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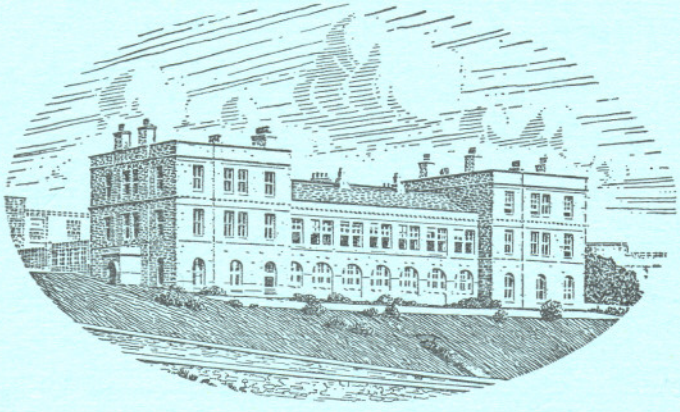

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SEA TEMPERATURES IN PLYMOUTH SOUND

By L. H. N. Cooper, D.Sc.

The Plymouth Laboratory

For ecological studies records of mean sea temperatures in and near Plymouth Sound are often required. The necessary data exist but have not been readily available in a convenient form.

Since 1898 sea temperature has been included amongst the observations made by the Plymouth City Meteorologist. In early days this was Mr Victor Prigg whose successors, Mr G. H. Ivory and Messrs G. H. Ivory and Partners continued the work until 1954. In that year the work was transferred to the Department of the Medical Officer of Health for Plymouth. The measurements on the Fahrenheit scale appear always to have been made at a depth of 1 fathom (1·8 m) at about the time of high water. They were made three times a week from the end of the Promenade Pier until it was destroyed by fire in March 1941 and afterwards from the end of the 'Banjo Pier' until 1955. For the last two years they have been taken from the Pier Head of Millbay Docks, 0·3 nautical mile (0·55 km) distant. The three positions have a very similar exposure to the tidal stream and the observers by a series of comparable observations assured themselves that no appreciable error was introduced by the changes.

Five-year running means (anomalies in ° C) for air temperatures on Plymouth Hoe have been published by Southward & Crisp (1954). In half a century they show a rise of more than 0·5° C. Monthly means of many meteorological measurements on Plymouth Hoe have been published for many years in the *Transactions of the Devonshire Association*, but sea temperatures have not been included. Annual means of sea temperature have appeared somewhat erratically in the Annual Reports of the Medical Officer of Health. Monthly means, although prepared, have never been published except for the 8 years 1912–19 (Orton, 1920).

Mr E. Voaden of Messrs G. H. Ivory and Partners and Mr D. W. A. Cole of the Department of the Medical Officer of Health have generously provided us with the monthly means for each month from January 1947 to December 1956. Converted to the Centigrade scale they are printed here as Table 1. For each month the 10-year mean has been computed. Monthly means averaged over the 55 years 1898–1953 have also been provided by Mr Voaden. To facilitate future computing, they are reported on both scales of temperature (Table 2). During the years 1912–19 the months January–September were colder and the months October–December were markedly warmer than the 55-year mean. In the years 1947–56, ten of the monthly means are well above

TABLE 1. MONTHLY MEAN SEA TEMPERATURES (° C) FOR PLYMOUTH SOUND 1947–1956
TOGETHER WITH MAXIMA AND MINIMA ATTAINED IN EACH MONTH

	Jan.			Feb.			Mar.			Apr.			May			June		
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
1947	8·3	9·1	6·2	5·3	6·5	4·3	6·4	8·3	4·7	9·6	11·6	8·0	11·2	14·1	8·4	13·6	15·3	12·2
1948	9·5	10·0	8·6	8·6	9·4	6·9	8·4	9·1	7·4	9·9	11·1	8·9	12·3	14·2	10·8	14·0	14·1	12·8
1949	9·5	9·9	8·8	9·3	9·7	8·2	9·2	10·1	8·8	10·4	11·3	9·3	12·1	13·2	10·7	14·6	17·1	13·0
1950	9·4	10·2	7·6	8·4	9·1	7·8	9·3	10·1	7·8	10·0	10·6	9·4	12·2	14·2	11·0	15·1	16·1	14·2
1951	8·2	8·9	7·7	7·9	8·2	7·6	8·1	8·3	7·6	8·9	9·9	8·2	10·7	12·2	9·5	13·7	14·7	12·3
1952	9·0	9·8	8·1	8·2	8·7	7·7	8·9	9·8	7·8	9·7	10·8	7·8	12·5	13·8	10·9	14·2	15·3	13·7
1953	8·5	8·9	7·9	7·8	8·6	7·1	8·4	9·6	8·0	9·6	10·3	8·9	11·9	13·2	10·3	13·2	14·6	11·4
1954	8·7	11·4	7·9	7·5	8·3	6·5	8·6	9·6	7·7	10·0	11·5	9·1	11·5	12·9	9·9	13·7	14·7	12·6
1955	8·6	9·4	7·3	7·9	8·9	6·7	6·9	7·8	6·3	8·6	9·5	7·2	10·2	11·8	9·4	13·2	15·0	12·2
1956	9·8	10·1	9·3	7·7	9·4	6·1	7·9	8·7	7·2	9·3	9·7	8·7	11·3	12·2	9·7	12·5	13·4	11·6
10-year average	8·95	—	—	7·86	—	—	8·21	—	—	9·60	—	—	11·59	—	—	13·78	—	—

	July			Aug.			Sept.			Oct.			Nov.			Dec.			
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Annual mean
1947	14·9	17·1	13·4	17·6	18·6	16·1	17·2	18·3	15·7	15·2	15·6	13·5	13·1	13·7	12·1	10·9	12·2	9·7	11·9
1948	14·7	17·0	13·3	16·0	16·9	15·3	15·4	16·7	14·7	14·3	15·4	12·7	12·3	13·0	11·3	10·7	11·9	9·0	12·2
1949	17·3	18·3	16·3	17·0	18·3	16·3	17·5	18·9	16·9	16·1	17·2	12·8	12·6	14·1	11·6	9·5	11·7	9·4	12·9
1950	15·7	16·6	14·6	16·4	17·1	15·4	15·7	16·1	14·2	13·8	14·6	12·4	11·5	12·4	10·6	9·3	10·8	7·6	12·2
1951	15·2	16·4	14·7	15·9	17·1	15·2	15·2	15·8	14·8	14·1	14·9	12·8	12·2	12·8	11·6	10·6	11·7	8·9	11·7
1952	15·7	16·7	14·9	16·4	17·2	15·6	15·2	16·9	14·3	13·4	14·3	12·9	11·6	13·2	9·4	9·1	9·4	8·7	12·0
1953	15·0	15·7	13·9	16·1	17·6	13·9	15·4	16·1	14·8	14·4	15·5	13·5	12·6	13·1	12·2	12·0	12·7	11·1	12·1
1954	13·8	14·3	13·3	14·4	15·5	13·7	15·2	16·8	13·9	14·2	14·8	13·6	12·1	13·2	10·8	10·9	11·8	9·7	11·7
1955	15·7	17·5	14·0	17·4	18·9	16·1	16·4	18·3	15·1	14·4	15·6	12·8	12·3	13·3	11·5	11·1	11·6	10·6	11·9
1956	14·6	15·6	13·7	15·1	15·5	14·4	15·0	15·6	14·2	14·4	15·4	13·3	12·3	13·3	11·2	10·6	11·3	9·4	11·8
10-year average	15·27	—	—	16·22	—	—	15·81	—	—	14·43	—	—	12·26	—	—	10·47	—	—	12·04

TABLE 2. AVERAGE MEAN MONTHLY TEMPERATURES (° C) OVER PERIODS OF YEARS

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
55-year average, ° F, 1898–1952	47·9	46·5	46·9	49·0	52·5	56·8	59·6	60·9	60·1	57·0	53·2	49·9
55-year average, ° C, 1898–1952	$8{\cdot}8_3$	$8{\cdot}0_6$	$8{\cdot}2_8$	$9{\cdot}4_4$	$11{\cdot}3_9$	$13{\cdot}7_8$	$15{\cdot}3_3$	$16{\cdot}0_6$	$15{\cdot}6_1$	$13{\cdot}8_9$	$11{\cdot}7_8$	$9{\cdot}9_4$
8-year average, ° C, 1912–19	$8{\cdot}6_1$	$7{\cdot}8_3$	$7{\cdot}9_4$	$9{\cdot}0_0$	$11{\cdot}2_2$	$13{\cdot}4_4$	$15{\cdot}0_0$	$15{\cdot}9_7$	$15{\cdot}5_3$	$14{\cdot}0_0$	$12{\cdot}0_0$	$10{\cdot}3_3$
10-year average, ° C, 1947–56	$8{\cdot}9_5$	$7{\cdot}8_6$	$8{\cdot}2_1$	$9{\cdot}6_0$	$11{\cdot}5_9$	$13{\cdot}7_8$	$15{\cdot}2_7$	$16{\cdot}2_2$	$15{\cdot}8_1$	$14{\cdot}4_3$	$12{\cdot}2_6$	$10{\cdot}4_7$
Difference, ° C, 1912–19 from 55-year average	$-0{\cdot}2_2$	$-0{\cdot}2_3$	$-0{\cdot}3_4$	$-0{\cdot}4_4$	$-0{\cdot}1_7$	$-0{\cdot}3_4$	$-0{\cdot}3_3$	$-0{\cdot}0_9$	$-0{\cdot}0_8$	$+0{\cdot}1_1$	$+0{\cdot}2_2$	$+0{\cdot}3_9$
Difference, ° C, 1947–56 from 55-year average	$+0{\cdot}1_2$	$-0{\cdot}2_9$	$-0{\cdot}0_7$	$+0{\cdot}1_6$	$+0{\cdot}2_0$	$0{\cdot}0_0$	$-0{\cdot}0_6$	$+0{\cdot}1_6$	$+0{\cdot}2_0$	$+0{\cdot}5_4$	$+0{\cdot}4_8$	$+0{\cdot}5_3$
Difference, ° C, 1947–56 from 1912 to 1919	$+0{\cdot}3_4$	$+0{\cdot}0_3$	$+0{\cdot}2_7$	$+0{\cdot}6_0$	$+0{\cdot}3_7$	$+0{\cdot}3_4$	$+0{\cdot}2_7$	$+0{\cdot}2_5$	$+0{\cdot}2_8$	$+0{\cdot}4_0$	$+0{\cdot}2_6$	$+0{\cdot}1_4$

the long-term average. If the exceptionally cold months of February and March 1947 are omitted the monthly means for February and March for the remaining nine years were both also warmer than average. The years 1947–56 (even with February and March 1947 included) were on average warmer in every month than the years 1912–19. This trend is now well known to climatologists.

Atkins & Jenkins (1952) have published monthly means for International Hydrographic Station E 1 (50° 02′ N., 4° 22′ W.) and drew attention to some exceptional years. They presented no evidence for long-term changes. From the same observations I also sought evidence for long-term changes and failed to find it. The cruises are made about once a month, and there are inevitably many sources of bias in the raw data. A systematic study of the E 1 records which will stand statistical criticism will be tedious but needs to be made. Until this is done, the thrice-weekly records from Plymouth Sound provide a better basis for long-term ecological comparisons than do the monthly records from Station E 1.

Of recent years there are indications that the composition of the fauna of the English Channel has changed due to an increase in the occurrence of southern, warmer-water forms. The rise of about 0·3° C, found in Plymouth Sound, may well be a sufficient explanation: if it is, there is no point in seeking an explanation in other terms (as, for example, Cooper, 1955). I prefer to believe for the present that we have here interlocking facets of a global change and that the simple explanation by itself is not sufficient.

SUMMARY

Monthly mean sea-water temperatures in Plymouth Sound, prepared by the Plymouth City Meteorologist, are presented as data for ecological studies in this laboratory. In the last 40 years there has been a rise in mean temperature of about 0·3° C.

REFERENCES

Atkins, W. R. G. & Jenkins, P. G., 1952. Note on sea temperatures in the English Channel, 1921 to 1949, and Plymouth sunshine and light. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 327–33.

Cooper, L. H. N., 1955. Hypotheses connecting fluctuations in Arctic climate with biological productivity of the English Channel. *Pap. mar. Biol. Oceanogr., Deep-Sea Res.*, Suppl. to Vol. 3, pp. 212–23.

Orton, J. H., 1920. Sea-temperature, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 339–66.

Southward, A. J. & Crisp, D. J., 1954. Recent changes in the distribution of the intertidal barnacles *Chthamalus stellatus* Poli and *Balanus balanoides* L. in the British Isles. *J. Anim. Ecol.*, Vol. 23, pp. 163–77.

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ON SOME SMALL *IANTHINA JANTHINA* (L.) STRANDED ON THE ISLES OF SCILLY, 1957

By DOUGLAS P. WILSON, D.Sc.
The Plymouth Laboratory

(Text-fig. 1)

In an earlier paper (Wilson & Wilson, 1956) there was described a large-scale stranding, on the shores of Cornwall and Devon in 1954, of the pelagic gastropod *Ianthina janthina* (L.). Most of the shells then collected were broken, but thirty-six were entire and measurements of these showed that, in spite of considerable variation in the proportion of width to height, the width is proportionately greater in young than in old shells. None of the 1954 shells was less than half an inch high, the majority being considerably higher. Two shells stranded at Sennen several years before, and the figure of one in a paper by Fowler (1947, as *I. planispirata* Adams & Reeve) were the only specimens less than half-an-inch high available for comparison with the larger ones. The measurements of these agreed with the general conclusion that as the shell grows it becomes proportionately less wide. In the past these variations in shell shape have been responsible for the erection of a number of different species. In order to establish the conclusion firmly it was desirable to examine a collection of young shells.

On 31 March 1957 Mrs M. Hicks wrote from St Agnes, Isles of Scilly, to say that on that day her husband had found eight small *Ianthina*, six of which had stranded alive on the afternoon's tide, the other two, one of them alive, earlier. Subsequently more were collected. On 8 April Miss Deborah Hicks (age 9) picked up about 130, some of them living, some apparently not. On 10 April about thirty-two more in the same general condition were obtained, and on 11 April another twenty-four, all apparently dead.

When examined in Plymouth all these specimens proved to be small *I. janthina* (L.), mostly of a shape formerly described as *I. planispirata* Adams & Reeve. Almost all were less than half an inch high, being generally much smaller, and most of the shells were unbroken. Such a large collection of small shells of this species probably does not exist elsewhere, and it is therefore of interest to record their measurements and to compare them with the lesser collection of large shells described last year.

As before, the shells were measured with calipers graduated in hundredths of an inch. These measurements are recorded in Table 1; the width/height ratios were again worked out. In the graph in Fig. 1 the ratio for each shell is

TABLE 1. MEASUREMENT OF SHELLS OF *IANTHINA JANTHINA* (L.)

No. of shells	Totals: each height	Height (in.)	Width (in.)	Ratio: width / height
1	1	0·22	0·28	1·27
2	5	0·23	0·30	1·30
2		0·23	0·31	1·35
1		0·23	0·32	1·39
1	5	0·24	0·31	1·29
1		0·24	0·33	1·38
3		0·24	0·34	1·42
1	4	0·25	0·33	1·32
2		0·25	0·34	1·36
1		0·25	0·37	1·48
1	5	0·26	0·34	1·31
1		0·26	0·35	1·35
1		0·26	0·36	1·38
2		0·26	0·37	1·42
3	13	0·27	0·33	1·22
1		0·27	0·35	1·30
1		0·27	0·36	1·33
4		0·27	0·37	1·37
2		0·27	0·38	1·41
2		0·27	0·39	1·44
3	8	0·28	0·36	1·29
1		0·28	0·37	1·32
2		0·28	0·38	1·36
1		0·28	0·39	1·39
1		0·28	0·40	1·43
1	10	0·29	0·35	1·21
2		0·29	0·36	1·24
2		0·29	0·37	1·27
1		0·29	0·38	1·31
1		0·29	0·39	1·34
2		0·29	0·40	1·38
1		0·29	0·41	1·41
2	13	0·30	0·38	1·27
5		0·30	0·39	1·30
3		0·30	0·40	1·33
2		0·30	0·41	1·37
1		0·30	0·42	1·40
1	12	0·31	0·38	1·23
5		0·31	0·40	1·29
3		0·31	0·41	1·32
2		0·31	0·42	1·36
1		0·31	0·43	1·39
1	15	0·32	0·40	1·25
3		0·32	0·41	1·28
5		0·32	0·42	1·31
4		0·32	0·43	1·34
1		0·32	0·44	1·38
1		0·32	0·48	1·50
1	7	0·33	0·40	1·21
3		0·33	0·42	1·27
3		0·33	0·43	1·30
2	8	0·34	0·40	1·18
2		0·34	0·43	1·26
1		0·34	0·44	1·29
2		0·34	0·45	1·32
1		0·34	0·46	1·35
2	6	0·35	0·44	1·26
1		0·35	0·45	1·29
3		0·35	0·46	1·31
2	5	0·36	0·45	1·25
3		0·36	0·47	1·31
1	6	0·37	0·46	1·24
1		0·37	0·47	1·27
2		0·37	0·48	1·30
1		0·37	0·49	1·32
1		0·37	0·50	1·35
2	4	0·38	0·47	1·24
1		0·38	0·48	1·26
1		0·38	0·49	1·29
1	1	0·39	0·51	1·31
0	0	0·40	—	—
1	5	0·41	0·52	1·27
1		0·41	0·53	1·29
1		0·41	0·54	1·32
1		0·41	0·55	1·34
1		0·41	0·57	1·39
1	3	0·42	0·54	1·29
2		0·42	0·57	1·36
2	3	0·43	0·55	1·28
1		0·43	0·59	1·37
1	3	0·44	0·56	1·27
1		0·44	0·57	1·30
1		0·44	0·58	1·32
1	7	0·45	0·54	1·20
1		0·45	0·55	1·22
2		0·45	0·56	1·24
2		0·45	0·60	1·33
1		0·45	0·62	1·38
1	5	0·46	0·58	1·26
2		0·46	0·59	1·28
1		0·46	0·60	1·30
1	2	0·47	0·57	1·21
1		0·47	0·60	1·28
1	1	0·48	0·60	1·25
1	1	0·49	0·59	1·20
3	5	0·50	0·64	1·28
1		0·50	0·65	1·30
1		0·50	0·66	1·32
1	2	0·51	0·64	1·26
1		0·51	0·67	1·31
1	1	0·52	0·69	1·33

plotted against the height. This graph is identical with that in Wilson & Wilson (1956, text-fig. 2) with these shells added. It will be seen that their positions on the graph are in good agreement with the line which was originally based on the fewer and mainly larger shells measured previously. The shells marked I and II have not only wide apertures but also spires flatter than most. The shells marked III have average spires but their apertures, and hence cross-sections of the coil, are noticeably narrow compared with most others; this gives these shells a tall appearance.

