for 1947 over the period of the year when they are most abundant—March and April.[1] 1st and 2nd stage nauplii (which have again been grouped together because the 1st stage moults to give the 2nd stage within a few hours of liberation) first appeared in any numbers during the 3-day period beginning 20 February. In 1946 they first appeared in number on 27 February, in 1945 on 4 March and in 1944 on 24 February, so that their appearance in the plankton in 1947 was earlier than in the preceding 3 years. The factors which govern the date on which the early larvae become common in plankton hauls are not known, but there is no direct relationship with water temperature.

3rd stage nauplii first appeared on 4 March; 4th stage nauplii on 7 March, 5th stage nauplii on 13 March, 6th stage nauplii on 16 March, and the first cyprids were recorded on 22 March. This suggests that again roughly 30 days are required between hatching and the appearance of the cyprid larva. This estimate may be compared with the sequence of events in a laboratory culture which was successfully reared through the complete larval sequence in July and August 1942 (Table IV).

TABLE IV. RATE OF DEVELOPMENT OF *B. CRENATUS* IN THE LABORATORY

Larval stage	Time of appearance (days after hatching)
Nauplius I	—
Nauplius II	1
Nauplius III	8
Nauplius IV	11
Nauplius V	13
Nauplius VI	?15
Cyprid	16

This laboratory record compares reasonably well with the estimated period taken from hatching to the appearance of the cyprid in the sea, when it is remembered that development in the laboratory was carried through at a temperature of 15° C. or more, and the sea temperatures prevailing during February and March 1947 were 4–5° C.

Herz (1933), in his account of the larval stages of *B. crenatus*, described eight naupliar stages. In the course of the present work it has not been possible to discover more than six naupliar stages, of which a detailed description will be given in a later paper. Bassindale (1936) has discussed the validity of the 2nd stage nauplius described by Herz. It would seem unlikely that two species of *Balanus* should differ in the number of their naupliar stages, when there are no differences of this nature between species belonging to different genera, as *B. balanoides*, *Verruca stroemia* and *Chthamalus stellatus* each have six naupliar stages (Bassindale, 1936). It is possible, however, that the number

[1] As an indication of the numbers of larvae taken, the following are the average daily hauls, over the period shown in Fig. 6, for each larval stage: Nauplius I+II, 695; Nauplius III, 545; Nauplius IV, 1195; Nauplius V, 1560; Nauplius VI, 955; Cyprid, 970.

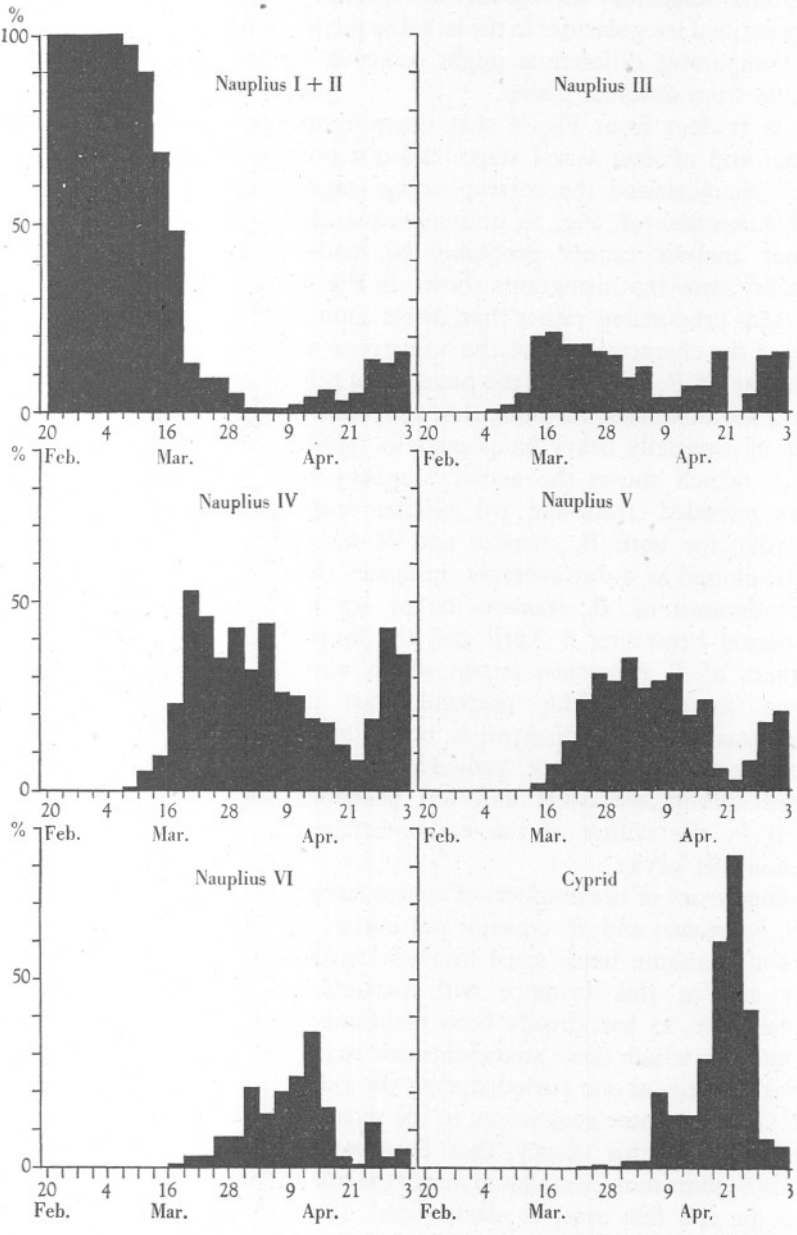

Fig. 6. Occurrence of the larval stages of *Balanus crenatus*, spring 1947. The numbers present, over 3-day periods, of each larval stage are expressed as a percentage of the total *B. crenatus* larvae present.

of naupliar stages may vary for the same species. Macdonald (1927), for example, has described irregularities in the larval sequence of *Meganyctiphanes norvegica*, and comparable differences might occur in the larval sequence of *Balanus crenatus* from different places.

It is evident from Fig. 6 that the proportions of 3rd stage *B. crenatus* nauplii and of later larval stages did not provide the sequence of maxima which characterized the corresponding stages of *B. balanoides* (cf. Fig. 2), and consequently further analysis cannot profitably be made. Further, since the histograms shown in Fig. 6 represent proportions rather than direct counts some of the characteristics of the occurrence of the larvae of *B. crenatus* in the plankton at this period are obscured, in particular, the occurrence of especially heavy hauls early in April. Fig. 7, which shows the actual numbers of larvae recorded (from the 3rd naupliar stage onwards), for both *B. crenatus* and *B. balanoides*, plotted as 3-day averages, indicates the preponderance of *B. crenatus* larvae up to the period beginning 6 April and the larger numbers of *B. balanoides* larvae which were present thereafter. This preponderance of *B. crenatus* larvae early in April is, on the basis of the restricted evidence provided by this limited survey, not usual and was probably caused by the failure of the earlier-hatched *B. balanoides* larvae.

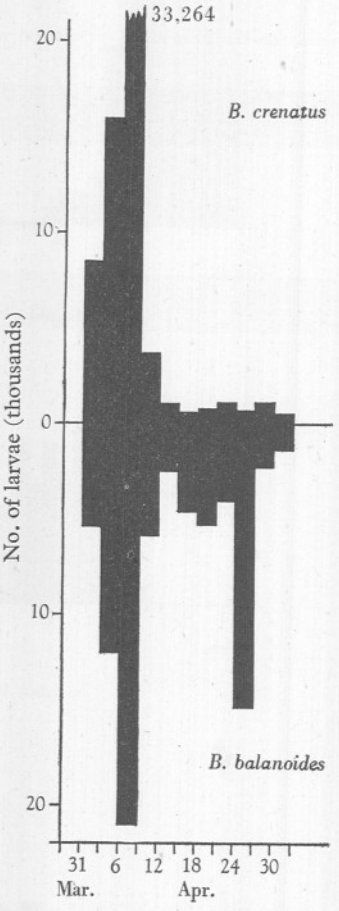

Fig. 7. Numbers of *Balanus crenatus* and *B. balanoides* larvae present in the plankton during April 1947 (shown as 3-day averages).

Comparisons of the numbers of cyprid larvae of *B. balanoides* and *B. crenatus* present in a series of plankton hauls must be made tentatively and in this instance with particular caution since, as has already been mentioned, the hauls on which these statements are based were made only at one period during the tidal cycle. There is some suggestion, in the records accumulated in this survey, that *B. crenatus* cyprids become more abundant later in the tide, i.e. as the ebb falls away to slack water. It is hoped to investigate this point more fully later but if the point is then substantiated, it is evident that the results just discussed may be erroneous in the emphasis laid on the abundance of the cyprids of *B. balanoides*. This point raises the whole question of the possibility of a relationship between larval

abundance and the time in the tidal cycle at which hauls were made. Some observations have been made which indicate that such variations do occur, but a fuller investigation, on a more strictly quantitative basis, is needed before the problem can be discussed further.

Earlier in this paper, an attempt was made to estimate the success of larval development of *B. balanoides* by comparing the numbers of cyprids and the numbers of 1st and 2nd stage nauplii. On this basis the February–March liberation of *B. crenatus* nauplii can be counted as successful, as the cyprids recorded early in April represent nearly 70% of the nauplii hatched at the end of February and early in March.

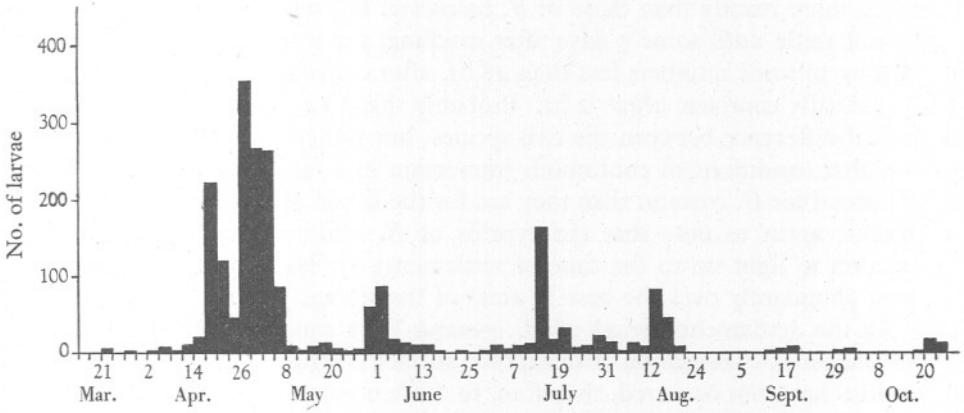

Fig. 8. Occurrence of cyprid larvae of *Balanus crenatus*, 1946. Numbers of larvae per haul in 3-day averages.

Examination of hauls later in the year shows that the larvae of *B. crenatus*, particularly the later-stage nauplii and the cyprids, are most common at intervals through the summer. Fig. 8 shows the recorded occurrence of the cyprids of *B. crenatus* from mid-March to the end of October 1946. Maxima occurred during April (and especially at the end of that month), at the end of May and in the middle of July and August. As these records are based on hauls made from Keppel Pier, these variations might have been caused by variation in tidal strength between springs and neaps[1], but as records for the settlement of this species show that this also was periodic, it would seem likely that these plankton records present a true picture of the situation. This periodicity in the reproduction and settlement of *B. crenatus* does not seem to have been recorded before, but it gives rise to marked differences in size between individuals which have settled, some early and some later in the season. On

[1] Spring tidal periods were: 3–6, 19–20 Feb.; 5–8, 19–22 Mar.; 5, 18 Apr.; 3, 15–18, 31 May; 13, 28 June; 12, 27 July; 11, 25 Aug.; 13–15, 24 Sept.; 13–15, 27 Oct., 1946 and 9, 22–26 Feb.; 10–12, 25 Mar.; 8, 23 Apr., 1947.

occasion, the assumption that *B. crenatus*, like *B. balanoides*, settles only at one period of the year has led to misinterpretations. For example, Foxon (1940) bases statements on the period for which the externa of *Sacculina carcini* had been in place on the occurrence of specimens of *Balanus crenatus* of two sizes on the carapace of the host. The crabs were collected in August, so it would seem quite possible that all the *B. crenatus* present had settled during the spring and summer of the same year, and did not, as Foxon supposes, belong to settlements of two successive years.

Settlement

On the basis of laboratory observations, the cyprids of *B. crenatus* settle much more readily than those of *B. balanoides* for, whereas the latter usually do not settle until some 5 days after catching, the cyprids of *B. crenatus* are settling in some numbers less than 48 hr. after collection and settlement may be virtually complete after 72 hr. Probably this does not represent a physiological difference between the two species, but rather is an expression of the fact that conditions of continuous immersion in a tank of sea water are less abnormal for *B. crenatus* than they are for the larvae of *B. balanoides*. It is of interest again to note that the cyprids of *B. crenatus* retain their positive reaction to light up to the time of settlement; the latter process takes place most abundantly over the best-lit areas of the storage tank.

As the settlement period of *B. crenatus* is so much longer than that of *B. balanoides*, colonization of an exposed surface is rarely so intense and opportunities have not occurred, therefore, to make observations on the conditions affecting settlement of *B. crenatus* comparable with those made for *B. balanoides*, though no indications have been noticed which would suggest that the reactions of the cyprid of *B. crenatus* to slimy or fouled surfaces differ markedly from those of the cyprid of *B. balanoides*.

As might be expected for a species with a normal vertical range lying mainly below low-water mark, *B. crenatus* settles in greater abundance at some distance below the water-line. Table V gives the density of settlement at various depths on a surface exposed from late June to late August 1945 which is typical of the vertical distribution of this species. In confirmation of the periodicity of settlement, this population was also distinctly bi-modal,

Table V. Vertical Distribution of *B. crenatus*

Depth below water-line (ft.)	Nos. (barnacles per sq.ft.)
0·5–1·5	1
1·5–2·5	10
2·5–3·5	8·5
3·5–4·5	32
4·5–5·5	62·5
5·5–6·5	120·5
6·5–7·5	128
7·5–8·5	100·5

consisting of a recent settlement—probably early August—and an earlier settlement, probably early July.