It appears that the shells comprise two main size-groups. The first of these contains 128 shells ranging in height from 0·22 to 0·39 in. with a peak (fifteen shells) at 0·32 in. The second group contains thirty-seven shells ranging in height from 0·41 to 0·52 in. with a peak (seven shells) at 0·45 in. These two groups are obvious in the graph (Fig. 1) on either side of height

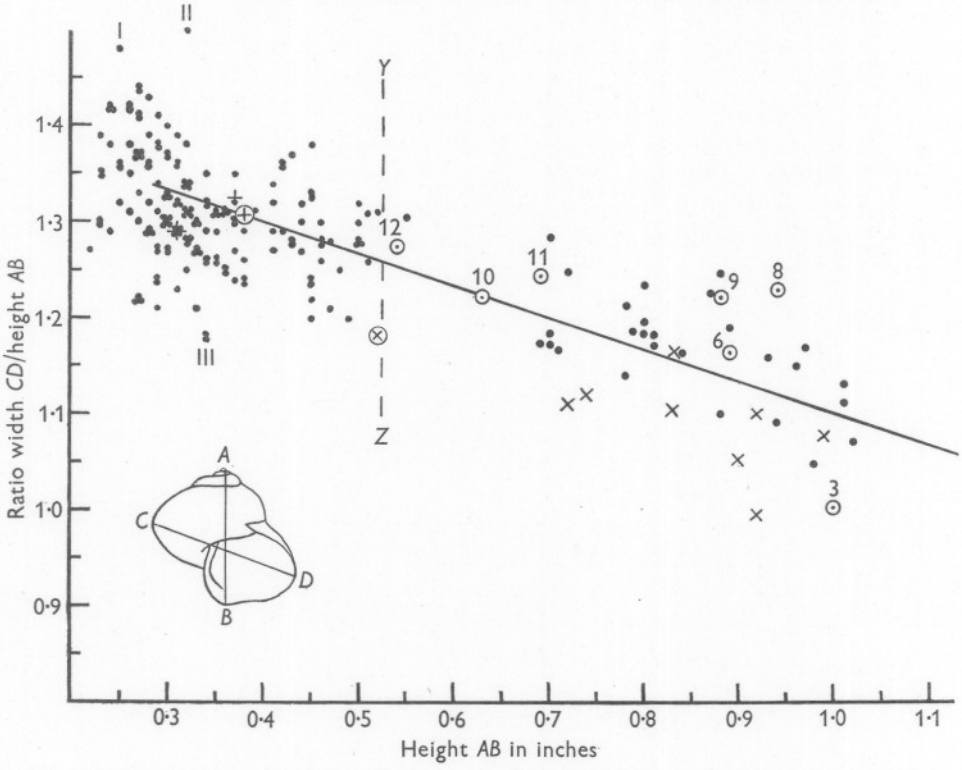

Fig. 1. Left-hand portion of the graph reproduced in Wilson & Wilson, 1956, p. 299, to which have been added, to the left of the broken line *YZ*, data for young *Ianthina janthina* shells stranded in the Isles of Scilly, March and April 1957. The graph shows the relation of shell width to shell height, expressed as ratio of width *CD* to height *AB*. Shells marked I–III are described in the text. For explanation of the remaining symbols see the legend to the original figure, reference as above.

0·40 in. There was no shell of that height in the collection. There is nothing to indicate what these groups represent, whether age, sex or other differences, but the presence of the peaks with almost regular falling off in numbers on either side (see Table 1) makes it likely that they are not purely fortuitous.

The new shells add appreciably to the data previously available, especially for small sizes, and confirm earlier conclusions concerning the effect of growth on the relations between width and height. It only remains to express grateful thanks to Mr and Mrs Hicks, and especially their daughter Deborah, for collecting the shells and forwarding them with such care.

REFERENCES

FOWLER, T. G. W., 1947. *Ianthina* in Cornwall. *J. Conch.*, Vol. 22, pt. 11, p. 167.

WILSON, D. P. & WILSON, M. A., 1956. A contribution to the biology of *Ianthina janthina* (L.). *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 291–305.

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A SINGLE-SOLUTION METHOD FOR THE DETERMINATION OF SOLUBLE PHOSPHATE IN SEA WATER

By J. MURPHY and J. P. RILEY
Department of Oceanography, The University, Liverpool

Of the reagents so far suggested for the reduction of phosphomolybdic acid to molybdenum blue in the determination of phosphate, only stannous chloride (Atkins, 1923; Harvey, 1948), metol (Burton & Riley, 1956), and ascorbic acid (Greenfield & Kalber, 1954), give colours of sufficient intensity to be used in the analysis of sea water.

Burton & Riley (1956) have recently reviewed the use of stannous chloride as a reducing agent in the molybdenum-blue method for the determination of phosphate in sea water. This reagent suffers from a number of disadvantages; in particular that (i) the time taken for development of maximum colour depends on the salinity of the water and its temperature, (ii) the colour fades quite rapidly, (iii) there is a large salt error, and (iv) different batches of molybdate reagent give different extinctions with the same amount of phosphorus.

These workers have proposed the use of metol (*p*-methyl-aminophenol sulphate) for the reduction of phosphomolybdic acid in the analysis of sea water. This reagent is free from most of the disadvantages of stannous chloride, but requires the development of colour to be carried out at 100° C.

The use of ascorbic acid for the reduction of phosphomolybdic acid was first reported by Ammon & Hinsberg (1936). Their method was modified by Lowry, Roberts, Leiner, Wu & Farr (1954), who used a greater concentration of ascorbic acid. Essentially the same procedure was employed by Chen, Toribara & Warner (1956) for the micro-determination of phosphorus in biological fluids. Greenfield & Kalber (1954) have suggested the use of ascorbic acid for the determination of phosphorus in sea water.

When this reductant was investigated, it was found possible to use a single reagent containing ammonium molybdate, ascorbic acid and sulphuric acid, instead of adding the acid molybdate and reducing agent separately, as in the stannous chloride method. The present paper describes the application of this mixed reagent to the determination of soluble phosphate in sea water.

The relative amounts of sulphuric acid and ammonium molybdate in the reagent exert a very marked effect on the formation of phospho- and silico-molybdic acids, and on the ease of reduction of these heteropoly acids and of

the molybdate ion itself. In order to establish the most suitable composition for the single-solution reagent, experiments were carried out varying the acid and molybdate concentrations systematically in the presence of a fixed amount of ascorbic acid. Aliquots (10 ml.) of distilled water, phosphate solution (5 μg PO_4^{3-}-P/ml.) and silicate solution (120 μg SiO_3^{2-}-Si/ml.) were treated with mixed reagents containing varied volumes of 2% ammonium molybdate and 4 N sulphuric acid, together with 5 ml. of 0·1 M ascorbic acid; the solutions were diluted to 50 ml. After 24 h their optical densities were measured at 827 mμ in a 1 cm cell. The results, which are given in Table 1, show that if low reagent blanks and freedom from interference by silicate are to be realized, the final concentrations of acid and molybdate in the solution should be approximately 0·40 N and 0·10% respectively. Variation of the final

TABLE 1. DETERMINATION OF OPTIMUM ACID AND MOLYBDATE CONCENTRATIONS FOR ESTIMATION OF PHOSPHATE USING ASCORBIC ACID

Optical densities measured at 827 mμ (1 cm cell).

ml. of 4 N sulphuric acid	ml. of 2 % ammonium molybdate: 1	2	3	4	5	7	10
2·5 Blank	0·010	—	0·024	—	>2·5	—	—
2·5 50 μg PO_4^{3-}-P	0·445	—	0·475	—	>2·5	—	—
2·5 1·2 mg SiO_3^{2-}-Si	0·074	—	>2·5	—	>2·5	—	—
5·0 Blank	0·006	0·006	0·006	0·016	0·018	0·025	>2·5
5·0 50 μg PO_4^{3-}-P	0·246	0·452	0·456	0·455	0·455	0·454	>2·5
5·0 1·2 mg SiO_3^{2-}-Si	0·017	0·041	0·043	0·204	0·609	1·59	>2·5
7·5 Blank	0·004	—	0·009	—	0·011	0·015	>2·5
7·5 50 μg PO_4^{3-}-P	0·061	—	0·441	—	0·442	0·477	>2·5
7·5 1·2 mg SiO_3^{2-}-Si	0·013	—	0·024	—	0·139	0·355	>2·5
10·0 Blank	0·005	—	0·007	—	0·012	0·008	0·005
10·0 50 μg PO_4^{3-}-P	0·005	—	0·301	—	0·450	0·438	0·440
10·0 1·2 mg SiO_3^{2-}-Si	0·010	—	0·012	—	0·017	0·018	0·022
15·0 Blank	0·003	—	0·005	—	0·011	0·015	0·017
15·0 50 μg PO_4^{3-}-P	0·006	—	0·038	—	0·170	0·392	0·447
15·0 1·2 mg SiO_3^{2-}-Si	0·006	—	0·011	—	0·015	0·019	0·028

ascorbic acid concentration in the range 0·004 to 0·03 M has no effect on the optical density attained. In all subsequent work 8 ml. of a reagent 2·5 N in sulphuric acid, 0·60% in ammonium molybdate, and 0·03 M in ascorbic acid was used for a final volume of 50 ml.

Using this reagent, colour development is complete in both sea water and distilled water after 24 h at 20° C, or after 30 min at 60° C. The colour once formed is stable for at least 60 h. The calibration curve is independent of changes in batches of reagents.

METHODS

All measurements of optical density were made with a Unicam S.P. 500 spectrophotometer modified to use 7·62 and 15·24 cm cells. Distilled water was used in the compensating cell.

Reagents

Sulphuric acid 5N. Dilute 70 ml. of concentrated sulphuric acid to 500 ml.

Ammonium molybdate (4%). Dissolve 20 g of ammonium molybdate A.R. in distilled water and dilute to 500 ml. Store the solution in a Pyrex glass reagent bottle.

Ascorbic acid 0·1M. Prepare a solution of 1·76 g of ascorbic acid in 100 ml. of distilled water. It is preferable to prepare this solution on the day it is needed, but if this is not possible, the solution may be preserved for about 10 days at 0° C.

Mixed reagent. Mix thoroughly 125 ml. of 5N sulphuric acid and 37·5 ml. of 4% ammonium molybdate solution. Add 75 ml. of ascorbic acid solution and dilute to 250 ml. The mixed reagent does not keep well and should be prepared within an hour of using.

Standard solutions

Stock phosphate solution. Dissolve 0·1757 g of potassium dihydrogen phosphate in distilled water and dilute to 1 l. This solution, which contains 40 mg PO_4^{3-}-P/l., should be preserved in a dark glass bottle.

Dilute phosphate solution. Prepare, by dilution of the stock phosphate solution, a solution containing 0·2 mg PO_4^{3-}-P/l. This standard solution should be prepared daily.

Treatment of apparatus

Allow apparatus, which is to be used in the determination, to stand overnight filled with concentrated sulphuric acid, and then rinse thoroughly. It is preferable to keep a set of graduated flasks to be used only for the determination of phosphate; after use, they should be washed well and kept filled with distilled water until required again. If this is done, the treatment with sulphuric acid is only required occasionally.

Determination of phosphate

Pipette 40 ml. of the sea-water sample into a 50 ml. graduated flask, add 8 ml. of the mixed reagent from a tilt measure and dilute to volume. Allow the colour to develop for 24 h at room temperature (20° C). Measure the optical density of the solution at 827 mμ. Determine the reagent blank in the same manner using freshly distilled water. Calibrate the method using 5, 10 and 20 ml. aliquots of the dilute standard phosphate (1, 2 and 4 μg. PO_4^{3-}-P respectively). To correct the calibration curve for use with sea water multiply the observed optical densities by the appropriate salt error factor (Table 3). The calibration curve remains constant and is independent of changes in the batches of reagents. The colour development may be carried out more rapidly at 60° C if desired. Transfer the solution to a loosely stoppered Pyrex flask (treated as described above) and heat it in a water bath at 60° C for 30 min. After cooling, measure the optical density of the solution at 827 mμ.

Beer's law and reproducibility

Calibration runs were carried out using 1–25 μg PO_4^{3-}-P in 40 ml. of distilled water. The colour was developed for 24 h at room temperature and the optical densities were then measured at 827 mμ in cells of appropriate length. The results, which are given in Table 2, show a satisfactory repro-

ducibility and show that Beer's law is obeyed up to a concentration of at least 0·5 p.p.m. PO_4^{3-}-P. Similar figures were obtained when the colour was developed at 60° or 90° C.

TABLE 2. DETERMINATION OF PHOSPHORUS

PO_4^{3-}-P μg	No. of determinations	Mean optical density* (7·62 cm cell)	Mean deviation (%)	Deviation from linearity (%)
1	4	0·134	0·6	+0·1
2	4	0·267	0·5	−0·3
3	4	0·403	0·3	+0·3
4	4	0·534	0·5	−0·3
5	3	0·671†	0·5	+0·2
10	3	1·342†	0·2	+0·2
15	3	2·019†	0·3	+0·5
20	3	2·667†	0·3	−0·4
25	3	3·330†	0·3	−0·5

* Less reagent blank.

† Measured in 1 cm cell and calculated for 7·62 cm cell.

TABLE 3. SALT ERROR CORRECTIONS

Sea water, Cl‰	0	5	10	15	19·3
Mean optical density for 25 μg, PO_4^{3-}-P*	0·437	0·424	0·420	0·417	0·419
Salt error, %	0	−2·9	−3·9	−4·6	−4·1
Correction factor	1·00	0·970	0·961	0·954	0·959

* 1 cm cell.

SALT ERROR

The effect of the salts of sea water on the intensity of the molybdenum-blue colour, developed by the mixed reagent, has been studied. Filtered sea water, low in phosphate, was diluted with distilled water to give sea waters having chlorinities of 5, 10, 15 and 19·3‰. Duplicate phosphate determinations were made on 40 ml. portions of these waters and on 40 ml. aliquots which had been enriched with 25 μg PO_4^{3-}-P. Colour development was carried out at room temperature. The results, which are summarized in Table 3, indicate that, as with the metol method, the salt error is not a linear function of chlorinity. For oceanic waters it is small compared with the salt error of the stannous chloride procedure (*ca.* 20%).

INTERFERENCES

The interference of several ions, known to interfere in molybdenum-blue methods for the determination of phosphorus, has been investigated using the ascorbic acid reagent. Experiments were performed using 40 ml. aliquots of distilled water containing these ions, both alone, and in the presence of 5 μg of PO_4^{3-}-P. The results (Table 4) indicate that of the elements capable of forming molybdenum-blue complexes, only arsenic interferes appreciably.

In every case the interference is much less than that found with either the stannous chloride or metol methods. The effects of these ions at their normal concentrations in sea water will be quite negligible. High concentrations of copper (500 p.p.m.) seriously reduce the intensity of colour formed, presumably by destruction of the ascorbic acid. Even as much as 500 p.p.m. of ferric iron has no effect. Since the colour development is rather prolonged and takes place in 0·24 N acid, it was thought that hydrolysis of the organic phosphorus compounds present in sea water might occur. This did not prove to be the case, however, since determinations carried out in distilled water, to which fairly readily hydrolysable organic phosphorus compounds had been added, gave no molybdenum-blue colour even after heating for several hours at 70° C.

TABLE 4. INTERFERENCES OF CERTAIN ELEMENTS

Element	Added as	Concentration of element (p.p.m.)	Optical density at 827 mμ (7·62 cm cell)		
			No added phosphate	5 μg PO_4^{3-}-P	Difference
—	—	—	0·055	0·710	0·655
Copper	$CuSO_4$	500	0·057	0·600	0·543
Iron	$FeCl_3$	50	0·056	0·715	0·659
Arsenic	Arsenate	1	0·107	0·814	0·707
Arsenic	Arsenite	0·1	0·059	0·715	0·656
Silicon	Silicate	10·0	0·057	0·710	0·653
Vanadium	Vanadate	500	0·085	0·743	0·658
Germanium	Germanate	1·0	0·057	0·715	0·658

SUMMARY

It has been found that a reagent containing sulphuric acid, ammonium molybdate and ascorbic acid may be used as a single-solution reagent for the determination of phosphate in sea water. Development of the molybdenum-blue colour is complete in 24 h at room temperature and in 30 min at 60° C; the colour is stable for at least 3 days. Beer's law is obeyed closely up to at least 500 μg PO_4^{3-}-P/l. The salt error is approximately 4% with sea water of chlorinity 19·3‰. The interference due to either arsenate or silicate at their concentrations in sea water is negligible.

REFERENCES

AMMON, R. & HINSBERG, K., 1936. Colorimetrische Phosphor- und Arsenbestimmung mit Ascorbinsäure. *Hoppe-Seyl. Z.*, Bd. 239, pp. 207–216.

ATKINS, W. R. G., 1923. The phosphate content of fresh and salt waters in its relationship to the growth of the algal plankton. *J. mar. biol. Ass., U.K.*, Vol. 13, pp. 119–59.

BURTON, J. D. & RILEY, J. P., 1956. Determination of soluble phosphate and total phosphorus in sea-water and of total phosphorus in marine muds. *Microchim. Acta*, 1956, p. 1350.

CHEN, P. S., TORIBARA, T. Y. & WARNER, H., 1956. Microdetermination of phosphorus. *Anal. Chem.*, Vol. 28, pp. 1756–8.

GREENFIELD, L. J. & KALBER, F. A., 1954. Inorganic phosphate measurement in seawater. *Bull. mar. Sci. Gulf Carib.*, Vol. 4, p. 323.

HARVEY, H. W., 1948. The estimation of phosphate and total phosphorus in seawaters. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 337–59.

LOWRY, O. H., ROBERTS, N. R., LEINER, K. Y., WU, M. L. & FARR, A. L., 1954. Quantitative histochemistry of brain. I. Chemical Methods. *J. biol. Chem.*, Vol. 207, pp. 1–16.

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THE FUNCTION OF THE HEART-BODY IN POLYCHAETES

By G. Y. KENNEDY
Cancer Research Unit, University of Sheffield,
and R. PHILLIPS DALES
Department of Zoology, Bedford College, London

(Text-figs. 1–9)

Loose, spongy intravasal tissues are common in polychaetes. In some this tissue is localized and forms a discrete plug or strand known as the heart-body. Such structures occur in the anterior part of the dorsal vessel in terebellids, ampharetids, cirratulids, amphictenids and flabelligerids.

Many authors have described the heart-body in different species and speculated about its function. Picton (1898) examined in some detail the heart-body in the cirratulid *Audouinia tentaculata* and in the terebellids *Polymnia nebulosa* and *Terebella meckelii*. He concluded that in *Polymnia* at least, the heart-body is formed by infolding from the epithelium on the outside of the vessel, so that it is comparable with the chloragogen cells which, in many polychaetes, clothe the outside of the vessels. Salensky (1884) working on *Terebella*, came to the same conclusion. Eisig (1887) also pointed out that such extravasal chloragogen cells are absent in species with intravasal heart-bodies, and compares the chloragosomes or yellow-green granules found in the chloragogen cells with the granules found in the heart-body. Romieu (1923) comes to similar conclusions. Fauvel (1897) concluded from his study of the ampharetids, that the heart-body served a valvular function, and suggested that it also removed and stored substances extracted from the blood, but was not directly related to the amoebocytes. Schneider (1897, 1899), on the other hand, concluded that the organ was related to the phagocytic and excretory systems in the amphictenid, *Pectinaria*. Picton (1898) concluded that the greenish granules were removed from the heart-body to the coelomic fluid, and from there to the outside by the nephridia, since the quantity of pigment did not appear to be greater in older worms.

In addition to the greenish or yellow granules in the heart-body, Picton (1898) also found fat, iron, and brownish 'chitinous bodies'. Iron was also detected by Bloch-Raphael (1939) and Romieu (1923), and its occurrence suggests an haematopoietic function. Schneider (1897), who also found iron in the heart-body of *Pectinaria*, claimed that this increased after injection of

iron saccharate into the coelom; Meyer (1887) also suggested, from his study of the cirratulid *Chaetozone*, that the heart-body is an haematopoietic organ.

The histological structure of the heart-body does not differ greatly in polychaetes belonging to different families, although it is variable in size and extent within the dorsal vessel, and may also increase with age (Ashworth, 1904). It is perhaps most extensive in the cirratulids, but seems best developed in the terebellids. The appearance of the heart-body of *Audouinia tentaculata* in transverse section is shown in Fig. 1. The organ in cirratulids extends throughout the region in which the dorsal vessel supplies vessels to the

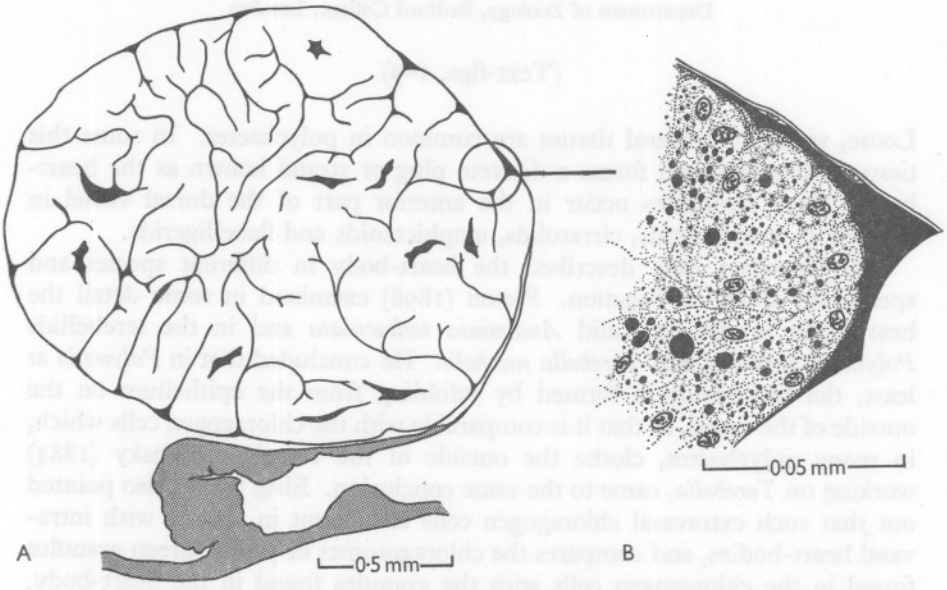

Fig. 1. *Audouinia tentaculata*, heart-body; A, section of whole organ, with gut-wall shaded; B, enlarged detail of part of A.

branchiae, and the general occurrence of heart-bodies in those polychaetes with specialized gill structures suggests that the organ has a mechanical function, as Picton (1898) concluded; certainly at systole the lumen of the dorsal vessel is completely obliterated by the wall of the vessel contracting on to the heart-body. It is perhaps not unreasonable to suppose that such loosely compacted tissue may have acquired other functions; a physiological parallel may be found in the vertebrate bone-marrow.

In the present work, pigment analyses have been made of the heart-bodies from the following species representing the main families of polychaetes in which discrete heart-bodies are found: *Amphitrite johnstoni* Malmgren, *Terebella lapidaria* L., *Lanice conchilega* (Pallas) (Terebellidae); *Audouinia tentaculata* (Montagu), *Cirratulus cirratus* (O. F. Müller) (Cirratulidae);

Melinna palmata (Grube) (Ampharetidae); and *Flabelligera affinis* Sars (Flabelligeridae). Unfortunately, insufficient specimens of *Pectinaria* were collected to make pigment examination of the heart-body possible. The heart-bodies of *Amphitrite johnstoni* and *Audouinia tentaculata* have also been examined histologically and histochemically. Similar chemical methods have been applied to other tissues in all these worms for comparison, and the work extended to *Polycirrus caliendrum* Clap. (Terebellidae), *Myxicola infundibulum* (Renier) (Sabellidae) and *Arenicola marina* L., none of which has a discrete heart-body, although there is a little intravasal tissue in the latter genera; *Polycirrus* has no vascular system. The extravasal tissue present in old *Arenicola* has also been examined histologically (Fig. 2), histochemically, and subjected to the same procedures to be described for *Amphitrite*.

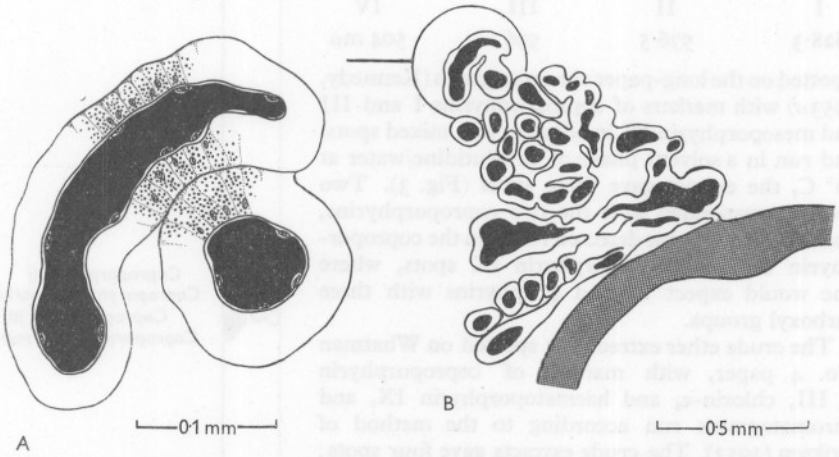

Fig. 2. *Arenicola marina*, extravasal tissue; A, detail of part of tissue; B, section of tissue, with gut wall shown shaded.

The work described in this paper was done at the Plymouth Laboratory, and we would like to express our appreciation of the facilities and help afforded to us by the Director and Staff, in particular Mr F. A. J. Armstrong for copper analysis; to Miss M. L. Weir for preparing the sections represented in Fig. 2, and to Mr A. C. Briggs for collecting material. We are also grateful to Mr W. H. Lockwood, of the Department of Chemical Pathology, University College Hospital Medical School, London, for some helpful discussion.

METHODS

The tissues from the animals examined were extracted by the methods described by Kennedy & Vevers (1953, 1954). Whenever possible, the pigments detected were isolated, melting points determined, and some

characteristic derivatives prepared. Absorption spectra were determined in the Beck–Hartridge Reversion Spectroscope, in the Unicam S.P. 500 Spectrophotometer, and in the Beck microspectroscope.

RESULTS

The main results are presented in Table 1.

Amphitrite johnstoni

Heart-body

Ether-acetic acid extracts from fifteen heart-bodies were washed with water containing some potassium acetate. The hypophase was bright yellow and showed a fine green fluorescence in u.v. light. The epiphase was red-fluorescent in u.v. light, and gave a spectrum (Hartridge):

I	II	III	IV
628·3	576·5	536	504 mμ

Spotted on the long-paper chromatograph (Kennedy, 1953*a*) with markers of coproporphyrins I and III and mesoporphyrin IX in adjacent and mixed spots, and run in a solvent phase of 2:6-lutidine/water at 26° C, the extract gave three spots (Fig. 3). Two spots corresponded with the two coproporphyrins, and another spot was detected between the coproporphyrin III and mesoporphyrin IX spots, where one would expect to find porphyrins with three carboxyl groups.

The crude ether extract was spotted on Whatman No. 4 paper, with markers of coproporphyrin I, III, chlorin-e_6 and haematoporphyrin IX, and chromatograms run according to the method of Eriksen (1953). The crude extracts gave four spots: two corresponded with those given by coproporphyrins I and III, there was one spot at the tricarboxylic level (see below, Band 3), and one dark-coloured non-fluorescent spot joining the latter with that given by coproporphyrin III (Fig. 4). This dark spot is probably a haematin.

Extracts of a further fifteen heart-bodies in methanol-sulphuric acid were diluted with an equal volume of water and extracted with chloroform. The chloroform extract was washed with 2 % sodium chloride, and then with water, and concentrated *in vacuo*. This solution gave a spectrum (Hartridge):

I	II	III	IV
626·3	573·5	540	506·2 mμ

After a further washing, the esterified pigment was spotted on Whatman No. 1 C.R.L. slotted paper, and chromatograms run according to the method of

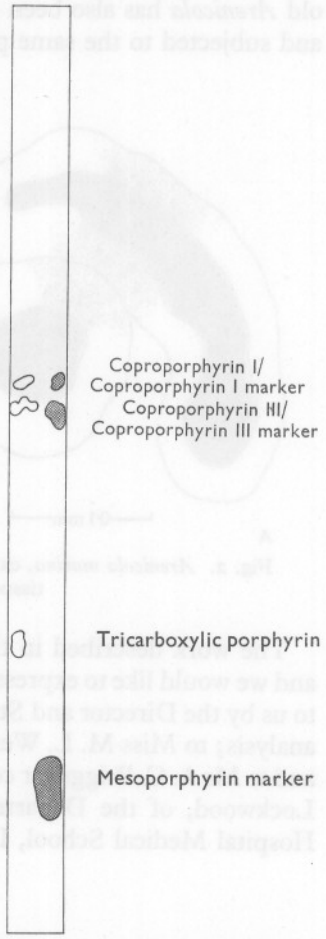

Fig. 3. Long-paper descending chromatogram of extract of *Amphitrite* heart-body, with adjacent markers.

Chu, Green & Chu (1951) for separating isomers of the coproporphyrins. Coproporphyrins I and III esters, and mesoporphyrin IX ester were used as markers. By this method, both the isomers I and III of coproporphyrin were found in the extract (Fig. 5). The chloroform solution of the pigment esters was then chromatographed on a column of magnesium oxide grade III (Nicholas, 1951) packed in chloroform. The chromatogram was developed with methanol-in-chloroform, 0·5, 1 and 2%, successively, and the fractions collected separately as the bands were eluted. Four fractions were obtained as follows:

Band 1. Brownish red fraction passing quickly down the column and out. Red-fluorescent in u.v. light. Hartridge spectrum:

I	II	III	IV
645	586	545	504 mμ (Chlorin-type)

TABLE 1. LOCALIZATION OF THE PIGMENTS IN THE TISSUES

	Uro I	Copro I	Copro III	3-COOH	Proto IX	Haematin	Yellow green-fluorescent	Blue-fluorescent
Amphitrite								
Heart-body	−	+	+	+	−	+	+	−
Coelomic cells (pink)	−	−	+	−	−	+	−	−
Coelomic cells (brown)	−	−	+	−	−	+	−	−
Blood	−	−	−	−	−	+ (Hb)	−	−
Body wall	−	−	+	−	−	+	+	−
Gut	−	−	−	−	−	−	−	−
Terebella								
Heart-body	−	−	+	−	−	−	−	−
Coelomic cells	−	−	−	−	−	+	−	−
Blood	−	−	−	−	−	+ (Hb)	−	−
Body wall	−	−	−	−	−	−	−	−
Gut	−	−	−	−	−	−	−	−
Lanice								
Heart-body	−	−	+	−	−	−	−	−
Gut	−	−	+	−	−	−	−	−
Cirratulus								
Heart-body	−	−	+	−	−	+	−	−
Body wall + gut	−	−	−	−	−	+	−	−
Audouinia								
Heart-body	−	+	+	−	−	−	−	+
Blood	−	+	+	−	−	+ (Hb)	−	−
Body wall	−	−	−	−	−	?	−	−
Gut	−	+	+	−	−	−	−	+
Melinna								
Heart-body	−	−	+	−	−	−	+	−
Body wall	−	−	−	−	−	−	−	−
Gut	−	−	−	−	−	−	−	−
Flabelligera								
Heart-body	−	−	+	−	−	−	−	+
Body wall	−	−	−	−	−	−	−	+
Gut	−	−	+	−	−	−	−	−
Polycirrus								
Whole body	−	−	+	−	−	−	−	−
Body wall	−	−	+	−	−	−	−	−
Gut	−	−	+	−	−	−	−	−
Myxicola								
Anterior nephridia	−	−	−	−	−	−	−	−
Body wall + gut	−	−	+	−	−	+	−	−
Arenicola								
Extravasal tissue	−	+	+	+	−	+	−	−
Body wall (dark)	−	+	+	+	−	+	+	−
Body wall (pink)	−	+	+	+	−	+	+	−

(Hb) = from haemoglobin.

In the spectrophotometer, only the band at 643 mμ was detected, probably owing to impurity. This spectrum resembles that given by dioxymesoporphyrin:

I	II	III	IV	
640	584	542	504 mμ	(chlorin type)

This pigment could be an artefact arising during manipulation of the pigments.

Band 2. A wide pink band, obviously the main pigment present. This was collected after elution with 0·5 % methanol in chloroform. This gave a spectrum in the Unicam:

I	II	III	IV	Soret	
622	569	535	501	405 mμ	(aetio-type)

This is the spectrum given by coproporphyrin.

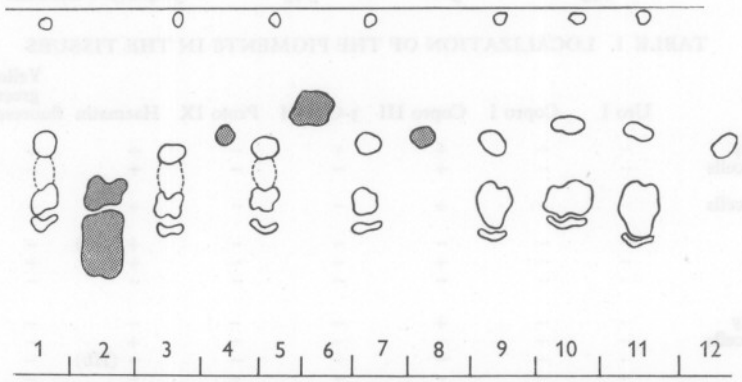

Fig. 4. Ascending paper chromatogram of *Amphitrite* heart-body and *Arenicola* tissue extracts. Solvent 2:6-lutidine/water at 26° C in atmosphere of NH_3. The small spots at the top are carotenoids; the other spots are as follows: (1) *Amphitrite* heart-body; (2) coproporphyrin III (lower) and coproporphyrin I; (3) *Amphitrite* heart-body ; (4) chlorin-e_6; (5) *Amphitrite* heart-body; (6) haematoporphyrin; (7) *Amphitrite* heart-body; (8) chlorin-e_6; (9) *Arenicola* extravasal tissue; (10) *Arenicola* body wall (light); (11) *Arenicola* body wall (dark); (12) *Amphitrite* heart-body 3-COOH spot.

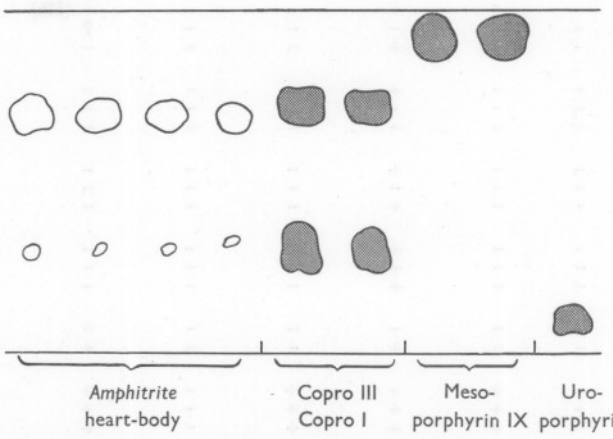

Fig. 5. Separation of porphyrin isomers by Chu, Green & Chu technique (1951).