From this table it is evident that settlement has been heavier some distance below the water-line; 75% of the settlement was limited to depths greater than 5 ft. It should, however, be emphasized that these data have been obtained from a floating object, since the surface was immersed from a raft.

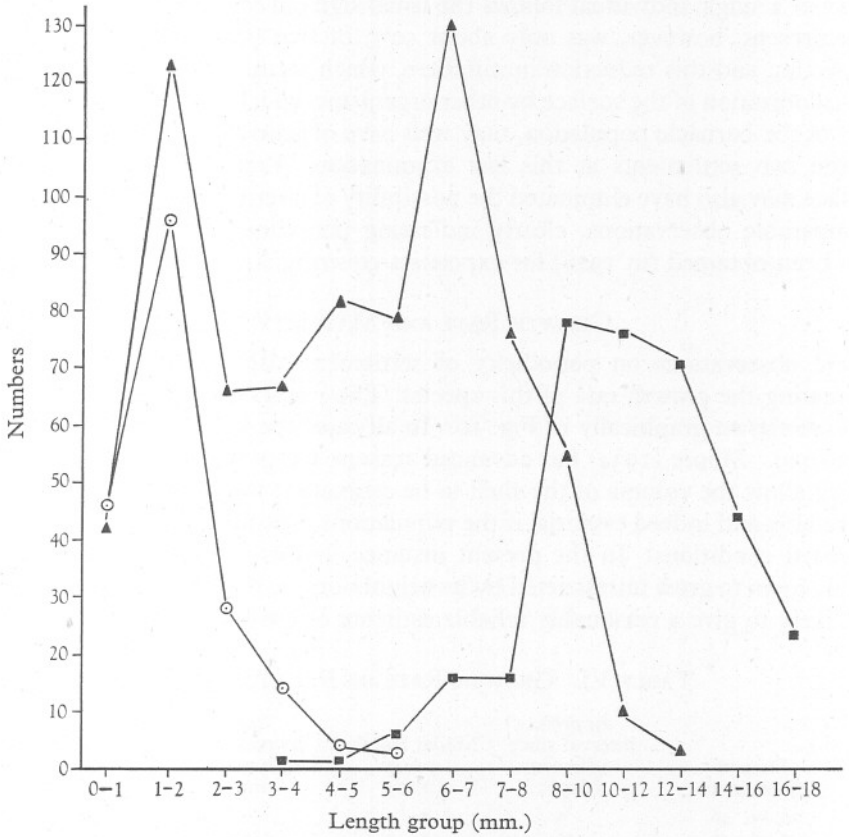

Fig. 9. Analyses of populations of *Balanus crenatus*, 1943.
—⊙— 26 June; —▲— 24 July; —■— 14 Sept.

The characteristic feature of the settlement of *B. crenatus* is its periodicity. Fig. 9 gives records of the measurements made on a population of *B. crenatus* which settled on a surface first exposed towards the end of May 1943. 32 days later 191 *B. crenatus* had settled on one face (4 sq.ft.), their modal length group (1–2 mm.) indicating that settlement had occurred shortly before the surface was examined. Roughly 2 months after immersion the population had risen to 735 over the same face and the length-group distribution was distinctly bi-modal; one mode occurring again in the 1–2 mm. basal length

group (and therefore representing a recent settlement) and the other (presumably representing the June settlement) in the 6–7 mm. group. Just over 2 months later, the surface was again examined and the population again proved to be uni-modal, with a rather ill-defined mode in the 8–10 mm. group. This single mode could have been produced by the merging of the two settlements so evident at the earlier inspection if it is assumed that the growth curve of a single individual follows the usual sigmoid form. The total population present, however, was only about 50% of that present at the previous inspection and this reduction in numbers, which seemed to have been due to the colonization of the surface by other organisms, which smothered and killed part of the barnacle population, may well have obscured the true relationship of the two settlements at this last examination. Prior colonization of the surface may also have eliminated the possibility of a settlement during August. Comparable observations, clearly indicating periodicity of settlement, have also been obtained (in 1946) for exposures covering April and May.

Growth Rate and Maturity

These observations on periodicity of settlement also provide a means of estimating the growth rate of this species. The results are given in Table VI and are shown graphically in Fig. 10. In all cases the length of the basis was measured. Moore (1934) has advanced reasons for preferring measurements which allow the volume of the shell to be estimated; this estimation is clearly preferable and indeed essential if the populations measured are growing under crowded conditions. In the present instance, however, each individual had ample room to grow unrestricted by its neighbours, so that basis measurements are likely to give a reasonably reliable estimate of growth rate.

Table VI. Growth Rate of *B. crenatus*

Date of examination	Approx. interval since settlement (days)	Modal length group (mm.)	Mean increase in length per day (mm.)	Maximum length group (mm.)
30. iv. 46	5	0– 1	—	—
5. vi. 46	40	5– 6	0·14	—
2. vii. 46	67	10–12	0·20	14–16
26. vi. 43	10	1– 2	0·15	—
24. vii. 43	39	6– 7	0·17	—
14. ix. 43	91	10–12	0·12	20–22
25. x. 46	?40	5– 6	0·14	—
17. xii. 46	?93	6– 7	0·02	—
25. i. 47	?132	6– 7	0	—
27. ii. 47	?165	6– 7	0	8–10
19. v. 47	?246	10–12	0·06	—
19. vi. 47	?277	14–16	0·13	18–20

Growth evidently takes place steadily through the summer months, but is arrested during the winter. There is no suggestion, for this species, of the

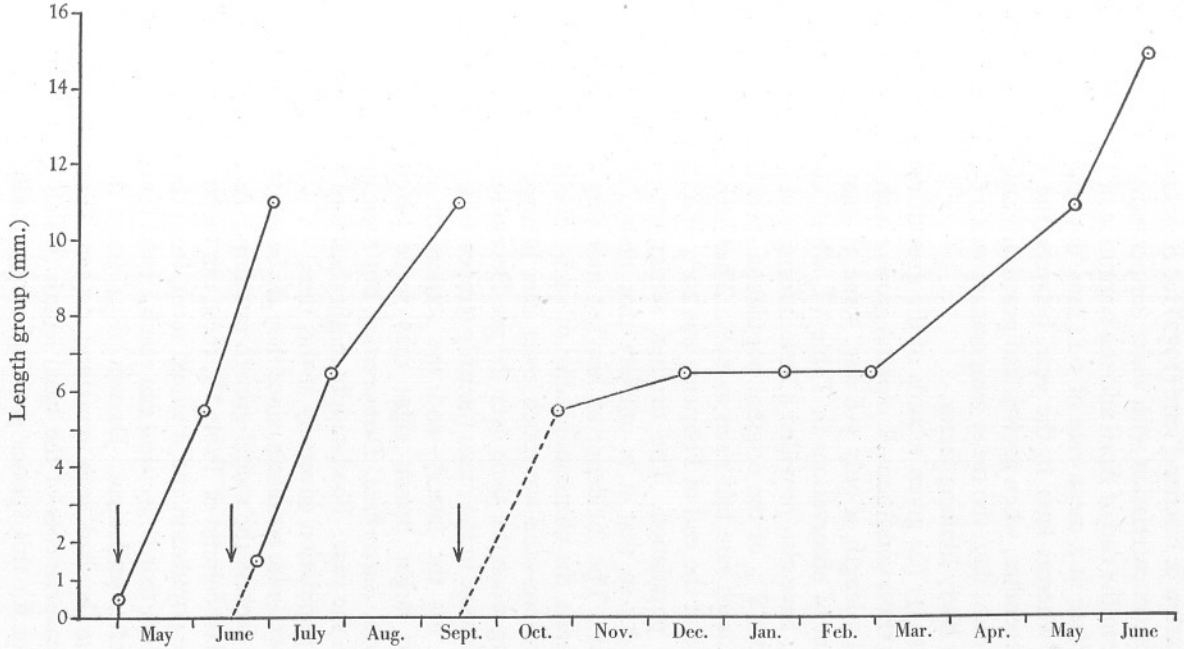

Fig. 10. Growth rate of *Balanus crenatus*. The arrows indicate the approximate date of settlement.

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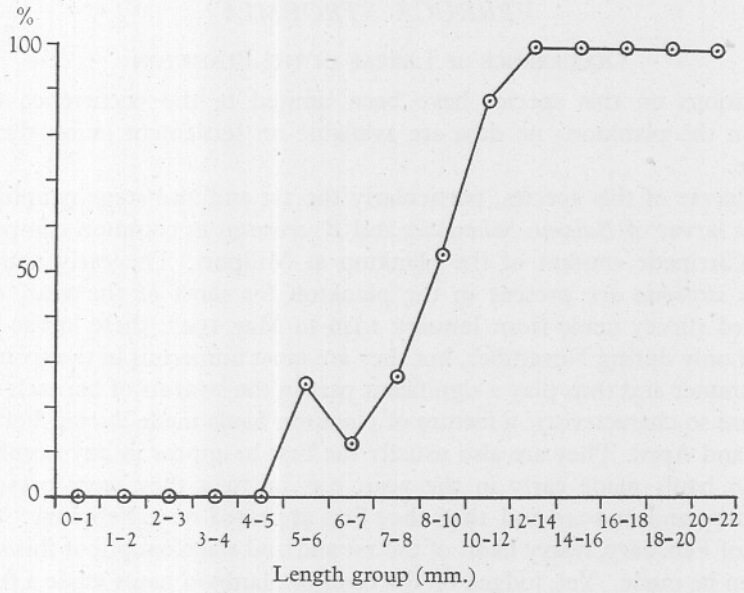

Fig. 11. Occurrence of developing ova in young *Balanus crenatus*, expressed as percentage of individuals containing them.

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Individuals which settle in the autumn are mature, as perhaps might be expected from the foregoing observation, the following spring. For example, a proportion (roughly 30%) of the population which settled in the autumn of 1946 (see Table VI) was examined in May 1947. 77% of these individuals contained developing ova at some stage, and of those in this condition roughly half contained larvae ready to hatch.

Moore (1934) finds that most of the 'lower- and middle-zone (*B. balanoides*) die in their 3rd year, but those from the top zone may live for 5 years or more'. Exact data of the length of life of *B. crenatus* are not available, but the impression gained is that the latter species has a shorter life than even the 'lower- and middle-zone' *B. balanoides*; it seems possible that a *B. crenatus* which settles in the spring, may die in the autumn of the following year. Present evidence suggests that it spawns twice and possibly three times during this period, once later in the summer of the year of settlement, once the following spring and possibly once again later that year.

VERRUCA STROEMIA

Occurrence of Larvae in the Plankton

Observations on this species have been limited to the occurrence of the larvae in the plankton; no data are available on settlement or on the adult barnacle.

The larvae of this species, particularly the 1st and 2nd stage nauplii, rank with the larvae of *Balanus balanoides* and *B. crenatus* as common components of the Cirripede content of the plankton at Millport. The early nauplii of *Verruca stroemia* are present in the plankton for most of the year, e.g. in a detailed survey made from January 1946 to May 1947, these larvae lacked a record only during November, but they are most numerous in the spring and early summer and thus play a significant part in the swarms of barnacle larvae which are so characteristic a feature of plankton hauls made during February, March and April. They are also usually the first to appear in any numbers in plankton hauls made early in the year, e.g. in 1944 they were present on 3 February and in 1945 and 1946 they first appeared on 2 February. By the middle of February, heavy hauls of the 1st and 2nd stage nauplii of this species can often be made. Yet, judged on the basis of plankton hauls made a few feet below the surface, these large numbers of 1st and 2nd stage nauplii which appear early in the year are not immediately followed by the appearance of later nauplii or cyprids, as with *Balanus balanoides* and *B. crenatus*. Fig. 12, which shows the proportion of (*a*) the 1st and 2nd stage nauplii and (*b*) nauplii from the 3rd stage onwards (plotted as 3-day averages) which occurred during the early months of 1947, emphasizes this point. From 20 February until 16 March the only stages present were the 1st and 2nd stage nauplii, the later naupliar stages (3rd stage onwards) did not assume any considerable proportions until 6 April, and cyprids did not appear until the period beginning 30 April.

Further, the period between 20 February and 16 March was characterized by a preponderance of 1st stage larvae—during this period nearly 46,000 1st stage nauplii were recorded, but only just over 9000 2nd stage nauplii. Nilsson-Cantell (1921) found at Bohuslän, W. Sweden, that the 1st naupliar

stage occupied about a week, and this prolonged preponderance of 1st stage nauplii in the Clyde plankton suggests that this stage has a longer life period than that of the corresponding stages of *B. balanoides* and *B. crenatus*. In the laboratory, however, the 1st stage nauplius of *Verruca stroemia* moults to give the 2nd stage within a few hours of hatching.

The general sequence of events just described for 1947 also characterized the planktonic occurrence of the larvae of this species for the other years over which this survey extended. Fig. 13 shows the results obtained in 1946. Again, heavy hauls of early larvae were taken early in the year (with a preponderance of 1st stage nauplii, though not quite so marked as in 1947), no heavy hauls of late stage nauplii were made until much later and no cyprids were recorded until towards the end of April.