Band 3. A third red-fluorescent band appeared which travelled much more slowly than the preceding band, and was eluted with 2% methanol in chloroform. This band gave a spectrum in the Unicam:

I	II	III	IV	Soret
622	572	538	503	404 mμ (aetio-type)

Band 4. Some pigment remained close to the top of the column and could not be eluted even with 5% methanol in chloroform. The column was pushed out and this band cut off with a razor blade. The brown material at the very top of the column was discarded, and the red-fluorescent material eluted with chloroform containing a trace of formic acid. The chloroform extract was washed with water containing a little potassium acetate and then with water to remove the formic acid. The remaining chloroform solution gave a spectrum in the Unicam:

I	II	III	IV	Soret
621	568	535	500	402 mμ (aetio-type)

This spectrum resembles that of coproporphyrin, and it is possible that this is unesterified pigment in view of its chromatographic behaviour, although free coproporphyrin is normally somewhat insoluble in chloroform.

Band 1 containing the pigment which resembles dioxymesoporphyrin could be, as already mentioned, an artefact due to oxidation during the extraction process. In the first paper chromatograms carried out, no spot at the 2-carboxyl level was obtained from the crude *Amphitrite* heart-body extracts. However, in later chromatograms a trace of red fluorescence at the 2-carboxyl level was in fact obtained. This would lend credence to the view that this pigment is indeed an oxidation artefact.

Band 2, which contains the bulk of the porphyrin present in the heart-body, was shown, by the technique of Chu *et al.* (1951), to be a mixture of coproporphyrins I and III, with the III isomer predominating (Fig. 5). The isomers were separated by the Chu *et al.* (1951) method, the spots of the isomers were cut out with scissors, and those of each isomer pooled. The porphyrin esters were eluted with chloroform-methanol mixture 100:2, and the methanol washed out with water containing some potassium acetate to prevent the coproporphyrin from passing into the water phase. After a further washing with water, the chloroform solutions were roughly dried by filtering through chloroform-soaked filter papers, and the pigments crystallized. Coproporphyrin III was crystallized by evaporating to a volume of 3 ml. and adding an equal volume of dry ether, stirring all the time. The mixture was placed in the ice chest for some hours, when clusters of coproporphyrin III tetramethyl ester were obtained (Rimington, 1939). The melting point after one recrystallization was 136° C. Vannotti (1954) gives the melting point of coproporphyrin III tetramethyl ester as 137° C. There was too little of the coproporphyrin I isomer for crystallization.

The pigment ester from Band 3 was hydrolysed by dissolving it in concentrated HCl and allowing it to stand at room temperature overnight. The HCl was removed in a vacuum desiccator over solid KOH, and the resultant pigment dissolved in a little 2:6-lutidine. The solution was spotted on to Whatman No. 4 paper with markers of chlorin-e_6, coproporphyrin I, coproporphyrin III, and haematoporphyrin, and the chromatograms were run at 26° C in 2:6-lutidine/water in an atmosphere of ammonia (Fig. 4). The pigment gave only one spot which travelled further than those of the tetracarboxylic coproporphyrin III, and took up a position approximating to that given by chlorin-e_6 which has three carboxyl groups. It will be noted that spots of the crude extract from *Amphitrite* heart-body also gave this spot, but in this case it did not

travel quite as far as the tricarboxylic chlorin spot. This is most likely due to retardation by the other pigments travelling behind it. From these data it is permissible to conclude that the pigment from Band 3 is a tricarboxylic porphyrin. The free porphyrin has an HCl number of 0·5–1·0.

Band 4 gave a very indeterminate spot on an ascending lutidine chromatogram, about the 4-carboxyl level, the fluorescence of which was not particularly intense. It would, therefore, seem that this is in fact some unesterified coproporphyrin.

The pigments obtained from the heart-body tissue were, therefore, coproporphyrins I and III, coproporphyrin III being the main pigment present, and a tricarboxylic porphyrin.

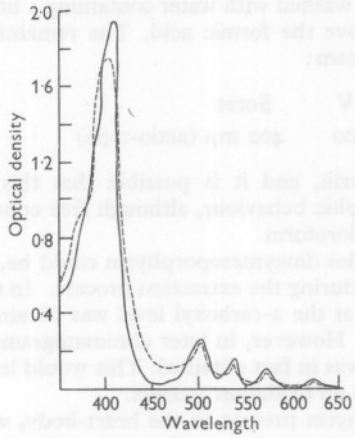

Fig. 6

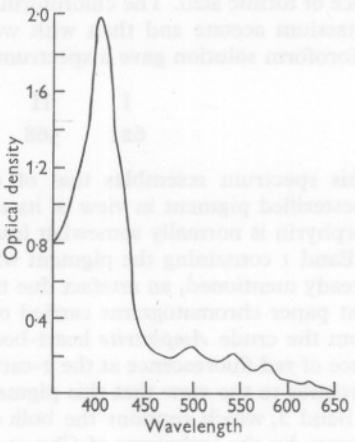

Fig. 7

Fig. 6. Absorption curves of main heart-body pigment ester (Band 2) in $CHCl_3$ (broken line) and of coproporphyrin I tetramethyl ester in $CHCl_3$ (continuous line).

Fig. 7. Absorption curve of tricarboxylic porphyrin ester (Band 3) in $CHCl_3$.

Remaining tissues

The coelomic cells, pink and brown (see Dales, 1957); the blood, the body wall, and the gut were all examined by the methods which have already been described for the heart-body. These results are summarized in Table 1, from which it will be seen that with the exception of the gut all these tissues contain haematin, shown to be protohaematin by histochemical tests. This haematin, treated with hydrazine hydrate gave a spectrum, Hartridge:

I	II
555	530 mμ

The haematin from the blood was certainly formed from the haemoglobin in the course of extraction.

Coproporphyrin III was present in the coelomic cells and the body wall in relatively small amounts as compared with that in the heart-body, and was entirely absent from the blood. The tricarboxylic pigment did not occur in any of these tissues.

The body wall and the heart-body contained a bright yellow intensely green-fluorescent pigment resembling in behaviour the pigment found in *Arenicola* and in holothurians. It is intended to investigate this pigment later.

Terebella lapidaria

Coproporphyrin III was the only porphyrin present, occurring only in the heart-body. Protohaematin was detected in the coelomic cells, and in extracts of the blood, in which case it was formed from haemoglobin.

Lanice conchilega

Coproporphyrin III was the only porphyrin detected, and occurred in the heart-body and the gut.

Cirratulus cirratus

Coproporphyrin III was detected in the heart-body together with protohaematin. Protohaematin was also detected in the body wall plus gut.

Audouinia tentaculata

The heart-body, the blood and the gut contained coproporphyrins I and III, with the III isomer predominating. Protohaematin was derived from the haemoglobin, in the blood extracts, and this pigment possibly occurred in the body wall. The heart-body and the gut also contained a colourless compound giving an intense blue fluorescence in u.v. light (c.f. *Flabelligera*).

Melinna palmata

Coproporphyrin III was detected in the heart-body, but not in the body wall or the gut. The heart-body in addition contained some of the yellow, green-fluorescent pigment found in *Amphitrite*.

Flabelligera affinis

The heart-body and the gut contained coproporphyrin III. Ether-acetic extracts of the heart-body and of the body wall when washed with water showed an intense bright blue fluorescence in u.v. light apparently masking or quenching that due to coproporphyrin. The residue was a dark green in colour, and was non-fluorescent. Chromatographed in the long paper apparatus in 2:6-lutidine/water at 23° C in an atmosphere of ammonia, an intensely blue-fluorescent spot was formed at R_F 0·6, which is at the 4-carboxyl level. Again, the fluorescence of the coproporphyrin seemed to be masked. On washing the ether-acetic acid extract with water, the blue-fluorescent pigment became hypophasic and could thereby conveniently be separated. The ether epiphase when concentrated *in vacuo* gave the spectrum (Hartridge) of coproporphyrin, and when examined chromatographically, only the III isomer could be detected. The water hypophase containing the blue-fluorescent pigment was extracted with *n*-butanol, when all the pigment went into the butanol phase. Evaporated to dryness *in vacuo* and redissolved in butanol, no definite absorption spectrum was given in the spectrophotometer (Fig. 8). This pigment is interesting in that it is extremely soluble in both fat solvents and in water, and it has a definite R_F value. Fox, Crane & McConnaughey (1948) described non-carotenoid blue-fluorescent chromolipoids from *Thoracophelia mucronata*. These pigments have single sharp maxima in the u.v. at from 310 to 300 mμ or below, but the pigment of *Flabelligera* does not seem to be of this type since it has no definite maxima, and is extremely soluble in water. It is possible that the pigment is related to fluorocyanine. Phaeophorbide-*a* was also detected in the ether epiphase, and the dark green non-fluorescent residue from the ether extraction when treated with mineral acids gave the absorption

spectrum of phaeophorbide-*a*. Copper was detected in the ash. The dark green non-fluorescent pigment in the residue must, therefore, be a copper phaeophorbide-*a*. This pigment has already been detected in the mollusc *Akera bullata* (Kennedy & Vevers, 1956).

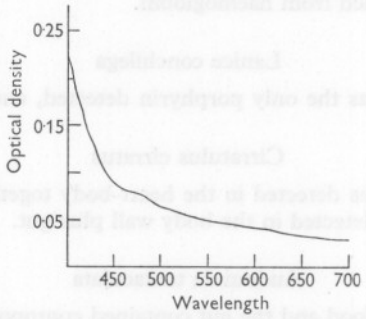

Fig. 8. Absorption curve of blue-fluorescent pigment from *Flabelligera* in *n*-butanol.

Polycirrus caliendrum

Coproporphyrin III was the only porphyrin detected, and occurred in extracts of the whole body, the body wall, and the gut. (*Polycirrus* is without vascular system, and hence heart-body.)

Myxicola infundibulum

Coproporphyrin III and chlorohaematin were found in the body wall plus gut, but not in the anterior nephridia. It is most likely that the haematin came from the body-wall tissue. Coproporphyrin III has been found previously in the gut (Kennedy & Vevers, 1954), and chlorohaematin can be prepared from the blood (Kennedy, 1953*b*).

Arenicola marina

Ashworth (1904) reports intravasal tissue increasing with age within the hearts of *Arenicola*. This was found in insufficient quantity for extraction. Extravascular tissue, also increasing with age, related to certain vessels from the hinder part of the intestine, occurs in larger amounts, and extracts were made of this tissue and of the body wall of pink and dark worms (Fig. 2).

Extravasal tissue was removed from a number of worms, homogenized, washed with sea water, and centrifuged. This process was repeated until the tissue was freed from blood. No absorption band was visible with the microspectroscope; the spectrum of oxyhaemoglobin was absent. After the addition of a drop of hydrazine hydrate, the pink colour of a haemochromogen appeared. This gave a two-banded spectrum in the microspectroscope at

I	II	
555	525 mμ	(order of intensity I–II)

This is the spectrum given by protohaemochromogen, indicating that protohaem is present in the tissue, most probably as haematin. A drop of 2 N-HCl, added to a smear of the extravasal tissue on a slide, produced the spectrum of acid haematin with a strong maximum of 660 mμ. This is characteristic of acid protohaematin. The smear of the extravasal tissue allowed to dry on a slide shows very little, if any, fluorescence

in u.v. light. A drop of ethanol, when added, caused the appearance almost immediately of a bright yellowish green fluorescence resembling that already described in *Amphitrite* and *Melinna*. This fluorescent pigment was washed off with ether, after which no fluorescence was apparent. A drop of formic acid produced a bright red fluorescence at once. Examined with the microspectroscope the absorption spectrum of coproporphyrin became apparent, with a slight shift of the bands towards the red due to formic acid. The non-appearance of coproporphyrin spectrum on examination of the fresh tissue may be due to the fact that the porphyrin is almost certainly present in combination with protein in the extravasal tissue. The addition of formic acid would not be sufficient to break down a haematin in the cold.

Fresh *Arenicola* blood gives a spectrum of oxyhaemoglobin:

I	II	
578	540 mμ	(Hartridge)

The addition of a drop of hydrazine hydrate produced the haemochromogen:

I	II	
555	525 mμ	(Hartridge)

and bubbling carbon monoxide through a sample of haemolysed blood produced a spectrum:

I	II	
570	540 mμ	(Hartridge)

of carboxyhaemoglobin. These experiments were sufficient to indicate that the blood contains a haemoglobin with protohaem as its prosthetic group. A drop of fresh blood treated with dilute HCl produced a brown coagulum. This did not fluoresce with u.v. light, even after the addition of formic acid, and this is conclusive proof that the blood does not contain a free porphyrin.

These experiments indicate that the porphyrin in the extravasal tissue does in fact come from that tissue and not from the blood. The tissue also contains protohaematin.

Coproporphyrin I and coproporphyrin III, with the III isomer predominating, were found in the extravasal tissue and the body wall of light and dark animals. All these tissues also contained the tricarboxylic porphyrin already described from the heart-body of *Amphitrite*, and in addition they contained protohaematin. The body wall of both dark and light animals also produced the yellow, green-fluorescent pigment described in *Amphitrite* and *Melinna*. Such a pigment has been described by Lignac (1945) and van Duijn, Havinga & Lignac (1951) in *Arenicola* and called arenicochrome. It may well be that it is this pigment which occurs in *Amphitrite* and *Melinna*. This will be the subject of a future investigation.

Ether-acetic acid extracts of the extravasal tissue and the body wall of dark and light animals were washed with water, concentrated and spotted on Whatman No. 4 filter paper with markers of coproporphyrin I and III, chlorin-e_6, and haematoporphyrin, and chromatograms were run using the ascending method of Eriksen (1953) (Fig. 4). In each case spots of coproporphyrin I and coproporphyrin III were obtained, coproporphyrin III predominating, together with a spot at the tricarboxylic level. The ether extracts were evaporated to dryness and the pigment esterified by adding a mixture of methanol 19 parts: concentrated sulphuric acid 1 part, and allowing the solution to stand at room temperature for 48 h. The esterified pigments were extracted by diluting with an equal volume of water and shaking with chloroform. After washing the chloroform extract with 2% sodium chloride followed by water, and drying the solution roughly by passing it through chloroform-soaked paper, the

pigments were chromatographed on a column of MgO, grade III (Nicholas, 1951). Apart from a dark brown band at the top of the column which did not move when the chromatogram was developed with the chloroform-methanol mixture as before, only one broad, pink, red-fluorescent band developed. When collected in chloroform this band gave an absorption spectrum (Hartridge):

I	II	III	IV
622·3	565·5	533	501 mμ

From the paper-chromatographic data and this spectrum, the pigment can be identified as coproporphyrin.

From the body wall of the dark animals a large amount of coproporphyrin isomers was isolated; much less, although still an appreciable quantity, could be extracted from the light (pink) animals. When crystallized as described for *Amphitrite*, the coproporphyrin III tetramethyl ester gave a melting point of 137·9° C. The coproporphyrin I again was present in too small a quantity to be isolated.

From this work it may be seen that coproporphyrin III is the predominating pigment in the heart-bodies of all the species examined. The tricarboxylic porphyrin was only detected in *Amphitrite* and *Arenicola* extravasal tissue, but this could be because much larger amounts of these worms were available, and only a few specimens of some of the other species could be obtained.

DISCUSSION

The chief interest in this work is centred in the presence of relatively large amounts of coproporphyrin III, together with traces of coproporphyrin I, a tricarboxylic porphyrin, and haematin in the heart-body. The fact that all these pigments can be isolated from the heart-body tissue is very strong evidence in support of the view expressed by Meyer (1887) that the heart-body is an haematopoietic organ. A possible scheme for the biosynthesis of

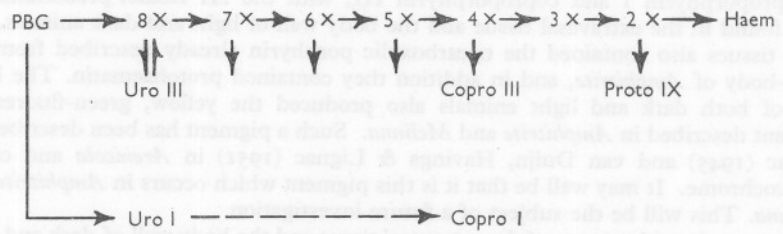

Fig. 9. A scheme for the biosynthesis of haem (Dresel, 1955).

haem is that of Dresel (1955) represented in Fig. 9. In this scheme porphobilinogen (PBG) is converted into an 8-carboxyl leuco-compound or prouroporphyrin III, which is then progressively decarboxylated each time to the III leuco-compound and finally dehydrogenated, with the incorporation of Fe leading to haem. Dresel (1955) considers that observations of porphyrins with from 8- to 2-carboxyl groups in many different biological situations lend support to such a scheme. If this system proceeds normally, only minute traces of free porphyrins are formed. However, in the porphyrias, where

aberrations of the biosynthetic process occur, free porphyrins are formed, that is to say, the leuco-compounds are oxidized to the coloured free porphyrins which then find their way into the tissues. Once these free porphyrins have been formed they apparently cannot play any further part in the synthesis of the haem molecule. Eriksen (1955) put forward some evidence for the conversion of free uroporphyrin and coproporphyrin to protoporphyrin in the biosynthetic cycle, but this has not been supported, and has been criticized by Schwartz & Ikeda (1955), by Shemin (1955) and by Dresel (1955). The metabolism of the series I porphyrins appears to follow a different pathway in which the free porphyrins, at any rate uroporphyrin I and coproporphyrin I, appear. Lockwood & Rimington (1957) have succeeded in purifying an enzyme which converts PBG to uroporphyrin, and for which they propose the name porphobilinogenase (PBGase). This enzyme produces uroporphyrin III from PBG apparently without trace of uroporphyrin I. If, however, the enzyme is heated for 15 min at 55° C, it produces from PBG at 37° C, uroporphyrin I, with a trace of uroporphyrin III, and no ether-soluble porphyrin is formed by either the heated or unheated enzyme. PBGase was prepared from chicken erythrocytes, and Lockwood (private communication) has shown that with Conger-eel erythrocytes, addition of PBG after haemolysis and incubation at room temperature produces large amounts of coproporphyrin III and protoporphyrin IX, with practically no uroporphyrin.

In the porphyrias, where the free porphyrins are formed, these may be both series I and series III according to the type of syndrome. In congenital porphyria, which is a gene-controlled disturbance of the haematopoietic system, quantities of uroporphyrin I and coproporphyrin I are formed, and Rimington & Booij (1957) found that a haemolysate prepared from the blood of a case of congenital porphyria produced considerable quantities of these porphyrins together with the series III isomers. Photosensitization occurs as well in congenital porphyria, and PBG is absent. In idiopathic porphyria, series III isomers predominate, and PBG is present. These findings give further support to Dresel's scheme (Fig. 9), and for the suggestion that series I isomers have a separate metabolic route. Booij & Rimington (1957) suggest that because a haemolysate of congenital porphyria blood incubated with PBG at 37° C yields a mixture of series I and III porphyrins, 'the defect is present in some at least of the circulating cells and is not confined to processes occurring in the bone marrow during early maturation of the erythrocytes'. All these studies have an important bearing on the present problem of the function of the polychaete heart-body, and on the occurrence of porphyrins in marine invertebrates. It is possible that in the polychaetes with haemoglobin which we have examined, there exists a kind of idiopathic porphyria in which the haem biosynthesis system is inefficient. In such a system the intermediates in the chain of reactions in Fig. 9, namely, coproporphyrin III, coproporphyrin I, and the tricarboxylic porphyrin, have been

deposited in the tissues in their oxidized form and their metabolism to haem cannot, consequently, be carried further. Kench, Langley & Wilkinson (1953) found that extracts of stools of a porphyria patient, when chromatographed on paper, gave the whole gamut of porphyrins from 8-carboxyl down to 2-carboxyl. In molluscs, if free porphyrin is present it is uroporphyrin I which occurs (Kennedy & Vevers, 1954; Kennedy, 1958). In molluscs which have a shell the porphyrin is laid down in the shell, very often in a definite pattern, and in those molluscs which are without shells or have very reduced shells, such as the tectibranchs and land slugs, if uroporphyrin I is present it occurs in the integument. The deposition of uroporphyrin in these molluscs seems to be closely connected with calcium metabolism, and indeed Turner (1937) associated the formation of uroporphyrin I which occurs in the bones of the Pennsylvanian fox-squirrel (*Sciurus niger*), with the megaloblasts of the bone marrow. It is significant that it is the black variety of the slug, *Arion ater* in which the greatest amount of uroporphyrin I occurs (Kennedy, 1958), the amount of porphyrin being closely parallel to the amount of melanin in the integument, so that in the very pale grey specimens, no uroporphyrin I is detectable. *Sciurus niger* has black hair, so that in these two widely differing animals an additional pigment is present, namely melanin, which protects these animals from the photosensitizing effects of the porphyrin. *Aplysia* and *Akera* which are more darkly coloured, have more uroporphyrin I than *Duvaucelia* which has a bright orange pigment; there was a much greater concentration of coproporphyrin III in the body wall of the dark coloured *Arenicola* and *Amphitrite* than in the lighter, pink specimens: the more darkly pigmented *Asterias* contained more protoporphyrin IX than the paler animals (Kennedy & Vevers, 1953) although protoporphyrin is not noteworthy for its photosensitizing action. Fischer & Zerweck (1924) have shown in mammals that photosensitivity to u.v. light increases with the number of carboxylic groups in the porphyrin molecule, the action of proto-, deutero- and meso-porphyrin is very slight, but copro- and uroporphyrin have an intense photosensitizing action. The exception to this is haematoporphyrin which has not been detected free in nature so far, which has the greatest photosensitizing effect of all, and which has two carboxyl groups and two hydroxyethyl groups.

CONCLUSIONS

These results suggest that the heart-bodies (when present) in polychaetes are haematopoietic tissues.

Types of porphyria comparable with those described in man occur in invertebrates, and this may explain the apparently random distribution of free porphyrins in marine animals. Further support is given to the view that porphyrin metabolism and calcium metabolism are closely associated. Since all animals in which photosensitizing porphyrins occur also have dark

pigments (frequently melanin, and sometimes carotenoid) the latter presumably have a protective function.

The ability of the heart-body and other polychaete tissues to form porphyrins from added PBG and δ-aminolaevulinic acid (ALA) will be the subject of a further investigation. It is of the first importance also to determine whether the heart-bodies contain PBGase. These experiments may lead to an understanding of the formation in the heart-bodies of the coproporphyrin III in such large amounts.

It is also of interest to note here that the suggestion of Picton (1898) that the heart-body is formed from an infolding of extravasal epithelia, is amply borne out by the equivalence, as shown by the porphyrins present, of the heart-bodies with the extravasal tissue in *Arenicola*, tissue frequently referred to by previous authors as 'chloragogen' (Romieu, 1923). It thus seems possible that such extravasal 'chloragogen' in polychaetes fulfils an haematopoietic role, without excluding the possibility that it participates in excretion or food storage as well (Dales, 1957). Romieu (1923) regarded the results of his histochemical and histological investigations as providing evidence for regarding the heart-body as a 'storage kidney', or intravascular 'chloragogue'. It is also intended to investigate this further. Eisig (1887) considered that the brown or brown-green pigment of the heart-body in *Terebella lapidaria* was a derivative of haemoglobin. Romieu (1923) did not agree with this, and did not elicit the reactions for haematin or biliverdin; he was of the opinion that this brown pigment was a 'urochrome', and compared it with chaetopterin, which he said was a modified chlorophyll, the comparison resting solely on its solubility in acetic acid and on its colour. Kennedy & Nicol (1958) have shown that the pigment hitherto known as chaetopterin is a mixture of phaeophorbides *a* and *b*, and as such could in fact be regarded as a modified chlorophyll. Experiments described in this present paper confirm Eisig's view that the brown granules in the heart-body (and also in the extravasal tissue of *Arenicola*) are a derivative of haemoglobin, in fact, haematin.

SUMMARY

The heart-bodies and some other tissues of a number of polychaetes have been examined chemically, and the porphyrin pigments from these tissues described. Coproporphyrin III was shown to be present in relatively large concentration in all the heart-bodies, together with traces of coproporphyrin I, a tricarboxylic porphyrin and protohaematin. The body wall of light (pink) and dark (brown) coloured *Arenicola* and of *Amphitrite* also contained these pigments in varying amounts. Other non-porphyrin pigments were also seen in the body wall of some animals, the most remarkable being a colourless compound with a bright blue-fluorescence occurring in *Flabelligera*. Evidence is presented which supports the view that the heart-body is an haematopoietic

organ, and the significance of this is discussed. Parallels are drawn between the occurrence of free porphyrins in marine animals and the occurrence of such pigments in porphyrias in man.

REFERENCES

ASHWORTH, J. H., 1904. Arenicola. *L.M.B.C. Memoirs*, No. 11, 118 pp. London: Williams and Norgate.

BLOCH-RAPHAEL, C., 1939. Localisation, formation et destruction de l'hémoglobine chez les Annélides Polychètes. *Ann. Inst. océanogr. Monaco*, T. 19, Fasc. 1, pp. 1–78.

BOOIJ, H. L. & RIMINGTON, C., 1957. Effect of pre-heating on porphyrin synthesis by red cells. *Biochem. J.*, Vol. 65, p. 4P.

CHU, J. C., GREEN, A. A. & CHU, E. J., 1951. Microchromatography of porphyrin esters. *J. biol. Chem.*, Vol. 190, pp. 643–6.

DALES, R. P., 1957. Preliminary observations on the role of the coelomic cells in food storage and transport in certain polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 91–110.

DRESEL, E. I. B., 1955. The biosynthesis of haem. *Ciba Foundation Symposium on Porphyrin Synthesis and Metabolism*. 308 pp. London: Churchill.

EISIG, H., 1887. Monographie der Capitelliden des Golfes von Neapel. *Fauna u. Flora Neapel*, Monogr. 16, 906 pp. Berlin: Friedländer.

ERIKSEN, L., 1953. Paper chromatography of porphyrin pigments. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 155–7.

—— 1955. The relation of free erythrocyte porphyrins to haemoglobin biosynthesis. *Ciba Foundation Symposium on Porphyrin Synthesis and Metabolism*. 308 pp. London: Churchill.

FAUVEL, P., 1897. Recherches sur les Ampharétiens, Annélides Polychètes sédentaires. *Bull. sci. Fr. Belg.*, T. 30, pp. 277–488.

FISCHER, H. & ZERWECK, W., 1924. Über der Haemfarbstoff. *Z. phys. Chem.*, Vol. 137, pp. 176.

FOX, D. L., CRANE, S. C. & MCCONNAUGHEY, B. H., 1948. A biochemical study of the marine annelid worm, *Thoracophelia mucronata*. *J. mar. Res.*, Vol. 8, pp. 567–85.

KENCH, J. E., LANGLEY, F. A. & WILKINSON, J. F., 1953. Biochemical and pathological studies on congenital porphyria. *Quart. J. Med.*, Vol. 22, pp. 285–94.

KENNEDY, G. Y., 1953*a*. Partition paper chromatography of the porphyrins. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 281–4.

—— 1953*b*. Chlorocruoroporphyrin: a simple method of preparation. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 365–6.

KENNEDY, G. Y. & VEVERS, H. G. 1953. The biology of *Asterias rubens* L. V. A porphyrin pigment in the integument. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 235–47.

—— —— 1954. The occurrence of porphyrins in certain marine invertebrates. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 663–76.

—— —— 1956. Porphyrin pigments in the tectibranch mollusc *Akera bullata* O. F. Müller. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 35–9.

LIGNAC, G. O. E., 1945. About arenicochrome and its possible significance as a mesocatalyst. *Proc. Acad. Sci. Amst.*, Vol. 48, pp. 406–10.

LOCKWOOD, W. H. & RIMINGTON, C., 1957. Purification of an enzyme converting porphobilinogen to uroporphyrin. *Biochem. J.*, Vol. 67, p. 8P.

MEYER, E., 1887. Studien über den Körperbau der Anneliden. *Mitt. zool. Sta. Neapel*, Bd. 7, pp. 592–741.

NICHOLAS, R. E. H., 1951. Chromatographic methods for the separation and identification of porphyrins. *Biochem. J.*, Vol. 48, pp. 309–13.

PICTON, L. J., 1898. On the heart-body and coelomic fluid of certain Polychaeta. *Quart. J. micr. Sci.*, Vol. 41, pp. 263–302.

RIMINGTON, C., 1939. A reinvestigation of turacin, the copper porphyrin pigment of certain birds belonging to the Musophagidae. *Proc. roy. Soc.*, B, Vol. 127, pp. 106–20.

RIMINGTON, C. & BOOIJ, H. L., 1957. Porphyrin biosynthesis in human red cells. *Biochem. J.*, Vol. 65, p. 3P.

ROMIEU, M., 1923. Recherches histophysiologiques sur le sang et le corps cardiaque des Annélides Polychètes. *Thés. Fac. Sci. Univ. Paris*, 336 pp., Paris: Doin.

SALENSKY, W., 1884. Études sur le développement des Annélides. Part III, *Pileolaria*, *Aricia* et *Terebella*. *Arch. biol., Liège*, Vol. 4, pp. 143–264.

SCHNEIDER, G., 1897. Über die Segmentalorgane und den Herzkörper einiger Polychäten. *Arb. d. Kaiserl. Naturf. Ges. St Petersburg*, Vol. 27, pp. 135–7.

—— 1899. Über Phagocytose und Excretion bei den Anneliden. *Z. wiss. Zool.*, Bd. 66, pp. 497–520.

SCHWARTZ, S. & IKEDA, K., 1955. Studies of porphyrin synthesis and interconnexion. *Ciba Foundation Symposium on Porphyrin Synthesis and Metabolism*. 308 pp. London: Churchill.

SHEMIN, D., 1955. Discussion. *Ciba Foundation Symposium on Porphyrin Synthesis and Metabolism*. 308 pp. London: Churchill.

TURNER, W. J., 1937. Studies on porphyria. I. Observations on the fox-squirrel, *Sciurus niger*. *J. biol. Chem.*, Vol. 118, pp. 519–30.

VAN DUIJN, P., HAVINGA, E. & LIGNAC, G. O. E., 1951. Arenicochrome, a new pigment from *Arenicola marina* L. *Experientia*, Vol. 7, pp. 376–7.

VANNOTTI, A., 1954. *Porphyrins*. 533 pp. London: Hilger and Watts.

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LUMINESCENCE IN POLYNOIDS

IV. MEASUREMENTS OF LIGHT INTENSITY

By J. A. C. NICOL

The Plymouth Laboratory

(Text-figs. 1–3)

INTRODUCTION

Many polynoid worms (family Aphroditidae) are luminescent. In recent papers, physiological and histological aspects of polynoid luminescence have been considered in some detail (Bonhomme, 1942; Nicol, 1953, 1954, 1957*a*, *b*). The light appears in the scales or elytra which cover the dorsal surface of the worm. The photocytes form a single epithelial layer on the lower surface of the elytrum. They are concentrated near the centre of the scale and, when the latter is pigmented, there is a clear area above the photogenic tissue through which the light escapes.

Luminescence in polynoids is under nervous control and is evoked by external stimulation. The elytra usually flash repetitively to a single stimulus, and the responses can be analysed by recording from single elytra. Flash duration ranges from 100 to 200 msec. There is much variation in flash intensity: initially, consecutive flashes become progressively brighter, owing to facilitation, but fatigue soon sets in and the flashes become fainter and finally die away.

The light of polynoids is emerald-green in colour. Spectral emission extends from about 450 to 680 mμ, with a maximum at about 515 mμ (Nicol, 1957*c*). The spectral emission curve is shown in Fig. 1. This information has been utilized to calculate the intensity of polynoid light, in the manner now to be described.

MATERIAL AND METHODS

The light of two species was measured, viz. *Lagisca extenuata* and *Acholoë astericola*. Elytra were removed from the animals under $MgCl_2$-narcosis, and were subsequently washed in sea water. A single elytrum was mounted in a Perspex chamber over a pair of silver electrodes (see Nicol, 1953). Flashing was induced by stimulating with electric shocks: these were square wave pulses, about 2 msec in duration, and up to 10 V in intensity.

The light was detected by a photomultiplier (E.M.I. type no. 6685), the spectral sensitivity of which had been determined by the National Physical Laboratory. The photocathode of the photomultiplier is 9 mm in diameter. Sensitivity is maximal in the violet, and falls off steadily at long wavelengths,

above 500 mμ (Fig. 2, curve A). The elytrum was positioned 2 cm beneath the photomultiplier, so that its upper surface lay beneath the centre of the photocathode, and was parallel to the face of the latter. The light-emitting region of the elytrum is less than 1 mm² in area, and can be regarded as a point-source.

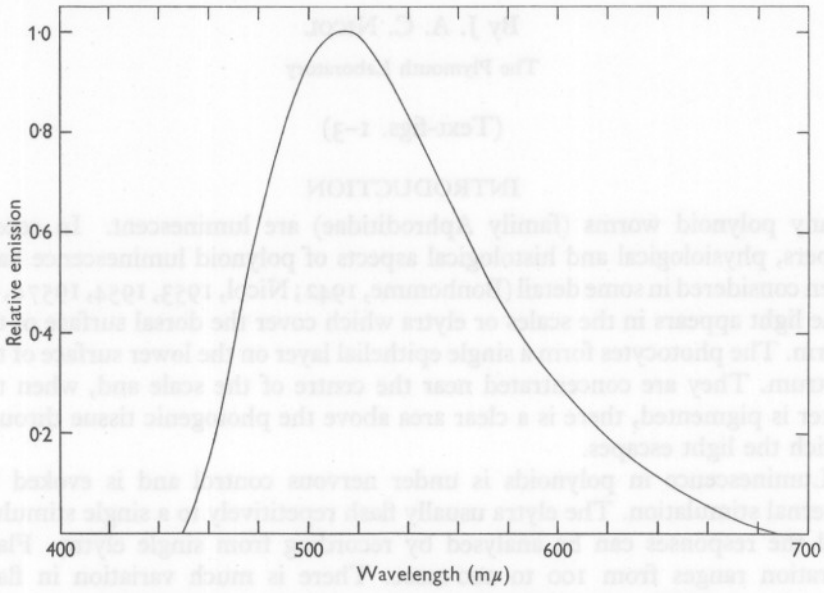

Fig. 1. Relative spectral emission curve of polynoid light (from Nicol, 1957*c*).

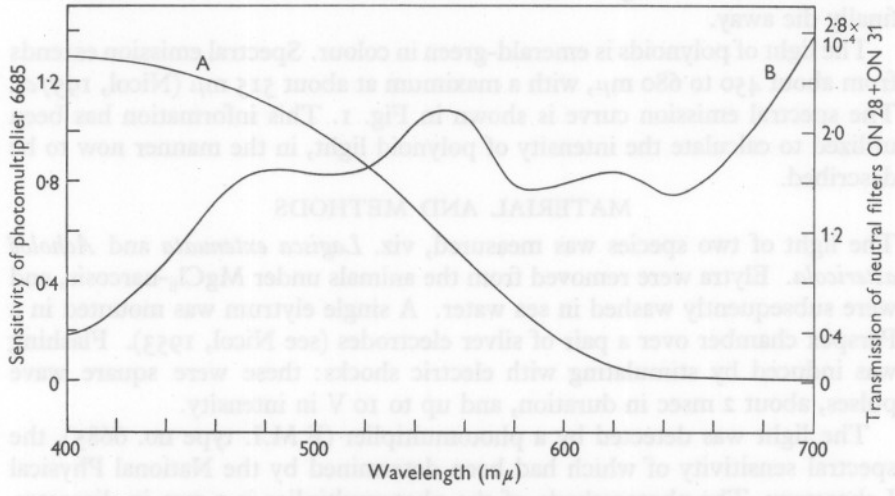

Fig. 2. Curve A: relative spectral sensitivity of photomultiplier type E.M.I. no. 6685. Curve B: combined transmission of two neutral glass filters (Chance ON28+ON31).