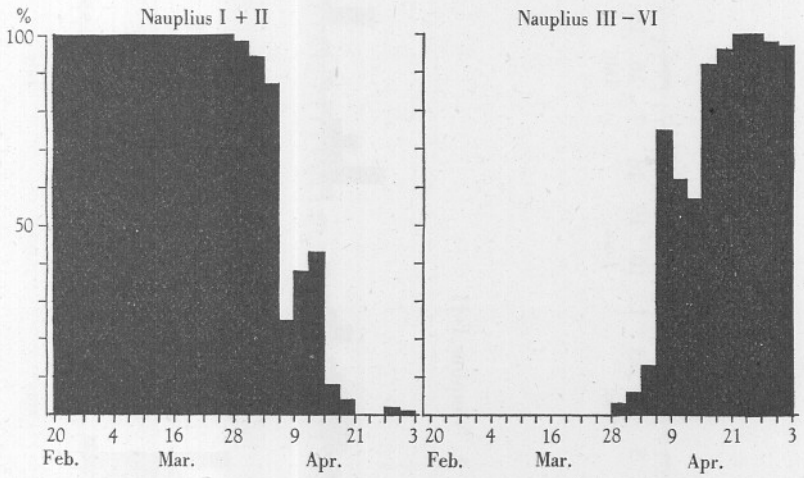


Fig. 12. Occurrence of the larval stages of *Verruca stroemia*, spring 1947. The numbers present, over 3-day periods, of each group of larval stages are expressed as a percentage of the total *V. stroemia* larvae present. (Cyprids were absent except for 2% during the very last period.)

In the absence of settlement data for *Verruca*, any suggested explanation of this situation must remain tentative, but there seem two outstanding possibilities. First, it is possible that only the earlier stages of naupliar development occur near the surface, the later-stage nauplii and the cyprids occurring in any numbers only in deeper water, so that the surface records for these stages are misleading. Secondly, it is possible that the record presented by these hauls is substantially correct and that the sequence of larval stages of *V. stroemia* extends over a period which is much longer than that of the two species of *Balanus* discussed earlier. If the first possibility is correct, it is difficult to see why the periods of abundance of early- and later-stage nauplii should approximate more closely later in the season, whereas if the second possibility is

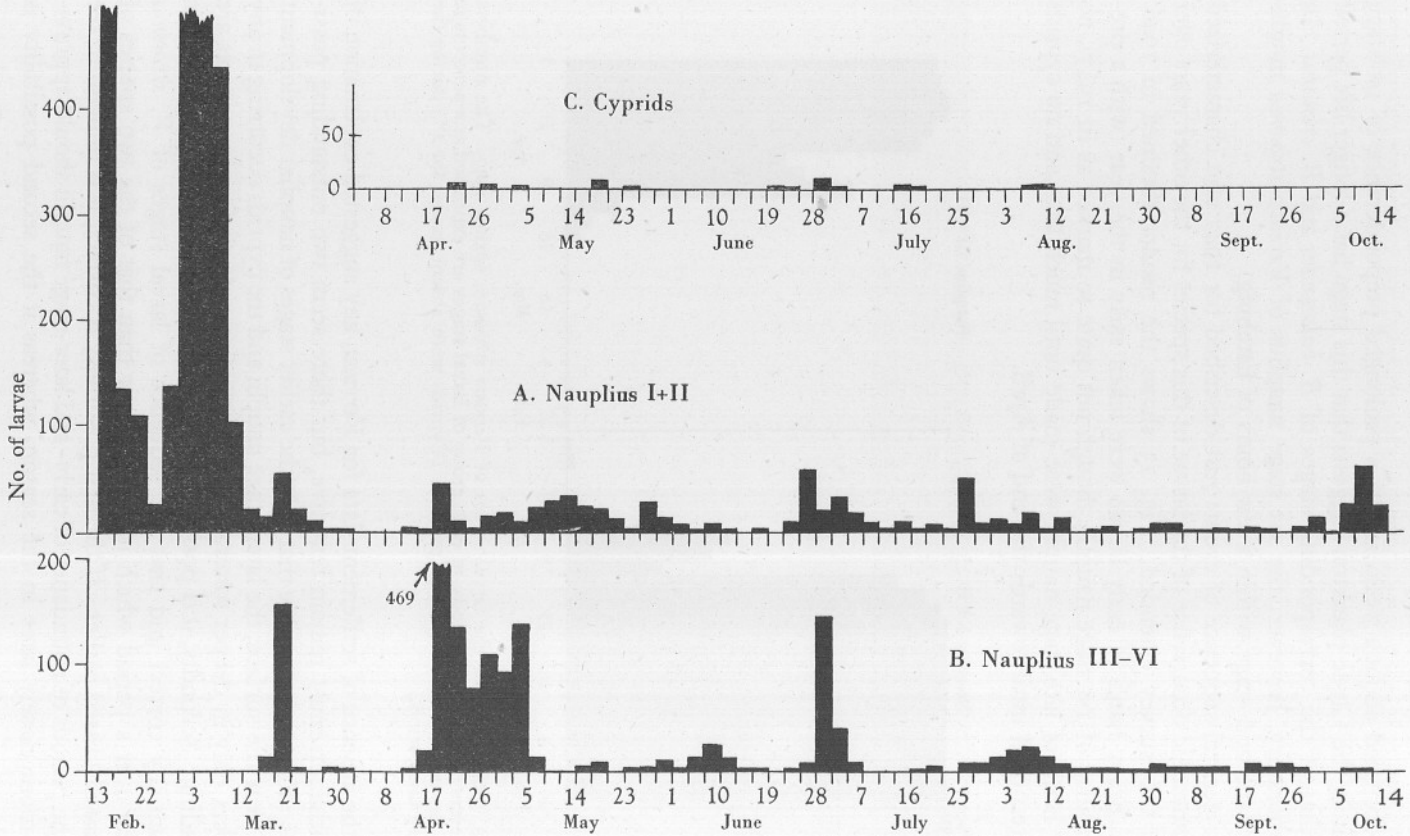


Fig. 13. Occurrence of the larval stages of *Verruca stroemia*, 1946, expressed as the number of larvae per haul, in 3-day averages. A, nauplii I and II. B, nauplii III–VI. C, cyprids.

correct a closer correspondence between periods of abundance later in the year might be expected, as rising water temperatures would presumably accelerate larval development. If the larval sequence does take place slowly early in the season, the early-stage larval maximum (Fig. 13 A) beginning on 13 February could be correlated with the later-stage maximum on 18 March (Fig. 13 B) and possibly with the cyprids recorded around 20 April (Fig. 13 C); the early-stage maximum on 28 February with the later-stage maximum beginning on 17 April and the cyprids occurring in the following month, suggesting that the full larval sequence, from hatching to the appearance of the cyprid, takes roughly 2 months at this season. Such links can only be tentatively suggested, particularly as the cyprid stages are never at all common. There are times during the spring and summer when the later-stage nauplii of *Verruca* are reasonably common (e.g. over 2000 occurred in one haul, made on 30 April 1947) but they do not seem to be followed by comparable hauls of the cyprid larva. Perhaps the latter only occurs in numbers in deeper water and, though slow development may be the explanation of the lack of immediate correspondence between the early- and later-stage nauplii of *Verruca*, surface hauls are not a reliable indication of the abundance of the cyprid larvae.

Though nothing is known of the settlement of this species, it can be predicted, from what is known of the occurrence of the nauplii, that it extends at least over the period April to September and there is a further suggestion that, like that of *Balanus crenatus*, it may be periodic. Fischer-Piette (1932), however, has recorded the appearance of young *Verruca* in abundance on the shore on 7 December and he therefore suggests that the approximate time of settlement lies between September and December. This is rather later than the present observations would suggest.

OTHER CIRRIPEDE LARVAE

The examination of plankton hauls made, for the most part daily, during 1946–47 has afforded an opportunity to assess the abundance of other forms. The only larvae that occurred at all regularly were the nauplii of *Sacculina* (probably *S. carcini*) and those of *Peltogaster paguri*. The *Peltogaster* larvae were at first puzzling, since they differ radically from the figures of the nauplius given by Boschma (1927), notably in possessing a shield-like expansion of the integument, such as Hoek (1909) figures for *P. purpureus*. They agree, however, with the re-description of the nauplius of *P. paguri* given by Reinhard (1946)[1]; and, furthermore, larvae identical with those found in the plankton have been obtained from specimens of *P. paguri* on *Eupagurus bernhardus* collected locally.

The records of these two nauplii are given in Table 7. *Peltogaster* nauplii were present in 10 out of the 14 months covered and it would seem possible

[1] Dr. Boschma kindly drew my attention to this paper.

that they are liberated all the year round. *Sacculina* larvae, however, proved to be more abundant during the winter months, from November to February or March.

TABLE VII. OCCURRENCE OF THE NAUPLII OF *PELTOGASTER* AND *SACCULINA*

Month	Nauplii recorded	
	Peltogaster	*Sacculina*
February (1946)	4	4
March	0	10
April	0	0
May	12	0
June	4	0
July	19	1
August	4	0
September	2	0
October	0	5
November	0	33
December	6	37
January (1947)	8	24
February	29	34
March	10	0

It is of interest to note that these records of the occurrence of *Sacculina* nauplii in the plankton confirm in part the conclusions drawn by Foxon (1940) about the breeding season of this parasite in the Clyde.

DISCUSSION

The descriptions given earlier in this paper of the seasonal occurrence of barnacle larvae in the plankton have clearly indicated their abundance during the months of February, March and April. Tables VIII and IX below set out the average hauls of barnacle larvae per day, for each month of the year, based on the records of this 4-year survey.

In both tables a dash indicates that no hauls were made during that particular month.

The sequence of larvae during the spring outburst is similar year by year. Early-stage nauplii of *Verruca stroemia* and early-stage nauplii of *Balanus crenatus* are the first to appear, usually during the latter part of February and early in March. Early-stage nauplii of *B. balanoides* first appear early in March and reach a maximum towards the middle of that month; the second half of March is characterized by the presence of large numbers of later-stage nauplii of *B. crenatus* and *B. balanoides* sometimes (e.g. in 1946) accompanied by smaller numbers of later-stage nauplii of *Verruca stroemia*. The end of March or the beginning of April sees the appearance of the first cyprid stages and during the first half of the latter month the cyprid of *Balanus balanoides* tends to predominate. During the second half of April the numbers of Cirripede

larvae usually decrease and by the end of that month, or early in May, they are reduced to the more modest proportions which they maintain, in general, for the rest of the summer. The numbers recorded after the end of October are negligible. Figs. 14–17 show the details of the records of the nauplii and cyprids of *B. balanoides*, *B. crenatus* and *Verruca stroemia* during the spring outburst in 2 successive years, 1946 and 1947.

TABLE VIII. ALL CIRRIPEDE NAUPLII MONTH BY MONTH

Expressed as the average number per daily haul

	Year			
Month	1944	1945	1946	1947
January	—	0	0	1
February	1716	21	607	419
March	2846	274	2034	13644
April	515	52	725	12199
May	7	—	136	.
June	—	—	50	.
July	—	—	25	.
August	—	—	21	.
September	—	—	9	.
October	—	—	30	.
November	—	—	0	.
December	—	—	1	.

TABLE IX. ALL CYPRID LARVAE MONTH BY MONTH

Expressed as the average number per daily haul

	Year			
Month	1944	1945	1946	1947
January	—	0	0	0
February	0	0	0	0
March	15	5	1	22
April	1491	137	110	2918
May	35	—	68	.
June	—	—	17	.
July	—	—	28	.
August	—	—	17	.
September	—	—	1	.
October	—	—	3	.
November	—	—	0	.
December	—	—	0	.

The figures given in Tables VIII and IX also emphasize the enormous variations that can occur from year to year. For detailed quantitative comparisons, the fishing methods used in this survey were crude, but even so, a difference such as that between the average catch of all stages of all Cirripede nauplii for March 1945 (274 nauplii) and that recorded for the corresponding month 2 years later (13,644 nauplii)—the latter practically fifty times the former—is great enough to be significant. Johnstone, Scott & Chadwick (1924) give figures for *Balanus balanoides* nauplii and cyprids for the period

1907–20, and it is of interest to find that even greater variations appear in their records, e.g. more than two thousand times the number of nauplii were recorded in March 1912 than in March 1907.

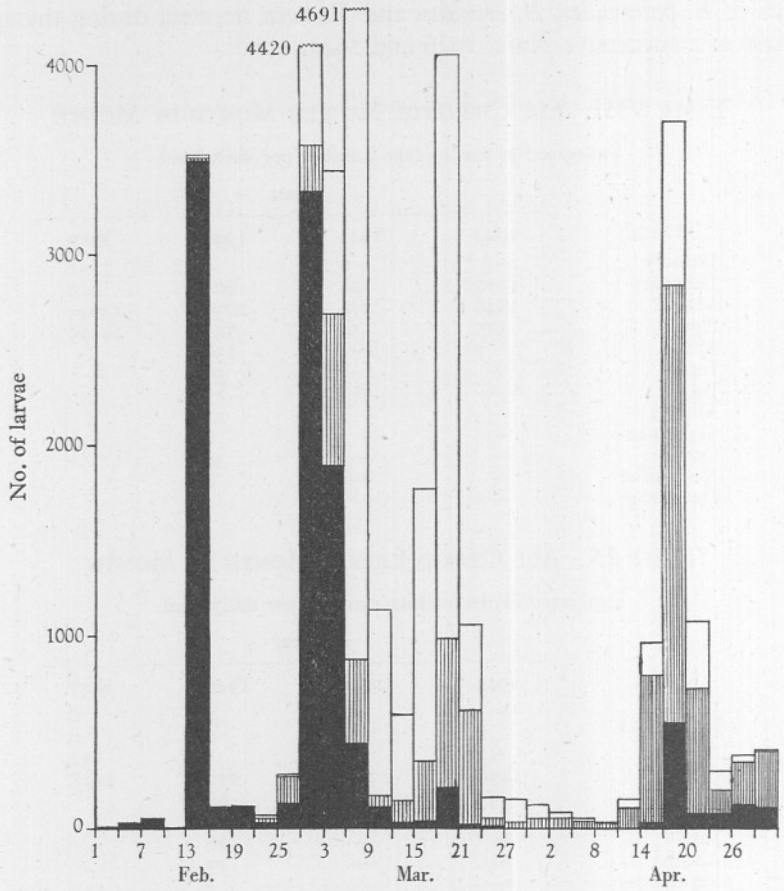


Fig. 14. Occurrence of Cirripede nauplii, Feb.–Apr. 1946. The shaded areas within each histogram indicate the numbers of the larvae of *Verruca stroemia*; the vertically hatched areas the numbers of *Balanus crenatus* larvae; the remaining areas the numbers of *B. balanoides* larvae.