The photomultiplier was connected to a cathode-ray oscilloscope, and photographic records of the responses were made on moving paper.

Measurements were made at ambient temperatures of 18–19° C.

CALIBRATION OF THE PHOTOMULTIPLIER

The sensitivity of the apparatus, that is of photomultiplier 6685 plus amplifier and C.R.O., was determined against a known light source. This was a substandard (tungsten) lamp, of colour temperature 2360° K, and energy output of 24·15 candelas. The lamp was calibrated by the National Physical Laboratory. It was placed on an optical bench 2 m from the face of the photomultiplier. Intensity was reduced by two neutral glass filters (Chance ON28 + ON31), the transmission characteristics of which were measured in a spectrophotometer (Unicam SP500) (see curve B, Fig. 2).

The flux of light from the substandard lamp at 2 m, falling on the surface of the photomultiplier perpendicular to the direction of the light beam, is

$$\int_{400}^{700} pK\mathcal{J}_\lambda V_\lambda \, d\lambda = pK \int_{400}^{700} \mathcal{J}_\lambda V_\lambda \, d\lambda = I/4 \times 10^4 \text{ lumens/cm}^2.$$

I is the intensity of the substandard lamp in candelas, p is a quantity for the particular experimental set-up, and K is the luminous efficiency of radiation, which is 682 lumens/W at 555 mμ (Walsh, 1953):

$$p = \frac{I}{4 \times 10^4} \times \frac{1}{682 \int_{400}^{700} \mathcal{J}_\lambda V_\lambda \, d\lambda} \text{ W/cm}^2/\text{m}\mu.$$

Let the light from the substandard lamp at 2 m, passing through the neutral filters, give a deflexion on the oscilloscope $= D_L$. Let T_λ be the combined transmission of the neutral filters, S_λ be the spectral sensitivity of the photomultiplier, and q be a constant such that $q/S_\lambda = \text{W/cm}^2$ of a given wavelength (λ) required to produce unit deflexion. Then

$$D_L = \int_{400}^{700} \frac{p\mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda}{q} = \frac{p}{q} \int_{400}^{700} \mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda$$

and

$$q = \frac{p}{D_L} \int_{400}^{700} \mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda \text{ W/cm}^2.$$

Values for V_λ were taken from the C.I.E. table for photopic vision (International Relative Luminous Efficiency of Radiation for Photopic Vision, table VI in Keitz, 1955). Values for $\mathcal{J}_\lambda$ were obtained from Skogland, 1929, for a lamp of colour temperature 2360° ($\mathcal{J} = 1$ at $\lambda = 590$ mμ).

To determine p and q, the following values were used. Intensity, I, of the substandard lamp is 24·15 candelas. With set amplification on the C.R.O.

(30 V/mm), and known voltage on the photomultiplier (1400 V), the deflexion produced by the light is 22·02 mm.

From this quantity, an estimate was made of the deflexion which would be produced at higher amplification, viz. 10 V/mm on the C.R.O., and photomultiplier voltage of 1600 V. This estimated deflexion was 227·36 mm.

$$\int_{400}^{700} \mathcal{J}_\lambda V_\lambda = 83{\cdot}52,$$

and $$p = \frac{24{\cdot}15}{4 \times 10^4} \times \frac{1}{682 \times 83{\cdot}52} = 1{\cdot}0599 \times 10^{-8} \text{ W/cm}^2/\text{m}\mu.$$

$$\int_{400}^{700} \mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda = 105{\cdot}1 \times 10^{-4}$$

and $$q = \frac{1{\cdot}0599 \times 10^{-8}}{227{\cdot}36} \times 105{\cdot}1 \times 10^{-4} = 0{\cdot}49 \times 10^{-12} \text{ W/cm}^2.$$

In order to make periodical checks on the sensitivity of the apparatus, an alternative light source was employed. This consisted of a stilbene phosphor irradiated by ^{60}Co (called, for brevity, Co source). The phosphor emitted a very faint blue light. Random emission by the phosphor gave a very broad beam-trace, which was smoothed out by putting a 0·1 μF condenser across the input of the oscilloscope. The Co source was set 2 cm from the face of the photomultiplier, and the oscilloscope deflexion was photographed.

MEASUREMENTS OF THE INTENSITY OF POLYNOID LIGHT

Records were obtained of the flashes of eight elytra of *Lagisca*, and of six elytra of *Acholoë*. Some examples are shown in Fig. 3. Various amplifications were used, and the measured deflexions were calculated on the basis of instrument-sensitivity occurring at a C.R.O. setting of 10 V/mm and 1600 V on the phototube. These calculated deflexions, D_s, for elytra at 2 cm distance, are shown in columns 2 of Tables 1 and 2. Column 3 shows calculated deflexions at a distance of 1 m, according to the inverse square law.

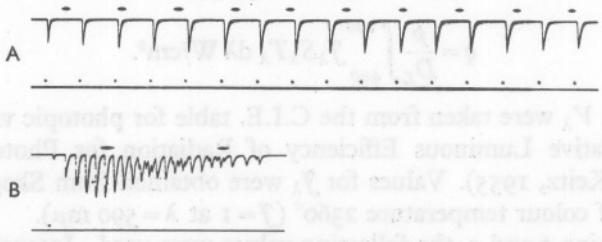

Fig. 3. Oscillograph records of the flashing of polynoid elytra. A, *Acholoë*, B, *Lagisca*. Time signal, above, 1/sec. Electrical stimuli on lower line.

The flashes of polynoids are much briefer than 1 sec. In order to calculate the energy of one flash, the response curve for a flash was averaged over 1 sec in terms of a maximal deflexion lasting 1 sec. These calculations gave factors, A, for response curves of: 0·02 for *Lagisca*, and 0·084 for *Acholoë*. The deflexions (D_s) for the responses of *Lagisca* and *Acholoë*, averaged over 1 sec, are shown in columns 4 of Tables 1 and 2 ($D = D_s A$).

TABLE 1. CALCULATION OF THE INTENSITY OF LIGHT EMITTED BY AN ELYTRUM OF *LAGISCA*

Elytrum and record	Maximal deflexion D_s at 2 cm (mm)	Calculated deflexion D_s at 1 m (mm)	$D\,(=A^{\star} \times D_s)$ at 1 m	Energy at 515 mμ in 1 flash, μJ/cm² at 1 m ($\times 10^{-12}$)	Total energy in 1 flash, μJ/cm² at 1 m ($\times 10^{-10}$)
2A	0·6	0·00024	0·0000048	0·03129	0·03309
B	0·9	0·00036	0·0000072	0·046944	0·04964
C	0·3	0·00012	0·0000024	0·015648	0·01655
D	0·55	0·00022	0·0000044	0·028688	0·03034
3A	4·1	0·00164	0·0000328	0·213856	0·22615
B	2·5	0·00100	0·0000200	0·13040	0·13790
C	16·5	0·00660	0·0001320	0·860640	0·91013
4A	0·9	0·00036	0·0000072	0·046944	0·04964
B	4·5	0·00180	0·0000360	0·234720	0·24822
C	52·5	0·02100	0·0004200	2·73840	2·89586
5A	6	0·00240	0·0000480	0·31296	0·33096
B	3·9	0·00156	0·0000312	0·203424	0·21512
6A	3	0·00120	0·0000240	0·15648	0·16548
B	66	0·02640	0·0005280	3·44256	3·64051
7A	10·8	0·00432	0·0000864	0·56332	0·59572
B	43·2	0·01728	0·0003456	2·253312	2·38286
8	6·48	0·002592	0·0000518	0·337997	0·35743
9A	8·64	0·003456	0·0000691	0·450662	0·47658
B	1·44	0·000576	0·0000115	0·075110	0·07943

$$\star\ A = \frac{\text{area of response curve for a single flash averaged over 1 sec}}{\text{maximal response} \times 1\ \text{sec}}.$$

TABLE 2. CALCULATION OF THE INTENSITY OF LIGHT EMITTED BY AN ELYTRUM OF *ACHOLOË*

Elytrum and record	Maximal deflexion D_s at 2 cm (mm)	Calculated deflexion D_s at 1 m ($\times 10^{-4}$) (mm)	$D\,(=A^{\star} \times D_s$ at 1 m) ($\times 10^{-4}$)	Energy at 515 mμ in 1 flash, μJ/cm² at 1 m ($\times 10^{-10}$)	Total energy in 1 flash, μJ/cm² at 1 m ($\times 10^{-8}$)
1A	1·824	7·296	0·6129	0·39961	0·42259
B	8·683	34·732	2·9175	1·90221	2·01159
2	0·917	3·668	0·3081	0·20089	0·21244
5A	1·643	6·572	0·5520	0·35994	0·38064
B	7·126	28·504	2·3943	1·56108	1·65084
8	1·376	5·504	0·4624	0·30148	0·31882
9A	1·099	4·396	0·3693	0·24078	0·25462
B	1·985	7·940	0·6670	0·43488	0·45989

* See Table 1 for definition.

A relative spectral emission curve for polynoid light is shown in Fig. 1, in which relative energy, E_λ, is plotted against λ (Nicol, 1957*c*). This curve can be put on an absolute basis in terms of a quantity r such that $E_\lambda r$ gives Watts/cm^2 of receptor surface/mμ under the experimental conditions specified.

$$D = \int \frac{rE_\lambda S_\lambda}{q}\,d\lambda, \qquad D = \frac{r}{q}\int E_\lambda S_\lambda\,d\lambda.$$

$$r = \frac{Dq}{\int E_\lambda S_\lambda\,d\lambda}\ \text{W/cm}^2/\text{m}\mu, \qquad \int_{400}^{700} E_\lambda S_\lambda\,d\lambda = 75{\cdot}1,$$

$$r = D \times \frac{0{\cdot}49 \times 10^{-12}}{75{\cdot}1} = D \times 0{\cdot}652 \times 10^{-14}\ \text{W/cm}^2/\text{m}\mu.$$

The energy at 515 mμ in 1 flash is equal to

$$r = D \times 0{\cdot}652 \times 10^{-14}\ \text{W/cm}^2/\text{m}\mu \text{ at 1 m.}$$

The total energy in 1 flash is equal to

$$r\int_{400}^{700} E_\lambda\,d\lambda = D \times 0{\cdot}652 \times 10^{-14}\int_{400}^{700} E_\lambda\,d\lambda\ \text{W/cm}^2 \text{ at 1 m.}$$

From integration of the curve in Fig. 1,

$$E_\lambda\,d\lambda = 105{\cdot}75.$$

Values for energy in 1 flash at 515 mμ, and for total energy in 1 flash, are shown in columns 5 and 6 of Tables 1 and 2. For *Lagisca* the total energy in a flash ranges from $0{\cdot}017 \times 10^{-10}$ to $3{\cdot}641 \times 10^{-10}$ μJ/cm^2 at 1 m. For *Acholoë*, the total energy in a flash ranges from $0{\cdot}212 \times 10^{-8}$ to $2{\cdot}012 \times 10^{-8}\mu$ J/cm^2 at 1 m.

These values were determined by measuring that light which was emitted in a cone having its axis perpendicular to the upper surface of the elytrum, and possessing a solid angle $w = 0{\cdot}16$ sterad.

The data and calculations of Tables 1 and 2 pertain to a single flash. Polynoid elytra usually flash repetitively, so that there may be 1 or many flashes in a given second; at fast rates there is often some degree of summation. A calculation for *Lagisca* gives the following results:

(*a*) Total energy in a single brief flash (maximal flash in a series of flashes): $0{\cdot}182 \times 10^{-9}$ μJ/cm^2 at 1 m.

(*b*) Total flux in a series of 13 flashes occurring in 1 sec (maximal flash intensity as in (*a*)): $2{\cdot}600 \times 10^{-9}$ μW/cm^2 at 1 m.

INTENSITY OF LIGHT EMITTED PER PHOTOCYTE

The photocytes lie in a single cellular layer. This arrangement permits an estimation of the light emitted per photocyte. Elytra were sectioned (at 7 μ), and the total number of photocytes counted. In elytrum no. 7 of *Lagisca* there were 1250 photocytes. Minimal and maximal values for the light emitted by a photocyte in 1 flash are: minimum, $0{\cdot}048 \times 10^{-12}$ μJ/cm^2 at 1 m; maximum, $0{\cdot}191 \times 10^{-12}$ μJ/cm^2 at 1 m.

COMMENT

Mean values for the intensity of light emitted in a single flash by an elytrum of *Lagisca* are $1{\cdot}8 \times 10^{-6}$ μJ/cm^2 at 1 cm, and $1{\cdot}8 \times 10^{-10}$ μJ/cm^2 at 1 m. Corresponding values for the flash of an elytrum of *Acholoë* are $1{\cdot}11 \times 10^{-4}$ μJ/cm^2 at 1 cm and $1{\cdot}11 \times 10^{-8}$ μJ/cm^2 at 1 m. These estimates are for air-paths.

Some estimations are available for the radiant flux emitted by other marine species. The luminescence of a ctenophore *Mnemiopsis leidyi*, 35 mm in diameter, was $0{\cdot}5 \times 10^{-4}$ to $> 0{\cdot}75 \times 10^{-4}$ μW/cm^2 at 50 cm (recalculated as $0{\cdot}125 \times 10^{-4}$ to $> 0{\cdot}187 \times 10^{-4}$ μW/cm^2 at 1 m in air) (Clarke & Backus, 1956). The light of *Euphausia pacifica* has a mean intensity of $1{\cdot}8 \times 10^{-3}$ μW/cm^2 at 1 cm. An adult *Euphausia pacifica* is about 20 mm long and has 10 light-emitting organs (photophores). The light intensity of *Pyrosoma atlantica* ranges from 8×10^{-3} to 4×10^{-2} μW/cm^2 at 1 cm. A colony 10 cm long emitted light of intensity $2{\cdot}5 \times 10^{-2}$ μW/cm^2 at 1 cm (Kampa & Boden, 1957).

The flashes of polynoids are shorter than 1 sec. If the light of these worms remained constant during 1 sec at maximal flash intensity, then the radiant flux for an elytrum of *Lagisca* and *Acholoë*, respectively, would be: $0{\cdot}9 \times 10^{-4}$ and $1{\cdot}31 \times 10^{-3}$ μW/cm^2 at 1 cm (in air). These values for radiant flux of polynoid elytra are of about the same order as that for *Euphausia*. Ctenophores and Pyrosomas are much bigger animals, with correspondingly larger light-emitting surfaces.

The light emitted in 1 flash by a single photocyte of *Lagisca* is $0{\cdot}12 \times 10^{-12}$ μJ/cm^2 receptor surface at 1 m. For a flash of 1 sec duration, the estimated radiant flux would be $1{\cdot}3 \times 10^{-12}$ μW/cm^2 receptor surface at 1 m. Harvey (1925) has estimated the total radiant flux emitted by a single bacterium of *Bacillus phosphorescens* to be $4{\cdot}95 \times 10^{-10}$ μW at $\lambda = 510$ mμ (the wavelength of maximal emission). Over the entire emission spectrum $\left(\text{i.e. } \int_{400}^{700} E_\lambda \, d\lambda\right)$, the radiant flux emitted by a single bacterium is about $0{\cdot}4 \times 10^{-12}$ μW/cm^2 receptor surface at 1 m. I have recently estimated the energy in a single brief flash from a cell of *Noctiluca miliaris* to be about $1{\cdot}1 \times 10^{-12}$ μJ/cm^2 receptor surface at 1 m (in air) (unpublished research). This is about ten times greater than the light output of a photocyte of *Lagisca*.

The threshold of the dark-adapted human eye for a flash having a duration $<0{\cdot}1$ sec is about 100 quanta at 510 mμ in a test field of 10′ (Graham & Margaria, 1935; Hecht, Shlaer, & Pirenne, 1942; Pirenne & Denton, 1952). When fully exposed, the human pupil has an area, mean $S=0{\cdot}5$ cm^2. Now, 1 quantum at 510 m$\mu = 4\times10^{-12}$ erg. For a just detectable flash, the human eye needs 100 quanta $= 4\times10^{-10}$ erg to fall in the pupil, area S.

The mean value for energy in a flash of *Lagisca* is $1{\cdot}8\times10^{-10}$ μJ/cm^2 receptor surface at 1 m, or $1{\cdot}8\times10^{-9}$ erg/cm^2 receptor surface at 1 m.

At x m, the equivalent energy, if all were concentrated at 510 mμ, in a polynoid flash

$$= \frac{1{\cdot}1\times10^{-9}}{x^2} \text{ erg/cm}^2 \text{ receptor surface}$$

$$= \frac{1{\cdot}1\times10^{-9}}{x^2} S \text{ erg/mean pupil area.}$$

The distance in air at which an average flash of *Lagisca* can be seen, by the dark-adapted human eye, is given by

$$4\times10^{-10} \text{ erg} = \frac{1{\cdot}1\times10^{-9}}{x^2} S \text{ erg/mean pupil area,}$$

$$x^2 = \frac{1{\cdot}1\times10^{-9}\times0{\cdot}5}{4\times10^{-10}} \text{ metres squared,}$$

$$x = 1{\cdot}2 \text{ m.}$$

I am indebted to Dr E. J. Denton for guidance in the calculations of light intensity. Part of the apparatus was purchased with grants from the Royal Society. The ^{60}Co-stilbene light source was devised and loaned by Dr G. N. Harding of the U.K. Atomic Energy Authority, to whom I am grateful.

SUMMARY

The light energy emitted in a flash by single elytra of two polynoid worms has been measured, viz. *Acholoë astericola* and *Lagisca extenuata*. Maximal emission occurs at 515 mμ. Mean values for light intensity per flash from 1 elytrum are: *Lagisca*, $1{\cdot}8\times10^{-10}$ μJ/cm^2 receptor surface at 1 m; *Acholoë*, $1{\cdot}11\times10^{-8}$ μJ/cm^2 receptor surface at 1 m. The light emitted in 1 flash by a single photocyte of *Lagisca* is $0{\cdot}12\times10^{-12}$ μJ/cm^2 receptor surface at 1 m. It is estimated that the light from 1 elytrum of *Lagisca* could be seen by the dark-adapted human eye at 1·2 m in air.

REFERENCES

BONHOMME, C., 1942. Recherches sur l'histologie de l'appareil lumineux des Polynoïnés. *Bull. Inst. océanogr. Monaco*, Vol. 39, No. 823, 8 pp.

CLARKE, G. L. & BACKUS, R. H., 1956. Measurements of light penetration in relation to vertical migration and records of luminescence of deep-sea animals. *Deep-Sea Res.*, Vol. 4, pp. 1–14.

GRAHAM, C. H. & MARGARIA, R., 1935. Area and the intensity–time relation in the peripheral retina. *Amer. J. Physiol.*, Vol. 113, pp. 299–305.

HARVEY, E. N., 1925. The total luminous efficiency of luminous bacteria. *J. gen. Physiol.*, Vol. 8, pp. 89–108.

HECHT, S., SHLAER, S. & PIRENNE, M. H., 1942. Energy, quanta and vision. *J. gen. Physiol.*, Vol. 25, pp. 819–40.

KAMPA, E. M. & BODEN, B. P., 1957. Light generation in a sonic-scattering layer. *Deep-Sea Res.*, Vol. 4, pp. 73–92.

KEITZ, H. A. E., 1955. *Light Calculations and Measurements*. Eindhoven: Philips's Technical Library.

NICOL, J. A. C., 1953. Luminescence in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 65–84.

—— 1954. The nervous control of luminescent responses in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 225–55.

—— 1957*a*. Luminescence in polynoids. II. Different modes of response in the elytra. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 261–9.

—— 1957*b*. Luminescence in polynoids. III. Propagation of excitation through the nerve cord. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 271–3.

—— 1957*c*. Spectral composition of the light of polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 529–38.

PIRENNE, M. H. & DENTON, E. J., 1952. Accuracy and sensitivity of the human eye. *Nature, Lond.*, Vol. 170, pp. 1039–42.

SKOGLAND, J. F., 1929. Tables of spectral energy distribution and luminosity for use in computing light transmissions and relative brightnesses from spectrophotometric data. *Misc. Publ. U.S. Dept. Commerce*, No. 86, 23 pp.

WALSH, J. W. T., 1953. *Photometry*. London: Constable.

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SPECTRAL COMPOSITION OF THE LIGHT OF *PHOLAS DACTYLUS* L.

By J. A. C. NICOL
The Plymouth Laboratory

(Text-figs. 1–6)

The light of the piddock *Pholas dactylus* L. appears bluish, but its spectral energy composition has not been determined. A spectral energy curve for the light of this species is desirable in order to calculate absolute energy content (radiant flux) and to relate it to pertinent visibility curves. To this end I have determined relative spectral energy approximately by means of a set of nine different spectral filters, covering the visual spectrum and the near ultra-violet and infra-red.

MATERIAL AND METHODS

Specimens of *Pholas dactylus* were obtained from the red sandstone at Exmouth, near low tide level. The animals were anaesthetized with iso-osmotic $MgCl_2$ and the luminous regions removed. These have been described and figured by Panceri and others (literature in Harvey, 1952). I selected the paired triangular organs in the mantle cavity and the siphonal cords for recording. The bits of tissue were washed in running sea water, and pinned out in black dishes.

The light from the pholads was analysed by means of spectral filters mounted in a rotating disc. The luminescent tissue was placed below the disc, the photomultiplier above, so that light passed through the various filters in turn as the disc revolved. Rotation of the disc was slow, about 1 revolution each 3 min. The photomultiplier was connected to a cathode-ray oscilloscope, and the deflexions were photographed on moving paper. Noise level was reduced by placing a 0·05 μF condenser across the input of the oscilloscope. The apparatus is described in more detail elsewhere (Nicol, 1957).

The photomultiplier was an E.M.I. tube, type no. 6685, with a blue-sensitive photocathode, the spectral sensitivity of which had been measured by the National Physical Laboratory. The disc contained the same spectral filters as the disc IV used for *Chaetopterus* (Nicol, 1957, p. 631), namely, in sequence, Ilford red 608, Ilford orange 607, Ilford yellow 606, Ilford yellow-green 605, Ilford blue-green 603 plus Chance neutral ON31, Ilford blue 602 plus Chance neutral ON32, Ilford violet 601 plus Chance neutral ON33, Chance deep purple OV1, with Ilford green 604 interposed between each.

Curves for the products of sensitivity of the photomultiplier (S_λ) times transmission of a filter (T_λ), plotted against λ, are given in Nicol (1957, fig. 5). In the same paper will be found values for $\eta_x = \int S_\lambda T_\lambda \mathrm{d}\lambda$, and mean representative wavelengths (mean λ) for these particular filters (x = filter).

Luminescence was evoked by electrical stimulation. Light is extracellular, and the response is slow, lasting several minutes. Temperatures were 18–19° C.

OBSERVATIONS

A photographic record of the responses obtained with the spectral filters is shown in Fig. 1. Responses obtained with the green filters No. 604 were used as reference levels. Let $D_{604(1)}$ and $D_{604(2)}$ be the measured deflexions of two contiguous green filters 604, and let D_x be the measured deflexion of a spectral filter placed between the two green filters. Then the corrected response is $2D_x/(D_{604(1)} + D_{604(2)}) = R_x$. By this means, changes of light-intensity at the source are compensated for.

First approximate results $R_x/\eta_x = Q_x$ are plotted in Fig. 2. The light is blue-green, with a peak at about 490 mμ. There is no emission below 410 mμ, and emission in the red is small.

To correct these first results for broad spectral transmission of the filters, the following procedure was adopted. Spectral energy levels ($E_{\lambda A}$) were taken from the curve in Fig. 2, and new curves were drawn for $E_{\lambda A} S_\lambda T_\lambda$ against λ (Fig. 4). Ratios were calculated:

$$\rho_1 = \frac{\int E_{\lambda A} S_\lambda T_\lambda \text{ filter } X}{\int E_{\lambda A} S_\lambda T_\lambda \text{ filter } 603}, \quad \rho_2 = \frac{R_x}{R_{603}},$$

and

$$\rho_2/\rho_1 = \rho_3.$$

The values for ρ_3 were then used to correct the first approximate results, thus $Q_x \rho_3$. Values for the latter are plotted against mean λ in Fig. 3, and a new

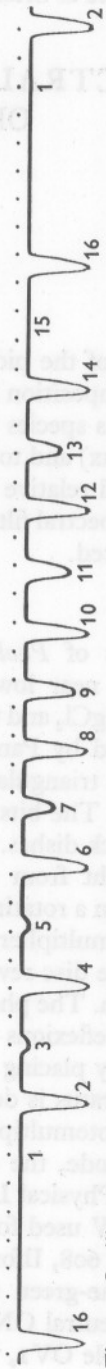

Fig. 1. Oscillograph record of the light of *Pholas* passed through spectral filters. Filters presented in the following order: 1, red 608; 2, green 604; 3, orange 607; 4, green 604; 5, yellow 606; 6, green 604; 7, yellow-green 605; 8, green 604; 9, blue-green 603 + Chance neutral ON31; 10, green 604; 11, blue 602 + Chance neutral ON32; 12, green 604; 13, violet 601 + Chance neutral ON33; 14, green 604; 15, Chance deep purple OV1; 16, green 604. Light falling on the photomultiplier causes a downward deflexion of the lower trace. Time scale above $\frac{1}{2}$ sec.

spectral energy curve is drawn for these values. The value of mean λ for each filter, used in Fig. 3, has been estimated from the centre of gravity of the individual curves $E_{\lambda A} S_\lambda T_\lambda$ of Fig. 4.

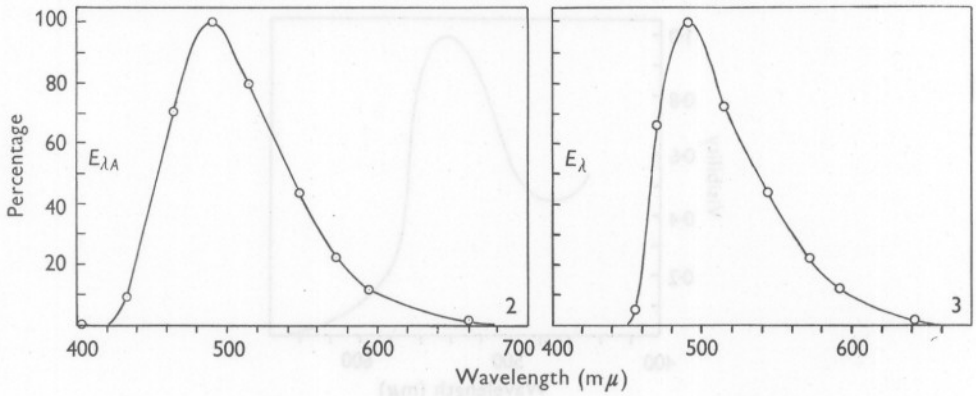

Fig. 2 Fig. 3

Figs. 2, 3. Spectral composition of the light of *Pholas dactylus*. Relative emission plotted against mean λ of the filters. Fig. 2, first approximate results. Fig. 3, corrected results (see text for details).

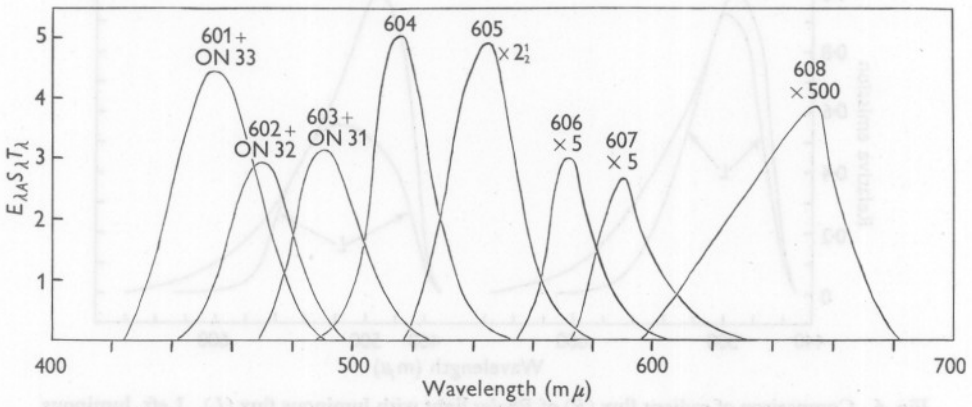

Fig 4. Plots of $E_{\lambda A} S_\lambda T_\lambda$ against λ. ($E_{\lambda A}$ = relative spectral emission (from Fig. 2); S_λ = relative spectral sensitivity of photomultiplier; T_λ = transmission of filter(s).)

COMMENT

The light of *Pholas* is blue-green. Spectral emission extends from about 440 to 670 mμ, with a maximum at about 490 mμ. The action spectrum of *Pholas* has been determined (Hecht, 1928), and it is reproduced in Fig. 5. This has a maximum at about 545 mμ, with evidence for another rise in the violet. Visibility and luminescence spectra, therefore, are quite dissimilar in shape, although all energy is emitted in a spectral range to which *Pholas* is

sensitive. 'Luminous flux' for *Pholas* vision and human scotopic vision have been calculated, and curves for these values plotted against λ are shown in Fig. 6. 'Luminous efficiencies' (total luminous flux ÷ total radiant flux) are

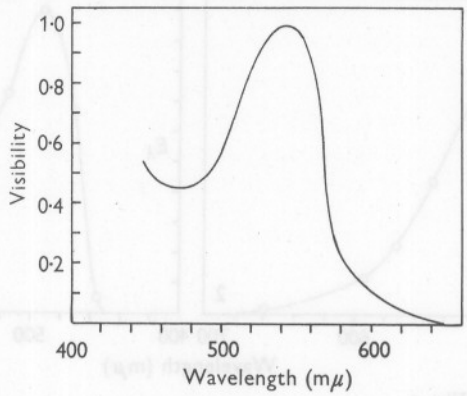

Fig. 5. Action spectrum of the light of *Pholas dactylus*. From Hecht, 1928.

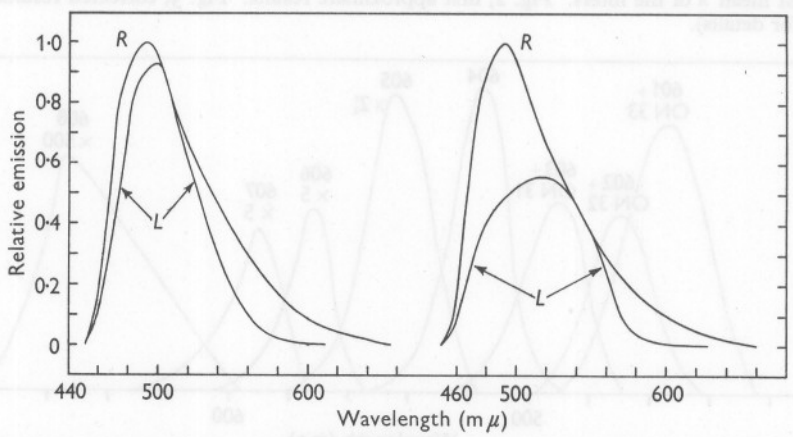

Fig. 6. Comparison of radiant flux (*R*) of *Pholas* light with luminous flux (*L*). Left, luminous flux based on human scotopic vision. Right, luminous flux based on Hecht's visibility curve for *Pholas*.

61% for *Pholas* visibility, and 76% for human scotopic vision. The use to which *Pholas* puts its light is unknown. Absolute threshold for photosensitivity and the intensity of *Pholas* luminescence have not yet been determined, so it is uncertain whether *Pholas* can detect or respond to its own light.

SUMMARY

A spectral emission curve for the light of *Pholas dactylus* has been determined by means of spectral filters and photomultiplier cell. Emission extends from about 440 to 670 mμ, with a maximum at about 490 mμ. The emission curve is compared with the action spectrum determined by Hecht.

REFERENCES

HARVEY, E. N., 1952. *Bioluminescence*. New York: Academic Press.

HECHT, S., 1928. The relation of time, intensity and wavelength in the photosensory system of *Pholas*. *J. gen. Physiol.*, Vol. 11, pp. 657–72.

NICOL, J. A. C., 1957. Spectral composition of the light of *Chaetopterus*. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 629–42.

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NOTE ON THE TEMPERATURE TOLERANCES OF SOME INTERTIDAL ANIMALS IN RELATION TO ENVIRONMENTAL TEMPERATURES AND GEOGRAPHICAL DISTRIBUTION

By A. J. SOUTHWARD

The Plymouth Laboratory

(Text-figs. 1–3)

It is generally acknowledged that temperature plays a large part in controlling the distribution of animals (see, for example, chapter 6 in Andrewartha & Birch, 1954). Often, however, other environmental factors may conceal or modify the influence of temperature. On the seashore, analysis of temperature effects is complicated by the presence of many other variables, such as those associated with the daily rhythms of emergence and submergence; it is particularly difficult to assess the relative importance of the temperature of the sea and of the air. Nevertheless, previous attempts have been made to correlate the distribution of some intertidal animals with temperature, the particular value of mean, extreme, seasonal or annual sea or air temperature chosen being that which fits best the distribution of the animal in question (e.g. Hutchins, 1947; Southward, 1950; Southward & Crisp, 1954). Unfortunately, there is little direct evidence for a causal relation between temperature and distribution. Some observations and experiments have been made on the influence of temperature on the breeding and development of a few marine animals (e.g. Orton, 1920; Runnström, 1929; Loosanoff, Miller & Smith, 1951), but until recently little was known of the temperatures that could be tolerated, especially in the adult stage. It is known that the adult stage of some marine animals may tolerate a wider range of temperature than the egg or larva (e.g. Vernon, 1899), but clearly the adult stage of an intertidal animal will be exposed to more extreme temperatures (effect of air temperature, sunlight, frost) than the planktonic larvae, or the egg masses developing in large or deep tide pools and the shelter of crevices and sea weed.

The experiments that have been carried out on the resistance of intertidal animals to temperature extremes have usually been designed to show the influence of high temperature on their vertical zonation (Huntsman & Sparks, 1924; Gowanloch & Hayes, 1926; Broekhuysen, 1940; Evans, 1948), or the effect of low temperature on ice-formation in the tissues (Kanwisher, 1955). It is therefore worth presenting the results of some laboratory experiments that were carried out to investigate the effect of temperature extremes on the

adults of eight species of intertidal animals and thus determine what influence, if any, extremes of temperature might have in nature on the distribution of the adult stage. The experiments were made concurrently with field investigations on the distribution of the same and other species, reported in part elsewhere in this Journal (Crisp & Southward, 1958, p. 157). In addition to laboratory experiments, attempts were made to assess temperatures in the intertidal zone, as distinct from sea or air temperatures, by measuring the tissue temperatures of some of the animals under various weather conditions on the shore.

The results of the work on temperature tolerance and on temperatures in the intertidal zone are presented separately below, and then discussed together in relation to the geographical distribution of the animals.

All temperatures are quoted in degrees Centigrade.