Direct comparisons between the records obtained in the present survey and those obtained by Johnstone, Scott & Chadwick (1924) are impossible since fishing conditions were entirely different in the two surveys. One point, however, seems worthy of mention. Johnstone, Scott & Chadwick (*loc. cit.* p. 66) drew attention to the great discrepancy between the numbers of nauplii and the numbers of cyprids caught, and attributed this to the destruction of the nauplii during their period of development and to the dispersal of the swarms.

consisting of a recent settlement—probably early August—and an earlier settlement, probably early July.

From this table it is evident that settlement has been heavier some distance below the water-line; 75% of the settlement was limited to depths greater than 5 ft. It should, however, be emphasized that these data have been obtained from a floating object, since the surface was immersed from a raft.

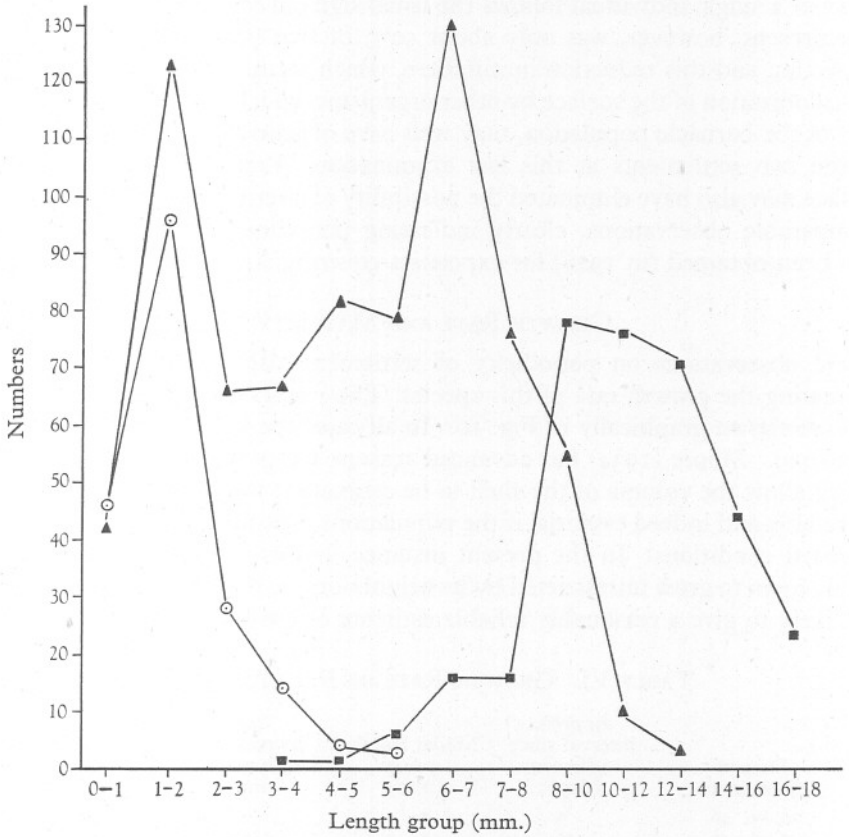


Fig. 9. Analyses of populations of *Balanus crenatus*, 1943.
—⊙— 26 June; —▲— 24 July; —■— 14 Sept.

The characteristic feature of the settlement of *B. crenatus* is its periodicity. Fig. 9 gives records of the measurements made on a population of *B. crenatus* which settled on a surface first exposed towards the end of May 1943. 32 days later 191 *B. crenatus* had settled on one face (4 sq.ft.), their modal length group (1–2 mm.) indicating that settlement had occurred shortly before the surface was examined. Roughly 2 months after immersion the population had risen to 735 over the same face and the length-group distribution was distinctly bi-modal; one mode occurring again in the 1–2 mm. basal length

group (and therefore representing a recent settlement) and the other (presumably representing the June settlement) in the 6–7 mm. group. Just over 2 months later, the surface was again examined and the population again proved to be uni-modal, with a rather ill-defined mode in the 8–10 mm. group. This single mode could have been produced by the merging of the two settlements so evident at the earlier inspection if it is assumed that the growth curve of a single individual follows the usual sigmoid form. The total population present, however, was only about 50% of that present at the previous inspection and this reduction in numbers, which seemed to have been due to the colonization of the surface by other organisms, which smothered and killed part of the barnacle population, may well have obscured the true relationship of the two settlements at this last examination. Prior colonization of the surface may also have eliminated the possibility of a settlement during August. Comparable observations, clearly indicating periodicity of settlement, have also been obtained (in 1946) for exposures covering April and May.

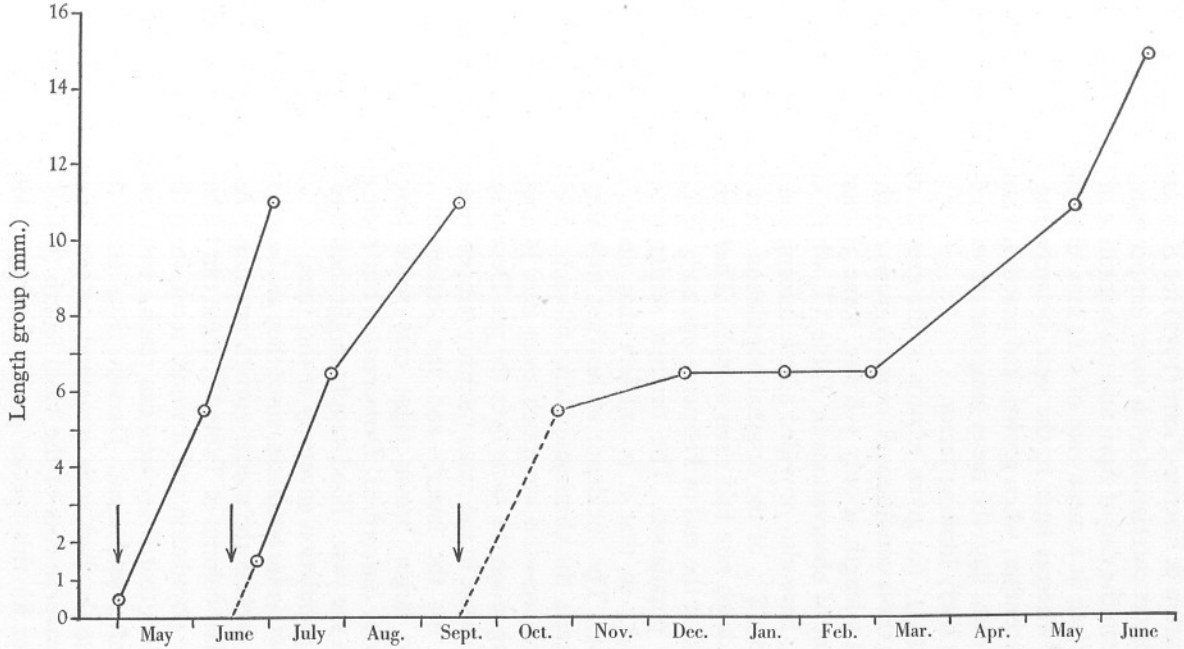
Growth Rate and Maturity

These observations on periodicity of settlement also provide a means of estimating the growth rate of this species. The results are given in Table VI and are shown graphically in Fig. 10. In all cases the length of the basis was measured. Moore (1934) has advanced reasons for preferring measurements which allow the volume of the shell to be estimated; this estimation is clearly preferable and indeed essential if the populations measured are growing under crowded conditions. In the present instance, however, each individual had ample room to grow unrestricted by its neighbours, so that basis measurements are likely to give a reasonably reliable estimate of growth rate.

Table VI. Growth Rate of *B. crenatus*

Date of examination	Approx. interval since settlement (days)	Modal length group (mm.)	Mean increase in length per day (mm.)	Maximum length group (mm.)
30. iv. 46	5	0– 1	—	—
5. vi. 46	40	5– 6	0·14	—
2. vii. 46	67	10–12	0·20	14–16
26. vi. 43	10	1– 2	0·15	—
24. vii. 43	39	6– 7	0·17	—
14. ix. 43	91	10–12	0·12	20–22
25. x. 46	?40	5– 6	0·14	—
17. xii. 46	?93	6– 7	0·02	—
25. i. 47	?132	6– 7	0	—
27. ii. 47	?165	6– 7	0	8–10
19. v. 47	?246	10–12	0·06	—
19. vi. 47	?277	14–16	0·13	18–20

Growth evidently takes place steadily through the summer months, but is arrested during the winter. There is no suggestion, for this species, of the



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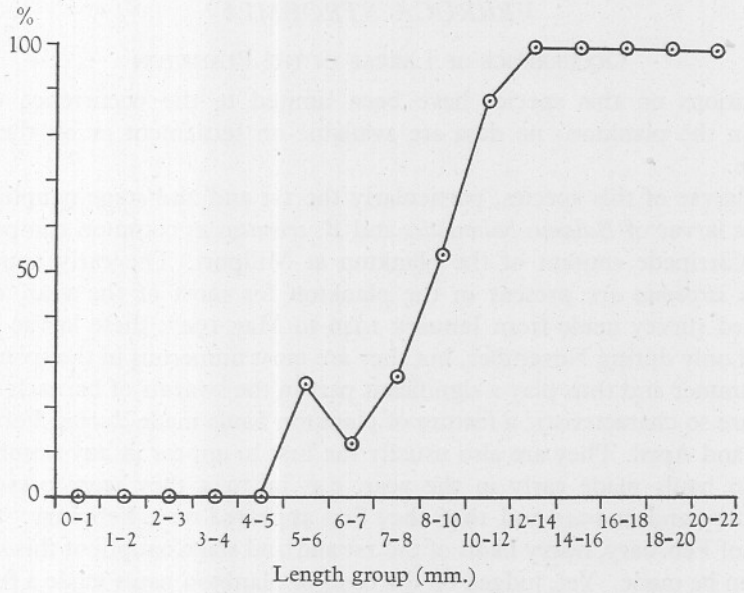

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Occurrence of Larvae in the Plankton

Observations on this species have been limited to the occurrence of the larvae in the plankton; no data are available on settlement or on the adult barnacle.

The larvae of this species, particularly the 1st and 2nd stage nauplii, rank with the larvae of *Balanus balanoides* and *B. crenatus* as common components of the Cirripede content of the plankton at Millport. The early nauplii of *Verruca stroemia* are present in the plankton for most of the year, e.g. in a detailed survey made from January 1946 to May 1947, these larvae lacked a record only during November, but they are most numerous in the spring and early summer and thus play a significant part in the swarms of barnacle larvae which are so characteristic a feature of plankton hauls made during February, March and April. They are also usually the first to appear in any numbers in plankton hauls made early in the year, e.g. in 1944 they were present on 3 February and in 1945 and 1946 they first appeared on 2 February. By the middle of February, heavy hauls of the 1st and 2nd stage nauplii of this species can often be made. Yet, judged on the basis of plankton hauls made a few feet below the surface, these large numbers of 1st and 2nd stage nauplii which appear early in the year are not immediately followed by the appearance of later nauplii or cyprids, as with *Balanus balanoides* and *B. crenatus*. Fig. 12, which shows the proportion of (*a*) the 1st and 2nd stage nauplii and (*b*) nauplii from the 3rd stage onwards (plotted as 3-day averages) which occurred during the early months of 1947, emphasizes this point. From 20 February until 16 March the only stages present were the 1st and 2nd stage nauplii, the later naupliar stages (3rd stage onwards) did not assume any considerable proportions until 6 April, and cyprids did not appear until the period beginning 30 April.

Further, the period between 20 February and 16 March was characterized by a preponderance of 1st stage larvae—during this period nearly 46,000 1st stage nauplii were recorded, but only just over 9000 2nd stage nauplii. Nilsson-Cantell (1921) found at Bohuslän, W. Sweden, that the 1st naupliar

stage occupied about a week, and this prolonged preponderance of 1st stage nauplii in the Clyde plankton suggests that this stage has a longer life period than that of the corresponding stages of *B. balanoides* and *B. crenatus*. In the laboratory, however, the 1st stage nauplius of *Verruca stroemia* moults to give the 2nd stage within a few hours of hatching.

The general sequence of events just described for 1947 also characterized the planktonic occurrence of the larvae of this species for the other years over which this survey extended. Fig. 13 shows the results obtained in 1946. Again, heavy hauls of early larvae were taken early in the year (with a preponderance of 1st stage nauplii, though not quite so marked as in 1947), no heavy hauls of late stage nauplii were made until much later and no cyprids were recorded until towards the end of April.

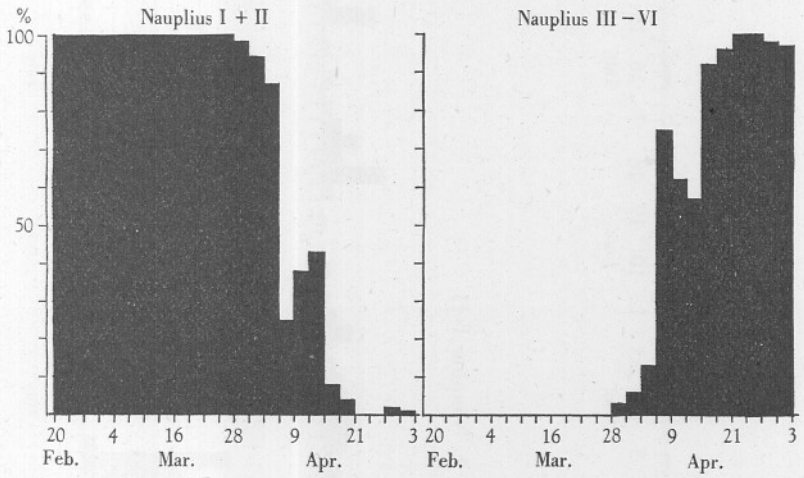

Fig. 12. Occurrence of the larval stages of *Verruca stroemia*, spring 1947. The numbers present, over 3-day periods, of each group of larval stages are expressed as a percentage of the total *V. stroemia* larvae present. (Cyprids were absent except for 2% during the very last period.)

In the absence of settlement data for *Verruca*, any suggested explanation of this situation must remain tentative, but there seem two outstanding possibilities. First, it is possible that only the earlier stages of naupliar development occur near the surface, the later-stage nauplii and the cyprids occurring in any numbers only in deeper water, so that the surface records for these stages are misleading. Secondly, it is possible that the record presented by these hauls is substantially correct and that the sequence of larval stages of *V. stroemia* extends over a period which is much longer than that of the two species of *Balanus* discussed earlier. If the first possibility is correct, it is difficult to see why the periods of abundance of early- and later-stage nauplii should approximate more closely later in the season, whereas if the second possibility is

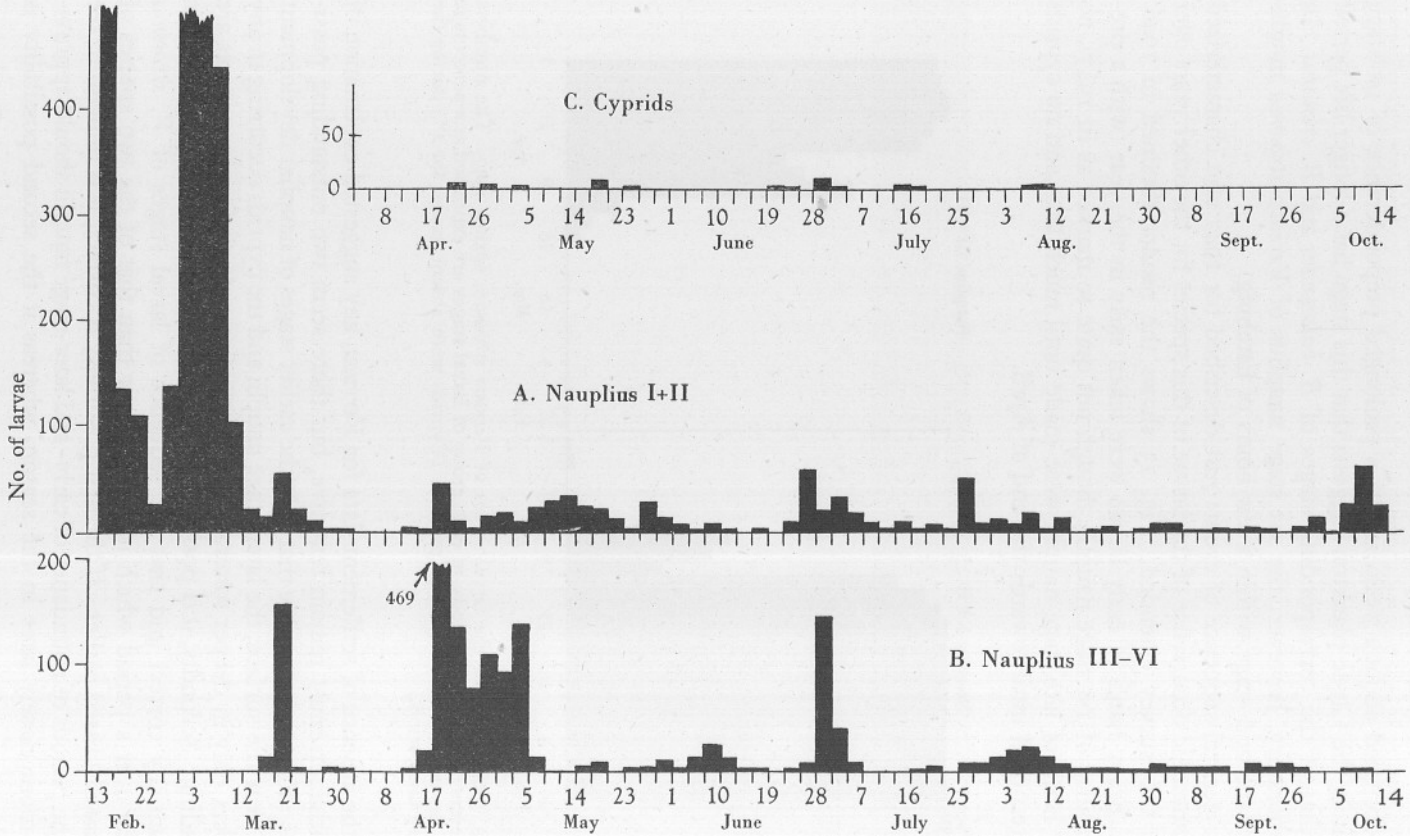

Fig. 13. Occurrence of the larval stages of *Verruca stroemia*, 1946, expressed as the number of larvae per haul, in 3-day averages. A, nauplii I and II. B, nauplii III–VI. C, cyprids.

correct a closer correspondence between periods of abundance later in the year might be expected, as rising water temperatures would presumably accelerate larval development. If the larval sequence does take place slowly early in the season, the early-stage larval maximum (Fig. 13 A) beginning on 13 February could be correlated with the later-stage maximum on 18 March (Fig. 13 B) and possibly with the cyprids recorded around 20 April (Fig. 13 C); the early-stage maximum on 28 February with the later-stage maximum beginning on 17 April and the cyprids occurring in the following month, suggesting that the full larval sequence, from hatching to the appearance of the cyprid, takes roughly 2 months at this season. Such links can only be tentatively suggested, particularly as the cyprid stages are never at all common. There are times during the spring and summer when the later-stage nauplii of *Verruca* are reasonably common (e.g. over 2000 occurred in one haul, made on 30 April 1947) but they do not seem to be followed by comparable hauls of the cyprid larva. Perhaps the latter only occurs in numbers in deeper water and, though slow development may be the explanation of the lack of immediate correspondence between the early- and later-stage nauplii of *Verruca*, surface hauls are not a reliable indication of the abundance of the cyprid larvae.

Though nothing is known of the settlement of this species, it can be predicted, from what is known of the occurrence of the nauplii, that it extends at least over the period April to September and there is a further suggestion that, like that of *Balanus crenatus*, it may be periodic. Fischer-Piette (1932), however, has recorded the appearance of young *Verruca* in abundance on the shore on 7 December and he therefore suggests that the approximate time of settlement lies between September and December. This is rather later than the present observations would suggest.

OTHER CIRRIPEDE LARVAE

The examination of plankton hauls made, for the most part daily, during 1946–47 has afforded an opportunity to assess the abundance of other forms. The only larvae that occurred at all regularly were the nauplii of *Sacculina* (probably *S. carcini*) and those of *Peltogaster paguri*. The *Peltogaster* larvae were at first puzzling, since they differ radically from the figures of the nauplius given by Boschma (1927), notably in possessing a shield-like expansion of the integument, such as Hoek (1909) figures for *P. purpureus*. They agree, however, with the re-description of the nauplius of *P. paguri* given by Reinhard (1946)[1]; and, furthermore, larvae identical with those found in the plankton have been obtained from specimens of *P. paguri* on *Eupagurus bernhardus* collected locally.

The records of these two nauplii are given in Table 7. *Peltogaster* nauplii were present in 10 out of the 14 months covered and it would seem possible

[1] Dr. Boschma kindly drew my attention to this paper.

that they are liberated all the year round. *Sacculina* larvae, however, proved to be more abundant during the winter months, from November to February or March.

TABLE VII. OCCURRENCE OF THE NAUPLII OF *PELTOGASTER* AND *SACCULINA*

Month	Nauplii recorded	
	Peltogaster	*Sacculina*
February (1946)	4	4
March	0	10
April	0	0
May	12	0
June	4	0
July	19	1
August	4	0
September	2	0
October	0	5
November	0	33
December	6	37
January (1947)	8	24
February	29	34
March	10	0

It is of interest to note that these records of the occurrence of *Sacculina* nauplii in the plankton confirm in part the conclusions drawn by Foxon (1940) about the breeding season of this parasite in the Clyde.

DISCUSSION

The descriptions given earlier in this paper of the seasonal occurrence of barnacle larvae in the plankton have clearly indicated their abundance during the months of February, March and April. Tables VIII and IX below set out the average hauls of barnacle larvae per day, for each month of the year, based on the records of this 4-year survey.

In both tables a dash indicates that no hauls were made during that particular month.

The sequence of larvae during the spring outburst is similar year by year. Early-stage nauplii of *Verruca stroemia* and early-stage nauplii of *Balanus crenatus* are the first to appear, usually during the latter part of February and early in March. Early-stage nauplii of *B. balanoides* first appear early in March and reach a maximum towards the middle of that month; the second half of March is characterized by the presence of large numbers of later-stage nauplii of *B. crenatus* and *B. balanoides* sometimes (e.g. in 1946) accompanied by smaller numbers of later-stage nauplii of *Verruca stroemia*. The end of March or the beginning of April sees the appearance of the first cyprid stages and during the first half of the latter month the cyprid of *Balanus balanoides* tends to predominate. During the second half of April the numbers of Cirripede

larvae usually decrease and by the end of that month, or early in May, they are reduced to the more modest proportions which they maintain, in general, for the rest of the summer. The numbers recorded after the end of October are negligible. Figs. 14–17 show the details of the records of the nauplii and cyprids of *B. balanoides*, *B. crenatus* and *Verruca stroemia* during the spring outburst in 2 successive years, 1946 and 1947.

TABLE VIII. ALL CIRRIPEDE NAUPLII MONTH BY MONTH

Expressed as the average number per daily haul

	Year			
Month	1944	1945	1946	1947
January	—	0	0	1
February	1716	21	607	419
March	2846	274	2034	13644
April	515	52	725	12199
May	7	—	136	.
June	—	—	50	.
July	—	—	25	.
August	—	—	21	.
September	—	—	9	.
October	—	—	30	.
November	—	—	0	.
December	—	—	1	.

TABLE IX. ALL CYPRID LARVAE MONTH BY MONTH

Expressed as the average number per daily haul

	Year			
Month	1944	1945	1946	1947
January	—	0	0	0
February	0	0	0	0
March	15	5	1	22
April	1491	137	110	2918
May	35	—	68	.
June	—	—	17	.
July	—	—	28	.
August	—	—	17	.
September	—	—	1	.
October	—	—	3	.
November	—	—	0	.
December	—	—	0	.

The figures given in Tables VIII and IX also emphasize the enormous variations that can occur from year to year. For detailed quantitative comparisons, the fishing methods used in this survey were crude, but even so, a difference such as that between the average catch of all stages of all Cirripede nauplii for March 1945 (274 nauplii) and that recorded for the corresponding month 2 years later (13,644 nauplii)—the latter practically fifty times the former—is great enough to be significant. Johnstone, Scott & Chadwick (1924) give figures for *Balanus balanoides* nauplii and cyprids for the period

1907–20, and it is of interest to find that even greater variations appear in their records, e.g. more than two thousand times the number of nauplii were recorded in March 1912 than in March 1907.

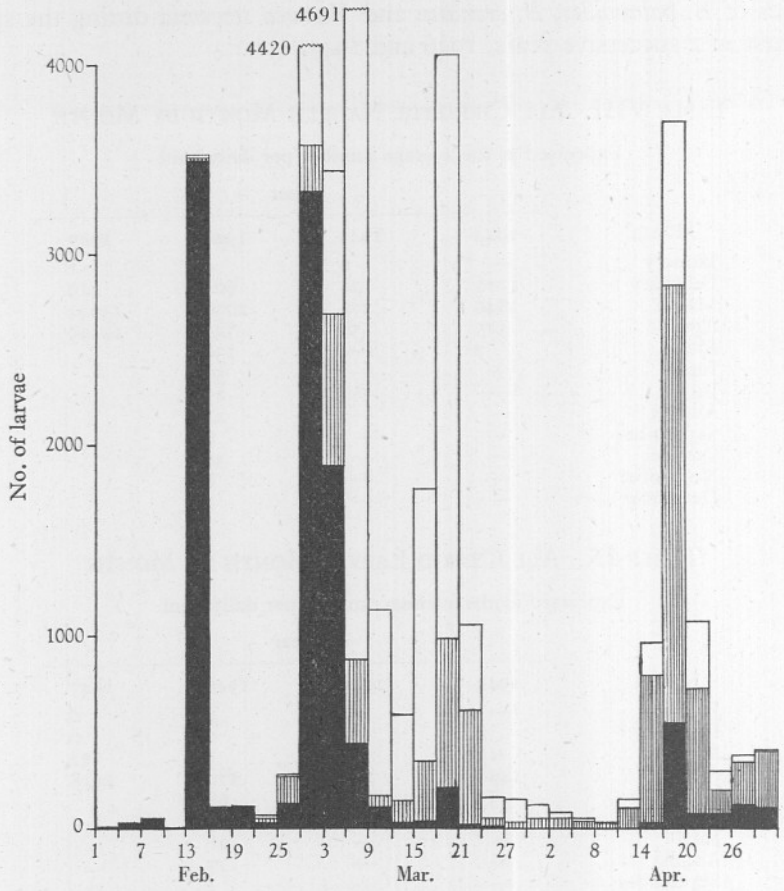

Fig. 14. Occurrence of Cirripede nauplii, Feb.–Apr. 1946. The shaded areas within each histogram indicate the numbers of the larvae of *Verruca stroemia*; the vertically hatched areas the numbers of *Balanus crenatus* larvae; the remaining areas the numbers of *B. balanoides* larvae.

Direct comparisons between the records obtained in the present survey and those obtained by Johnstone, Scott & Chadwick (1924) are impossible since fishing conditions were entirely different in the two surveys. One point, however, seems worthy of mention. Johnstone, Scott & Chadwick (*loc. cit.* p. 66) drew attention to the great discrepancy between the numbers of nauplii and the numbers of cyprids caught, and attributed this to the destruction of the nauplii during their period of development and to the dispersal of the swarms.

The discrepancy recorded for Port Erin is certainly very great (mean monthly haul of nauplii (in March) 35,224; mean monthly haul of cyprids (in May) 70), much greater than that of the present records, where the mean monthly haul of nauplii in March was 140,985 and the mean monthly haul of cyprids in April was 34,920. (The present records refer, of course, to the larvae of all three

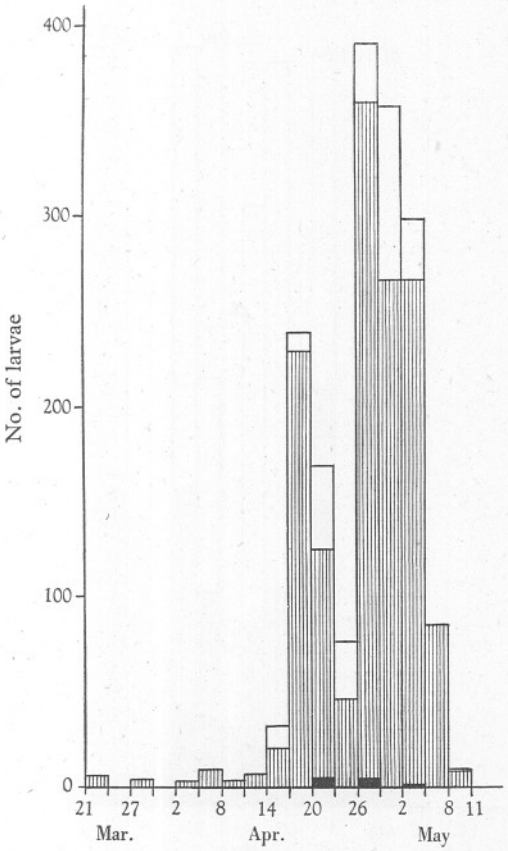

Fig. 15. Occurrence of cyprid larvae, Mar.–May 1946. Shading as in Fig. 14.

species of barnacle but, although Johnstone, Scott & Chadwick's account refers to their larvae as those of *B. balanoides*, their specific identity was merely inferred from the abundance of this species on the neighbouring shores; it would seem probable that larvae of other species of barnacle were present.) The present series of hauls were all made within a few feet of the shore and perhaps this may be the reason for the smaller discrepancy between the numbers of nauplii and the numbers of cyprids in the present instance, as it is possible that the cyprids tend to occur in their greatest numbers close inshore.

Some observations have been made on the dates when settlement of *B. balanoides* was first observed at different places. Fish (1925) states that in 1923 the first cyprids of this species appeared at Woods Hole on 8 February, that they were particularly abundant on 5 and 6 March and that they declined in numbers during April. He adds that the season in 1923 coincided exactly with that in 1900. This record is in general agreement with that of Grave (1933), who

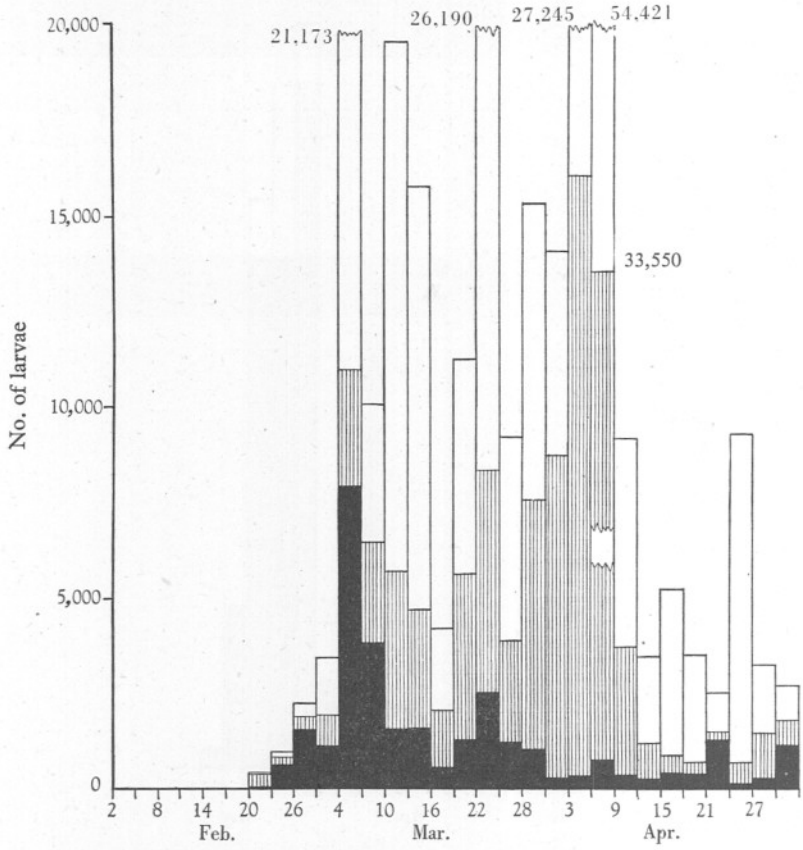

Fig. 16. Occurrence of Cirripede nauplii, Feb.–Apr. 1947. Shading as in Fig. 14.

states that metamorphosis takes place between 15 February and 15 March. Moore (1935*a*) comments on the surprisingly small variation in the date on which the first settled cyprids were observed on the rocks at Port Erin. In 1932 this was 26 April, in 1933 and 1934 on 25 April. Runnström (1925) mentions that the first cyprids were observed in Liverpool Bay on 6 April and that these larvae reached their maximum on about 15 April.

Hatton & Fischer-Piette (1932), however, found considerable variation both in the date when settled cyprids were first recorded and in the length of the

settlement period at St Malo. In 1930 the larvae first settled between 29 March and 2 April and settlement continued for 6 weeks, whereas in 1931 settlement first occurred between 15–21 February and 4–8 March, and this process continued for 3 months.

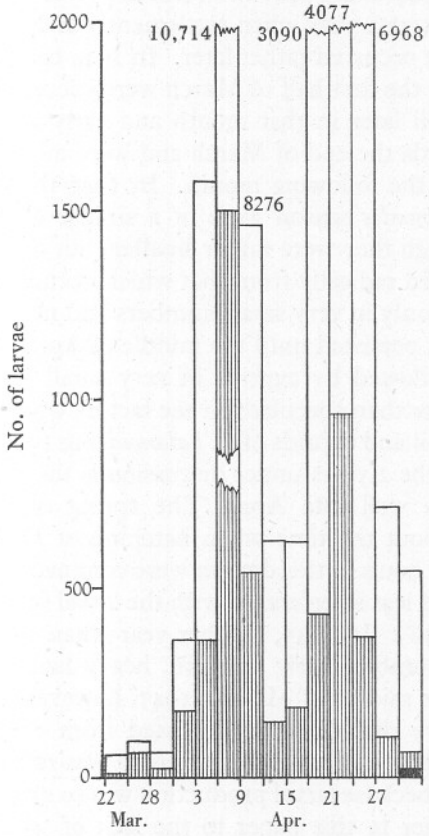

Fig. 17. Occurrence of cyprid larvae, Mar.–Apr. 1947. Shading as in Fig. 14.

The dates on which settlement was first observed and the period of settlement also varied to some extent in the present instance. Details are given below.

Year	Cyprids	Settlement
1944	First abundant on 3 April	Throughout April
1945	First abundant on 2 April	Limited to the first 2 weeks of April
1946	A few present from 14 April to the end of the month	Light settlement over last 2 weeks of April
1947	Some cyprids present throughout April; only abundant later in month	Light settlement throughout April; slightly heavier towards the end of the month

Thus it would appear that, in some places, settlement of *B. balanoides* occurs with some regularity on a given date (e.g. Port Erin, Woods Hole)

whereas at others (St Malo, Millport) variations can occur from year to year. Characteristics have been observed in the course of the present survey which suggest a possible explanation of these differences.

Fig. 18 compares the general features of the planktonic occurrence of *B. balanoides* for 1944 and 1946; the former a year when heavy settlement occurred and the latter a year when settlement was light and when, in comparison with 1944, it occurred rather later. In 1944 heavy hauls of 1st and 2nd stage nauplii during the first half of March were succeeded by moderate hauls of later-stage nauplii later in that month and early in April. Cyprid larvae first appeared towards the end of March and were moderately abundant until towards the end of the following month. In 1946 the hauls over the earlier part of the *B. balanoides* season were of a similar order to those recorded 2 years earlier (though they were rather smaller) but the situation towards the end of March differed radically from that which occurred in 1944. Late-stage larvae were present only in very small numbers and no cyprid larvae appeared at all. This situation persisted until the middle of April when some later-stage nauplii appeared followed by cyprids in very small numbers. It would be dangerous to do more than speculate on the factors which virtually obliterated the later-stage nauplii and cyprids of *B. balanoides* in 1946, but the outstanding difference between the 2 years under discussion is the persistence, in 1946, of heavy diatom hauls well into April. The spring outburst of *Skeletonema* usually occurs at about the time when hatching of *Balanus balanoides* takes place; in 1944 heavy hauls of this diatom were confined to a short period in the middle of March and less interference with the larval sequence of *B. balanoides* was apparent (Fig. 18). In 1945, another year when cyprids of *B. balanoides* appeared in some numbers early in April, heavy hauls of *Skeletonema* were again confined to the middle of March. 1947, however, resembled 1946 more closely in that heavy diatom hauls persisted from early March until early April; it seems possible that settlement in 1947 was rather heavier than in the preceding year only because larval production was so much heavier. Reference has been made earlier in this paper to the lack of 'success' of the *Balanus balanoides* larvae which hatched early in March compared with those which hatched towards the end of the month; it may be surmised that the full development of the earlier 'brood' was appreciably upset by the abundance of diatoms in the water.

It would therefore seem possible that the marked regularity with which settlement occurs at Port Erin is correlated with the absence of heavy diatom hauls there since, according to Johnstone, Scott & Chadwick (1924), the heaviest diatom hauls do not occur there until May.

The absence of zooplankton in the presence of abundant phytoplankton is a phenomenon which has received a good deal of attention in recent years (Hardy & Gunther, 1935; Harvey *et al.* 1935; Lucas, 1936). It is therefore of interest to note this example provided by the larval stages of *B. balanoides*, of

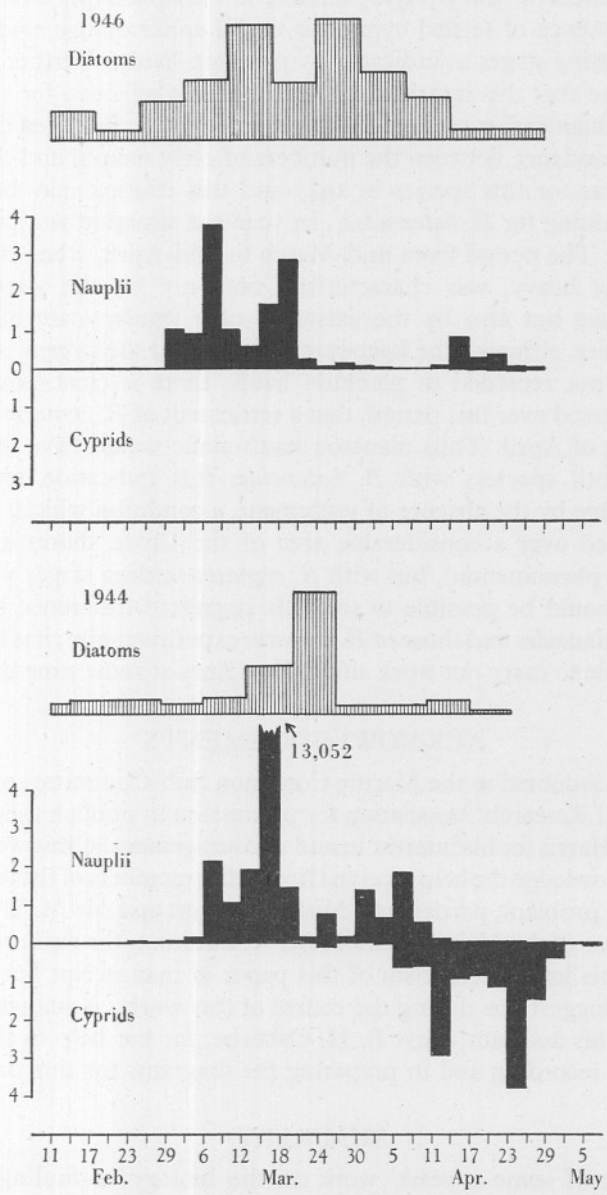

Fig. 18. Occurrence of larval stages of *Balanus balanoides*, 1944 (below) and 1946 (above), compared with estimates of diatom density in the two years. Histograms of the numbers of nauplii present over 3-day periods are shown extending upwards, those of the numbers of cyprids present extending downwards, from the base line. Numbers of nauplii and cyprids expressed in thousands; diatom abundance on an arbitrary scale.

the apparent effect of heavy phytoplankton on a zooplankton species, particularly as the absence of settled cyprids is useful confirmatory evidence of the absence of settling stages as indicated by plankton hauls. Further, it is also of interest to note that this interference seems far less effective for the larvae of *B. crenatus*. In an earlier section of this paper attention has been drawn to the closer correspondence between the numbers of early nauplii and the numbers of cyprid larvae for this species in 1947 and this relation may be compared with that obtaining for *B. balanoides*. In 1946 the situation was perhaps even more striking. The period from mid-March to mid-April, when diatom hauls were generally heavy, was characterized not only by the virtual absence of *B. balanoides* but also by the existence of a similar state of affairs for *B. crenatus*. Yet, although the later-stage nauplii and the cyprid larvae of this species were not recorded in plankton hauls, there is clear evidence, from surfaces immersed over this period, that a settlement of *B. crenatus* occurred at the beginning of April. Thus plankton hauls alone would have indicated the absence of both species: with *B. balanoides* this indication was probably correct (to judge by the absence of settlement, a condition which, it should be noted, obtained over a considerable area of the Clyde shores and was not purely a local phenomenon), but with *B. crenatus* settling stages were, in fact, present. It should be possible to test this suggested difference between the larvae of *B. balanoides* and those of *B. crenatus* experimentally; it is hoped that it will be possible to carry out work along these lines at some time in the future.

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SUMMARY

In the course of some 4 years' work on the biology of fouling organisms, observations have been made on aspects of the general biology of *Balanus balanoides*, *B. crenatus* and *Verruca stroemia*.

Observations on the state of development of the ova within the mantle cavity of *B. balanoides* and of the occurrence of the larvae in the plankton indicate that hatching takes place during the first fortnight in March, that the

later-stage nauplii are abundant during the second half of that month and that the cyprid larvae may become abundant during April. The larval sequence is not always completed successfully; conditions which seem inimical are discussed.

Factors which seem to be of importance in affecting settlement of *B. balanoides* are discussed. The presence of other organisms is an adverse factor, though the potency of this factor is probably not the same for all organisms; the presence of slime can prevent settlement and, though evidence can be quoted in support of heavier settlement on shaded surfaces, it is emphasized that the operation of other factors may have brought about this result. The proportion of the tidal cycle during which current speeds are not too high to prevent settlement seems likely to be important.

Provided that other conditions are favourable *B. balanoides* settles readily on surfaces continuously immersed and individuals which settle under these conditions grow more rapidly, at least for the first few months after settlement, than their contemporaries on the shore.

The larvae of *B. crenatus* appear in the plankton rather earlier than those of *B. balanoides*, but the time taken to complete the larval sequence from the 1st stage nauplius to the cyprid is roughly the same as that for the latter species, namely 1 month. In *B. crenatus*, however, hatching is not limited to a short period during March but continues at intervals through the summer. There are indications that the successful completion of the larval sequence of this species is less affected by an abundance of phytoplankton than is that of *B. balanoides*.

Settlement of *B. crenatus* is periodic and individuals which settle early in the spring can attain practically their full size (28–30 mm. basal length) during the same season. Growth during the summer takes place at average rates which vary from 0·1 to 0·2 mm. increase in basal length per day. Growth is negligible during the winter months.

Specimens of *B. crenatus* which settle early in April contain developing ova by the beginning of July, so that spring-settled forms can produce larvae which themselves may settle before the end of the summer.

Observations on *Verruca stroemia* have been limited to the occurrence of the larvae in the plankton. Like those of *Balanus crenatus*, the larvae of *Verruca stroemia* are present at intervals through the summer. Early-stage nauplii of this species may dominate the plankton early in the year, but no correspondingly heavy hauls of later-stage nauplii have been recorded. Possible explanations of this discrepancy are discussed. The cyprid never occurs in any numbers in surface hauls.

Incidental observations on the planktonic occurrence of the nauplii of *Sacculina* (probably *S. carcini*) and of *Peltogaster paguri* are given.

Details are given of the abundance of all Cirripede larvae in the plankton for the years 1944–47 which emphasize the extent of variation that can occur from year to year.

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THE EFFECT OF NARCOTICS ON THE ENDOGENOUS RESPIRATION AND SUCCINATE OXIDATION IN OYSTER MUSCLE

By George Frederick Humphrey

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(Text-figs. 1–6)

Introduction

The adductor muscles of Lamellibranchs present unusual features in their physiology. Thus it has been known for some time (Marceau, 1909) that they are able to support relatively large tensions, and the morphological differentiation into nacreous and vitreous parts in some species has led various workers (Parnas, 1910; Boyland, 1928; and Kobayashi, 1929) to attempt to correlate differences in metabolism with these properties. Also, Riesser (1933) has investigated, by the usual pharmacological techniques, the action of a number of compounds on invertebrate muscle, and concluded that these smooth muscles behaved similarly to cross-striated frog muscle. More recently, some features of the glycolytic mechanism (Humphrey, 1943) and of the succinoxidase system (Humphrey, 1947) in the adductor muscles of *Saxostrea commercialis* have been established. It was shown that succinic acid was able to increase the oxygen consumption considerably, and the effects of various inhibitors on the oxygen uptake in the presence and absence of succinic acid were determined.

In continuation of this, it was thought desirable to determine the effects of other classes of inhibitors on the oxygen consumption, and, with this object in view, the action of narcotics was studied. The effect of narcotics on the oxidation of succinic acid was also determined, since succinic acid plays such a central role in tissue metabolism (Szent-Györgyi, 1937). Also, Quastel & Wheatley (1933) have found that, in general, the oxidation of succinic acid by brain tissue is, unlike the oxidation of numerous other substrates, insensitive to the presence of narcotics; it therefore becomes important to determine if such insensitivity occurs with the muscle taken from *Saxostrea*.

Materials and Methods

Specimens of *Saxostrea commercialis* were obtained from the C.S.I.R. Experimental Farm at George's River, Sydney, Australia; only adult animals, 2–3 years old, were used.

The narcotics were used as neutralized aqueous solutions. Salicylamide was prepared by the method of Anschütz (1919); the other compounds were chemically pure commercial specimens.

Cytochrome was prepared from pig heart by the method of Keilin & Hartree (1937), but was dialysed against distilled water (Potter, 1941).

The adductor muscles, immediately after dissection, were homogenized at a 30% concentration with water in the instrument previously described (Humphrey, 1946). The desired amount of narcotic in 2 ml. of water was placed in a manometer flask, together with neutralized sodium succinate (to give a final concentration of 0·05 M) if the oxidation of this substance was being studied. 2 ml. of the muscle suspension was then pipetted into the flask, and the oxygen consumption measured under the conditions already given (Humphrey, 1946). When used, cytochrome was added directly to the main compartment of the manometer flask, to give a final concentration of 10^{-5} M.

Results

The main results are set out in Figs. 1–6, and Table I. Two or three concentrations of each narcotic were studied, depending on the solubility of the compound in question. Included with each set was a control with water in place of narcotic, and oxygen consumption was always recorded at 1 and 2 hr. Using the 2 hr. reading, the inhibition was then calculated as a percentage of the control, and the mean of several values graphed against the concentration of narcotic or the logarithm of the concentration.

Table I. Effect of Urethanes, Salicylamide, and Chloral Hydrate

Inhibition (%) by	...	Endogenous	Endogenous + cytochrome	Succinic acid	Succinic acid + cytochrome
Urethane	0·001 M	6	5	15	9
	0·01 M	18	15	21	14
	0·05 M	−6	−6	30	17
Phenyl urethane	0·001 M	30	39	33	20
	0·005 M	47	56	54	60
Salicylamide	0·001 M	10	15	29	33
	0·01 M	50	54	63	70
Chloral hydrate	0·001 M	25	15	13	10
	0·01 M	50	38	64	41

On the whole, straight lines are obtained when the logarithmic plotting is used, but sometimes (Figs. 5 and 6) the inhibition is directly proportional to the concentration. These differences may be compared with the sigmoid curves obtained by Jowett & Quastel (1937). Replacement of the carboxyl group of benzoic acid with an acid amide group always increases the inhibition, but the reverse is true for salicylic acid and salicylamide. At equal concentrations, phenyl urethane is a better inhibitor than ethyl urethane, and the latter, at a concentration of 0·05 M, causes a slight increase in the oxygen consumption.

All the narcotics inhibit succinate oxidation, and usually more strongly than the endogenous respiration. On the whole, the addition of cytochrome does

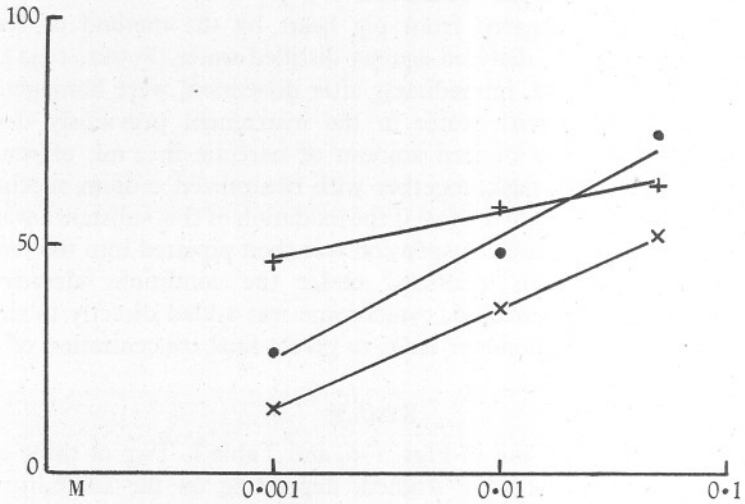

Fig. 1. Effect on endogenous oxygen consumption. Ordinates are percentage inhibition; abscissae, molarity of narcotic. • benzamide; + salicylate; × benzoic acid.

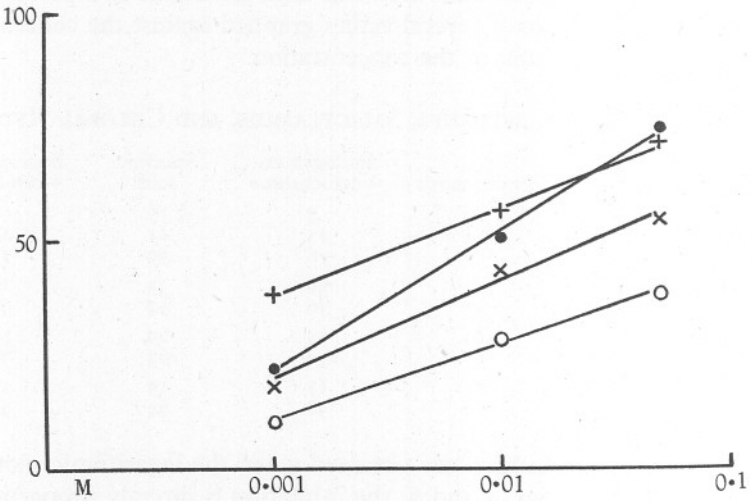

Fig. 2. Effect on endogenous oxygen consumption in the presence of cytochrome. Ordinates are percentage inhibition; abscissae, molarity of narcotic. • benzamide; + salicylate; × benzoate; ○ morphine.

not affect the inhibition of the endogenous or succinate respiration; marked exceptions are the effects on the endogenous respiration of 0·025 M barbitone, where the inhibition drops from 48 to 20% (Fig. 5), and 0·05 M morphine,

where 67% changes to 40% after the addition of cytochrome (Fig. 6). The variations in inhibition caused by the addition of cytochrome and/or succinate

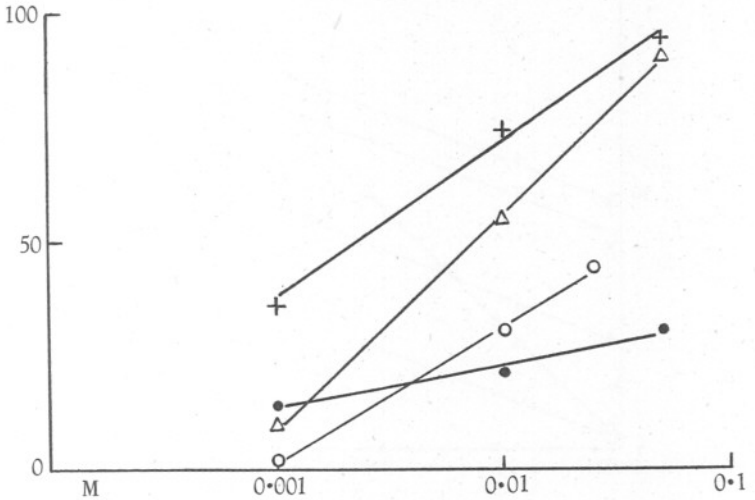

Fig. 3. Effect on succinate oxidation. Ordinates are percentage inhibition; abscissae' molarity of narcotic. + salicylate; △ caffeine; ○ barbitone; • urethane.

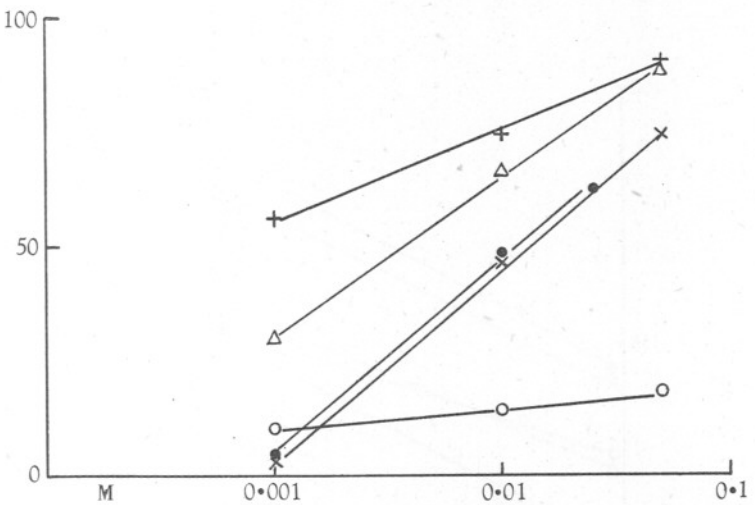

Fig. 4. Effect on succinate oxidation in the presence of cytochrome. Ordinates are percentage inhibition; abscissae, molarity of narcotic. + salicylate; △ caffeine; × benzoate; • barbitone; ○ urethane.

may be due to the fact that in the endogenous respiration several systems are functioning at more or less similar rates, whereas, after these additions, certain pathways become more prominent.

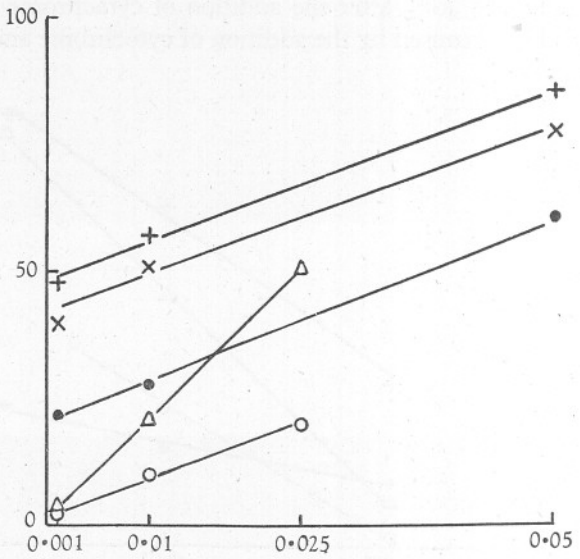

Fig. 5. Effect on endogenous oxygen consumption. Ordinates are percentage inhibition; abscissae, molarity of narcotic. + caffeine in the presence of cytochrome; × caffeine; △ barbitone; • morphine; ○ barbitone in the presence of cytochrome.

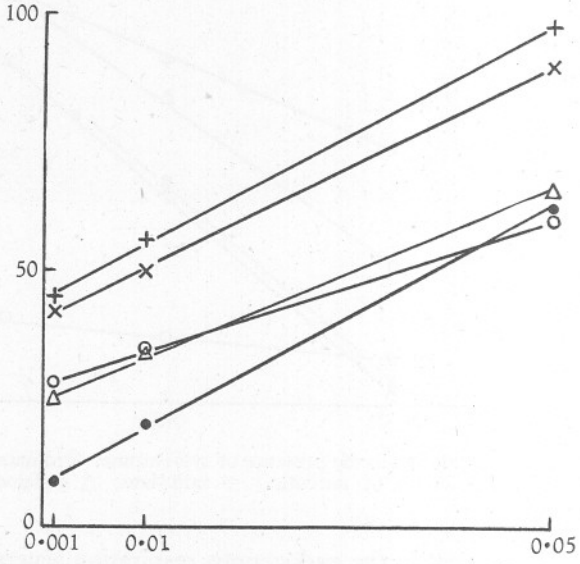

Fig. 6. Effect on succinate oxidation. Ordinates are percentage inhibition; abscissae, molarity of narcotic. + benzamide in the presence of cytochrome; × benzamide; △ benzoate; • morphine; ○ morphine in the presence of cytochrome.

It was not possible to increase the inhibition by incubation of the tissue suspension and narcotic for 30 min. before tipping in the succinate from a side arm. Similar incubation of the tissue and succinate, followed by the addition of narcotic, gave slightly less inhibition. This could be interpreted by assuming that there was substrate-narcotic competition for a definite site of attachment in the suspension.

Discussion

Keys (1937) has stated that it is a 'fundamental fact that narcotized cells no longer show or transmit irritable impulses... in narcotic doses, true narcotics do not seriously interfere with the total resting metabolism of the cell'. Since it is not known what a 'narcotic dose' for *Saxostrea* is, it is not possible to deny or affirm the latter part of this statement. Also, in general, it is desirable to reserve the concept of narcosis for the action of certain classes of substances on whole organisms, or cell preparations which approximate closely to physiological conditions. The system studied here can be regarded as 'intermediate' (Clark, 1937) and the use of the term narcotic must be regarded as a convenient way of designating a type of compounds.

The most widely accepted explanation of the action of narcotics on cells is the Meyer-Overton theory which seeks to correlate the activity with the solubility of the narcotic in lipoid phases. However, this does not give an explanation of the phenomena observed with partially isolated enzyme systems. Michaelis & Quastel (1941) investigated the effect of chloretone on various dehydrogenase systems, and came to the conclusion that this narcotic has its effect mainly on flavoprotein, or an unknown component intermediate between flavoprotein and cytochrome oxidase. Östergren (1944) suggests that narcotics can associate with the lipophilic side chain of polypeptides and change the folding pattern; perhaps this is how chloretone affects the flavoprotein. However, there is no conclusive evidence for these theories, and the action of a given narcotic seems to vary from one organism to another, suggesting that there is more involved than a relatively simple relationship between narcotic and chemical grouping.

Endogenous Oxygen Consumption

Urethanes have been used extensively on different organs and isolated respiratory systems. Warburg (1914) showed that phenyl urethane was a more powerful inhibitor of erythrocyte respiration than ethyl urethane. Krogh (1915) demonstrated that ethyl urethane is suitable for narcotizing various marine animals; in general 0·05–0·1 M was sufficient. Navez, Crawford, Benedict & Dubois (1941), working on the oxygen consumption of the heart of *Venus mercenaria*, showed that 0·01 M urethane caused an increase of 20%. Moog & Spiegelman (1942) found that neither ethyl nor phenyl urethane affected the oxygen uptake of stems cut from *Tubularia*. However, Barnes

(1944) obtained an inhibition of the respiration of developing eggs of *Rana pipiens* with 0·2 M urethane. Nothing similar to the break in activity-concentration relationship found with *Saxostrea* muscle has been previously observed in animal tissue.

Cheney (1946) obtained 60% inhibition of the oxygen uptake of fertilized *Arbacia* eggs in the presence of 0·1 M caffeine. He concluded, with little direct evidence, that caffeine acts on the cytochrome oxidase. On the whole, caffeine is one of the most effective narcotics for inhibiting the endogenous respiration of *Saxostrea* muscle.

Regarding the effects of the narcotics studied here, there has not yet been reported any other information with which it would be possible directly to compare the findings obtained with *Saxostrea*. However, Shideman & Seevers (1941), using rabbit muscle, found that 0·00017–0·0017 M morphine increased the oxygen consumption. No such acceleration was observed with *Saxostrea* muscle when 0·001–0·05 M morphine was used.

Oxidation of Succinic Acid

Assuming that added succinic acid causes an increase of about 300% in the oxygen consumption (Humphrey, 1947), an examination of Figs. 1–6 reveals that the oxidation of succinic acid is inhibited by all the narcotics used. Only with 0·001 M benzoic acid and 0·001 M chloral hydrate is the oxidation uninhibited, and this only in the presence of added cytochrome. These findings are in direct contrast to those of Quastel & Wheatley (1933), Jowett & Quastel (1937) and Seevers & Shideman (1941), who found that, for vertebrate tissue, the oxidation of succinic acid is unaffected by a large number of narcotics when these are present in concentrations of about 0·01 M.

Also, Greig (1946), basing her arguments on the work of Jowett & Quastel (1937) and Michaelis & Quastel (1941), i.e. on the insensitivity of the oxidation of succinate to narcotics, showed that the site of action of nembutal is the flavoprotein-cytochrome *b*-link of the respiratory chain; these experiments were with yeast and brain and cannot be directly compared with observations on invertebrate tissue.

Conclusions

As might be expected, the effects of narcotics on *Saxostrea* muscle differ quantitatively from those obtained with vertebrate muscle. In addition, there are two qualitative differences; namely, the break in activity-concentration relationship shown with urethane, and also the inhibition of the oxidation of succinic acid. In these two respects, *Saxostrea* muscle is distinguished from other tissues.

However, it is not possible to conclude that the role of succinic acid in the metabolism of the adductor muscle differs fundamentally from that in other muscle. Also, the changes in narcotic effect due to the presence of added

cytochrome are not sufficiently definite to indicate that the metabolic pattern is changed by added cytochrome. With regard to the site of action of the narcotics used, definite conclusions must await an elucidation of the main respiratory pathways operating in this invertebrate tissue.

Summary

The action of the following compounds on homogenates of the adductor muscles of *Saxostrea commercialis* was studied: urethane, phenyl urethane, chloral hydrate, salicylic acid, morphine, caffeine, barbitone, benzoic acid, salicylamide and benzamide.

It was found that, except for 0·05 M urethane, the endogenous oxygen consumption was reduced by all these compounds when present in concentrations ranging from 0·001 to 0·05 M. The oxidation of succinic acid was partially inhibited by these substances.

These findings are discussed in relation to similar studies on other tissues.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

The Pedal Sucker and Anal Gland of some British Stenoglossa

By **Vera Fretter**

Proc. Malacol. Soc., Vol. 27, 1946, pp. 126–30

In *Ocenebra erinacea* (L.), *Nucella lapillus* (L.) and *Urosalpinx cinerea* (Say) the foot possesses a small disk-shaped, retractile sucker, situated mid ventrally, immediately behind the anterior pedal gland. Its movement is controlled by muscles and blood pressure, which keep it turgid when protruded. It is covered by ciliated glandular epithelium. The amount of secretion is scanty and has no solvent effect on mollusc shells. The sucker grips the shell of the prey below the spot where the proboscis is working and seems to steady its base.

The rectal gland of these carnivorous stenoglossa develops as a simple diverticulum from the gut, and appears to act as an accessory kidney. The epithelium abstracts excretory matter from the blood and builds it up into masses which can be easily passed from the body. V.F.

Marine Seismic Prospecting

By **M. N. Hill** and **P. L. Willmore**

Nature, Vol. 159, 1947, p. 707

Considerable progress was made before the war in the use of seismic refraction shooting for the study of submarine geology. Two ships were used in this work, one of which fired the charges at varying distances from the second, on which was the recording gear connected to geophones on the sea bed. The difficulty of placing these instruments on the sea bed in depths greater than 100 fathoms limited the work to the continental shelf. As a result of this limitation, experiments were undertaken during the winter of 1947 to ascertain whether hydrophones at a depth of about 100 ft. compared favourably in their response with geophones laid on the bottom. The difficulties involved in obtaining two ships for this work were overcome by using a buoy for firing the charges, or, for the shorter shots, by trailing the charges on a long buoyant electric cable.

The conclusions from this work were first, that a hydrophone gave a response at least as good as that of a geophone, and secondly, that the charge-firing buoy was unsatisfactory since the instant of detonation was uncontrollable after the buoy had been launched, and because of the necessarily complicated safety precautions. For long range work it would therefore be necessary either to use two ships, or to attach the hydrophone to a radio buoy which would transmit the sounds to the firing ship. M.N.H.

Contribution to the Study of the Sponges

By Margaret W. Jepps

Proc. Roy. Soc., London, B, Vol. 134, 1947, pp. 408–17

The old observations of H. J. Carter and others on the contractile vacuoles of sponge cells have been questioned in recent years. The presence of contractile vacuoles is here established in both amoebocytes and choanocytes of the fresh-water sponges; but they have not been seen in the cells of several marine sponges, though figured by some of the older authors. Methods are described for observing the vacuoles, and incidentally other activities of the cells also.

Young sponges grown from gemmules were very useful in these studies and it was found possible to have them throughout the winter months by a simple process of vernalization.

Attention is drawn to the importance of these observations in any consideration of the relationships of the Porifera to the Protozoa and to the Metazoa respectively. M.W.J.

Giant Nerve Fibre of *Myxicola infundibulum* (Grube)

By J. A. C. Nicol and J. Z. Young

Nature, Vol. 158, 1946, p. 167

It has been known for some time that the Sabellid *Myxicola* possess very large nerve fibres, and investigations confirm that these may reach 1 mm. in diameter in the contracted state of the worm. The fibre extends throughout the length of the ventral cord and is a single unit maintained by many cell bodies scattered along its length. The fibre originates in the supra-oesophageal ganglia from two especially large cells. Branches given off along its length proceed to the muscles. There is a connective tissue sheath not containing any visible myelin.

Evidently the fibre constitutes a final common path by which afferent impulses from any part of the body activate all the longitudinal muscle fibres, producing the quick movements and withdrawal characteristic of the animal. J.Z.Y.

The Mechanics and Innervation of the Starfish Tube Foot-Ampulla System

By J. E. Smith

Phil. Trans. Roy. Soc., London, B, Vol. 232, 1946, pp. 279–310

A tube foot with its attached ampulla is a reciprocally acting self-contained system. The ampulla has a fluid capacity just adequate to produce, on contraction, the observed maximal degree of protraction of the foot. There is no evidence that water enters or leaves the system by way of the radial water canals during extension or withdrawal of the foot. Further aspects of the mechanics of movement of the foot and ampulla are considered in relation to the arrangement and properties of their constituent muscles and connective tissue.

An account, based on the results of *intra-vitam* methylene blue staining, is given of the nervous arcs that supply the muscles of the system. The sensory and associative fibres of the foot are described and their connexions with the association tracts of the radial nerve cord followed. The foot and ampulla muscles are innervated by axons whose cell bodies lie in one or other of two centres situated in the foot, near the entrance to the ampulla neck. One centre, laterally situated, appears to be excited directly through the association pathways of the foot; the more medial centre, on the other hand, has central connexions with the radial nerve cord through a system of neurones whose cell bodies lie above the cord in the floor of the radial perihaemal canal.

Some implications of the double innervation (central and peripheral) of the foot-ampulla are discussed. J.E.S.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888. The cost of the building and its equipment was £12,000 and, since that date, a new library and further laboratory accommodation have been added at an expenditure of over £23,000.

The Association is maintained by subscriptions and donations from private members, scientific societies and public bodies, and from universities and other educational institutions; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the Laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. An account of the Laboratory and the scope of the work undertaken there will be found in Vol. xv (p. 735) of this *Journal*.

The Laboratory is open throughout the year and its work is carried out under the supervision of a Director and with a fully qualified research staff. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology and physiology. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by a research vessel and by a motor boat and these also collect the specimens required in the Laboratory.

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Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the Laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the Laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the Library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

CONTENTS

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