TEMPERATURE TOLERANCE

The temperatures tolerated by an animal fall into three successively wider ranges. The narrowest range of temperature is that over which the animal feeds, moves about and shows normal respiratory movements or behaviour; a somewhat wider range of temperature is tolerated without loss of irritability, though the animal may not carry out any spontaneous activity; an even wider range of temperature can be borne before the animal dies (Fig. 1). The

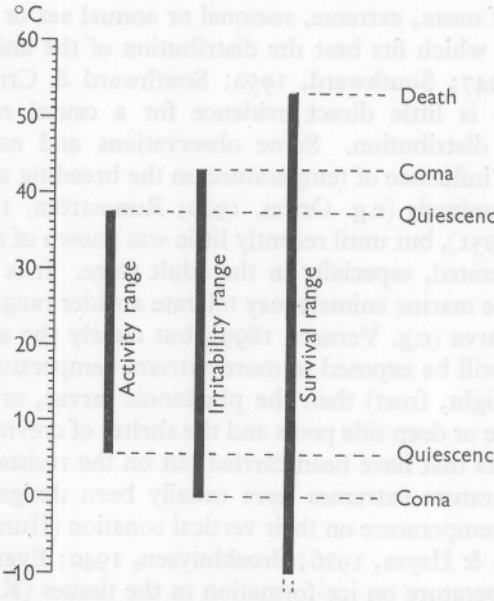

Fig. 1. Example of the three ranges of temperature tolerance of an animal. The barnacle *Chthamalus stellatus*; details of activity range and irritability range taken from Southward (1955). The lower death limit has not been ascertained.

temperature at which normal activity ceases may be termed the quiescence point, while the temperature at which irritability is temporarily lost is usually termed the point of coma. For the present purposes emphasis was placed on survival rather than ability to carry on normal activities, and the widest range of temperature tolerated has been determined. Some observations were made on the temperature limits for normal activity in a few species, but the results have been reported elsewhere (Southward, 1955, 1957).

There is no general agreement on the criteria used to determine lethal temperatures. I have followed Evans (1948) in using the same criterion for each species, namely the failure to respond to pricking with a needle within 12 h of return to normal temperature. Some investigators have used different criteria for each species (Orr, 1955), a procedure which may be justified where animals of very different habitat or habit are being compared. There is further disagreement over the proportion of a sample that need be killed to provide an estimate of the lethal point of the species or population. It has sometimes been said that total mortality of the sample has most biological meaning (cf. Edwards, 1946). If, however, a normal distribution of varying individual resistance to temperature is assumed it might be expected that a large sample would give a relatively wide range of tolerance before 100% mortality was reached, and it is more logical to take 50% mortality as an estimate of the population resistance. This criterion was used in the present work, but it was found that with the samples taken there was little difference between the 50 and 100% points (see Table 2, p. 55).

When the influence of duration on the resistance of an animal to extreme temperatures is being considered it is difficult to decide on the procedure to be adopted. Heilbrunn (1943) favours the construction of time-temperature curves showing the time taken to die at various temperatures, and Orr (1955) has worked on these lines. The latter, however, removed the animals directly from normal temperatures to high temperatures, without allowing time for conduction of heat to the internal organs. It is probably for this reason that the curves given (Orr, 1955) flatten out near the upper limits of temperature tolerance, the region between 0 and 10 min on the graph representing the time taken for equilibration between the tissues and the environment (compare Fig. 2 of this paper). The time taken to reach equilibrium must vary with each species. I have followed Huntsman & Sparks (1924), Broekhuysen (1940) and Evans (1948) in taking the animals in water at their normal temperature and heating them slowly. Apparatus limitations prevented a satisfactorily controlled slow cooling in the low-temperature experiments.

METHODS

The species of barnacles and molluscs used in the experiments are listed in Table 1, together with details of their normal zonation on the shore and their geographical distribution. As far as possible the animals were collected from

the same area of shore for each set of experiments, and used within 1–2 days. The species that had to be collected from more distant shores, however, were sometimes kept under sea-water circulation in the laboratory for up to 2 weeks before use, and there were obvious chances of acclimatization to conditions different from those on the shore.

The experiments were carried out during the winter of 1953–4; it was not possible to repeat the experiments on all species during the following summer, as originally planned. However, the seasonal differences in high-temperature tolerance found in the species that were investigated were not significant in relation to the temperature intervals (0·5°) used.

TABLE 1. THE SPECIES INVESTIGATED

Species	Locality at which species were collected	Tide-level at which sp. taken	Normal zonation of species	Distributional status (from Crisp & Southward, 1958)
Barnacles				
Chthamalus stellatus (Poli)	Rum Bay and Wembury Beach	M.H.W.N.	Midlittoral, from M.H.W.S. to M.L.W.N.	Southern, not in North Sea or eastern Channel
Balanus balanoides (L.)	Brixham	M.T.L.	Midlittoral, from M.H.W.N. to M.L.W.N.	Northern
B. perforatus (Bruguière)	Wembury Beach	M.L.W.N.	Lower midlittoral, M.T.L. to M.L.W.S.	Southern, in S.W. England and S. Wales only
Elminius modestus Darwin	Hen Point	M.L.W.N.	Midlittoral and infralittoral M.H.W.N. to shallow water	Immigrant from Australasia
Top-shells				
Monodonta lineata (da Costa)	Rum Bay and Wembury Beach	M.H.W.N.	Midlittoral, M.H.W.S. to M.L.W.N.	Southern, not in Scotland, North Sea, or eastern Channel
Gibbula umbilicalis (da Costa)	Rum Bay and Wembury Beach	M.H.W.N.	Midlittoral, M.H.W.N. to M.L.W.N.	Southern, not in North Sea or eastern Channel
G. cineraria (L.)	Rum Bay and Wembury Beach	M.L.W.N.	Infralittoral, M.L.W.N. to shallow water	Northern
Calliostoma zizyphinum (L.)	Rum Bay and Wembury Beach	M.L.W.N.	Infralittoral, M.L.W.N. to shallow water	Northern

High temperature

In testing tolerance of high temperature the animals were taken at room temperature (15–20°) in a small vessel of water, and the temperature raised slowly by heating the vessel in a water bath. For the molluscs the rate of heating was adjusted to 1° per 5 min, a value adopted by previous workers. For the barnacles, which were smaller, a rate of heating of 1° per min was found to allow sufficient time for penetration; tests with thermocouples showed no lag greater than 0·1° between the water and the tissues.

It will be realized that these experiments represent rather unnatural conditions, for on the shore the animals would experience such high temperatures only out of the water, when they might be subjected to desiccation also. Since the experiments were designed to show purely temperature effects their unnatural character could not be avoided. It is possible that some species may be more resistant than others when drying effects are added to those of heat.

For what may be termed the instantaneous lethal points, batches of three to ten animals, the number depending on the physical size and abundance of the species, were removed from the vessel at certain temperature intervals,

which were narrowed down to 0·5° in successive experiments, and allowed to recover in water at room temperature. The temperature at which more than half a batch failed to recover later within 12 h, and respond to pricking with a needle (by movement of the foot or valves), was regarded as the lethal temperature. When the instantaneous lethal point had been determined the effect of duration of high temperatures was studied. The temperature was raised as before and batches of animals removed at fixed temperatures (30°, 37°, 40° and 50°) to vessels of water maintained at the same temperature (plus or minus 1°) in an incubator; the fixed temperatures chosen for each species depending on the position of its instantaneous lethal point. Batches of animals were removed from the incubator at fixed intervals of time, allowed to recover in sea water at room temperature, and tested for recovery as before. For experiments of more than 1 day's duration the sea water in the incubators was made up with glass-distilled water to replace losses due to evaporation. In all experiments the water was aerated, and kept stirred, with compressed air.

Low temperature

The experiments on low temperature tolerance were hindered by difficulties over temperature control, and it was not possible to measure the instantaneous lethals. The animals were taken from water at room temperature, mopped dry on the outside of the shell with a clean cloth and placed in a dry finger-bowl covered by a sheet of glass. The finger-bowls were stacked in a small ice-cream conservator, set to 0°, −5° or −10° by means of a thermostat that was normally capable of controlling the temperature to plus or minus 1°. It was considered that under these circumstances heat transfer would be slow enough to prevent differences of tissue temperature between large and small animals, though the rate of change might be greater than that used for molluscs at high temperature.[1] Batches of animals were removed from the freezer at fixed intervals, and allowed to recover in sea water at room temperature. Those that failed to recover within 12 h and respond to pricking with a needle were considered dead. Some experiments were tried with the animals in sea water, but there were discrepancies between different sets, probably owing to the insulating properties of the ice that formed.

RESULTS

The results of the temperature tolerance experiments are given in Table 2. Clearly there was a much greater tolerance among the sessile barnacles than among the mobile top-shells, and the results for the two groups must be considered separately.

[1] Relative humidity was approx. 50%.

Barnacles

Tolerance of high temperature in barnacles seems related both to the distribution of the species and to the normal habitat on the shore. Thus of the two southern forms, *Chthamalus stellatus*, which can occur at much higher levels on the shore, was more resistant at all temperatures, and was the only animal tested to show an instantaneous lethal temperature above 50°. The other southern species, *Balanus perforatus*, is not normally found above mid-tide level, and its temperature tolerance was little better than the northern species, *B. balanoides*, which can live at higher levels on the shore. The immigrant Australasian species, *Elminius modestus*, was more resistant to high temperatures than either native species of *Balanus*; it can sometimes occur at higher levels on the shore than either.

The barnacles were remarkably tolerant of low temperatures. All four species recovered after several weeks at zero, even though they experienced chill coma after an hour's exposure: it was not possible to determine the 50% lethal point in the time available. At lower temperatures, −5° and −10°, *B. perforatus* was much less tolerant than the others. There was little difference in resistance of the other species at −10°, but at −5° the immigrant species *Elminius* was least tolerant, while the northern form *Balanus balanoides* was nearly twice as resistant as *Chthamalus stellatus*. Most of these differences are in accord with the natural distribution of the species. The relatively low resistance of *Elminius* is surprising in view of its present abundance on the cold east coast of England, where the southern species do not occur. It is interesting to record that in another series of experiments a specimen of *Monodonta lineata* was found dead after 72 h exposure at −5°, with a dead *Elminius* and a living *Chthamalus* on its shell.

Top-shells

In the molluscs tested temperature tolerance was more clearly related to vertical distribution on the shore than to geographical distribution. Thus, the two southern forms, *Monodonta lineata* and *Gibbula umbilicalis*, which occur higher up the shore, were more tolerant of both high and low temperatures than the two northern forms, *G. cineraria* and *Calliostoma zizyphinum*, which occur only at low water. In fact, for all four species the temperature tolerances increased in proportion to the relative capacity to withstand exposure out of the water. At −10° little difference in resistance was found; the value of 2–3 h given in the table is probably the time required for equilibration between the tissues and the air at this low temperature. The relatively low resistance of the low-water species to temperatures below 0° and above 30° is interesting, but in view of the normal habitat of the species may not be very important.

TABLE 2. THE TEMPERATURE TOLERANCES OF FOUR SPECIES OF BARNACLES AND FOUR SPECIES OF TOP-SHELLS

(Figures in brackets from Evans, 1948.)

Species	Time in hours to reach 50 % mortality at temperatures (° C) of								Point of heat coma (50 %) in ° C	Lethal point (50 %) in ° C	Lethal point (100 %) in ° C
	−10	−5	0	30	35	37	40	50			
Chthamalus stellatus	12–24	72–120	∞	—	—	—	29–30	½	43	52·5	53·7
Elminius modestus	12–24	48–72	∞	—	—	7½	5¼	—	36–38	48·3	49·5
Balanus perforatus	< 3	< 22	∞	—	—	—	3–5	—	38–40	45·5	47·0
B. balanoides	12–24	120–190	∞	—	—	¾	¾	—	35–37	44·3	45·3
Monodonta lineata	2–3	6–24	138–179	72–100	—	—	3 (6–6¼)	—	— (38–39)	45·0 (34·8)	45·3
Gibbula umbilicalis	2–3	16	30–79	24–72	— (10)	2	¾–1 (¾–1)	—	— (33–34)	41·8 (42·1)	42·0
G. cineraria	2–3	2–3	12–30	3¾ (5–5¼)	—	— (1¾–1½)	—	—	— (34–35)	35·5 (36·2)	36·0
Calliostoma zizyphinum	2–3	2–3	12–24	3½	—	—	—	—	33–34	34·5	34·8

TABLE 3. HEAT TOLERANCES OF SOME OTHER INTERTIDAL MOLLUSCS

(Data condensed and re-arranged from Evans, 1948.)

Species	Point of heat coma in ° C	Lethal point (50 %), ° C	Times in hours to 50 % mortality at temperatures (° C) of 30	35	40	Normal zonation	Distributional status (from Crisp & Southward, 1958)
Littorina neritoides	38	46·3	—	—	14–15	Supralittoral fringe and midlittoral	Southern, not in S. North Sea or eastern Channel
L. littorea	39	46·0	—	—	11½–12	Midlittoral	Intermediate, all round Britain
L. saxatilis	37	45·0	—	—	9½–10	Supralittoral fringe and midlittoral	Northern, all round Britain
L. obtusata	36	44·3	—	—	4¼–4½	Midlittoral	Intermediate, all round Britain
Patella depressa	37–38	43·3	—	—	3½–3¾	Midlittoral	Southern, S.W. England and Wales only
P. vulgata	37	42·8	—	10	2¾–3	Midlittoral	Intermediate, all round Britain
P. aspera	37	41·7	—	8½–9	¾–1	Lower midlittoral and infralittoral fringe	Southern, not in S. North Sea or eastern Channel

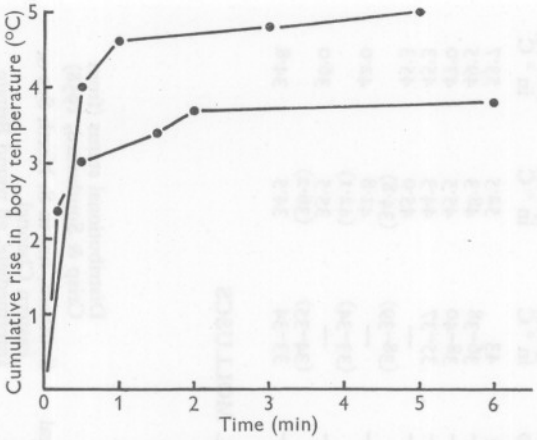

Fig. 2. Results of two experiments showing the time taken for equilibration between the tissues and the environment in a top-shell. A thermocouple was sealed inside the shell with dental cement, and the animal (15 mm diam.) transferred directly from room air temperature to sea water some 4 to 5° warmer.

Other molluscs

Time did not permit any experiments on further species of intertidal animals. However, the present results for top-shells were very little different from those recorded by Evans (1948) for the same species from Cardigan Bay (see Table 2); it seems permissible, therefore, to review Evans's results for other molluscs from the more general point of view of distribution. The heat tolerances of several littorinids and limpets from Cardigan Bay, as determined by Evans, are given in Table 3. In these animals, as in barnacles, the temperature tolerance seems connected with the geographical distribution of the species. However, the distributional differences of the vertical zonation are less marked than in barnacles and the differences in temperature tolerance seem likewise less obvious. Of the species of *Littorina*, *L. saxatilis* and *L. obtusata* are of somewhat more northern character than *L. neritoides*. This may explain the lower heat tolerance of the two former species, but it must be noted that all occur at least as far south as Portugal. Apparently, however, the littorinid most tolerant to heat, *L. neritoides*, is found the farthest south, and occurs in North Africa. No doubt the habitat of *L. obtusata*, which is normally found among fucoids, may be connected with its intolerance of high temperature.

Among the limpets, the most southerly species, *Patella depressa*, which is found only on a limited part of the south-west coasts of Britain, was found by Evans to be the most resistant to high temperatures. The least tolerance of high temperature was shown by *P. aspera*, which, though it has a more southerly distribution than *P. vulgata*, is very much a low-water species and occurs above mid-tide level only where the shore is wetted, and cooled, by considerable wave action.

Some of the above species of littorinids are found in northern Russia, where their tolerance of low temperature has been noted by Gurjanova, Sachs & Uschakov (1930). The most northern of them, *Littorina saxatilis*, was most tolerant of low temperature, and survived more than 27 h in air at $-9{\cdot}4°$, compared with about 4 h by *L. obtusata*; *L. littorea* was killed in 3 h at $-8{\cdot}8°$. The same workers found that *Balanus balanoides* survived 48 h at $-9{\cdot}4°$; compared with the present results this suggests the species may possess greater tolerance in more northerly latitudes.

TEMPERATURES IN THE INTERTIDAL ZONE

The most important temperatures on the shore, from a biological point of view, are those experienced by the animals themselves. Even though the normal inhabitants of the zone are all poikilothermic, it cannot be assumed that they adopt the temperature of the sea or the temperature of the air directly they are exposed to these media. In air, when the animals are exposed to fluctuating humidity and heating up by sunlight, it cannot be assumed that their tissue temperatures ever agree with meteorological values of air temperature. With terrestrial arthropods the effects of sunlight on body temperature are remarkable (Parry, 1951; Edney, 1953).

As measurements of the animal's body temperature cannot be made very often, it is necessary to try to relate them in a general way to meteorological values of sea and air temperature and to sunlight and humidity, before any conclusions can be made regarding temperature trends in the intertidal zone. In the present work, observations were made on 13 days over a period of 10 months in 1953–4. No very hot weather occurred during this period, and it was not possible to use the apparatus in rain, but a variety of other weather conditions was observed.

METHODS

The apparatus was planned after making some preliminary measurements at Port Erin, Isle of Man. The temperatures of the animals, of tide pools and of the air close to the ground were obtained with fine-wire thermocouples. Copper/constantan couples were finally chosen because of their ease of manipulation, even though the e.m.f. provided is lower than with chromium alloys. The measuring parts of the couples were made of 10 cm of 36 B & S gauge, insulated with thinned cellulose lacquer, and were connected to the galvanometer by flexible leads, 0·5 m long, of 50 strands 36 B & S insulated with PVC sleeving. The e.m.f. was measured against a reference junction in a Thermos-flask of ice and water (temperature 0·1–0·2° by mercury thermometer), and calibrated empirically against a certificated mercury thermometer. A galvanometer of taut suspension reflecting type[1] was used with an arrange-

[1] Tinsley type 5195 temperature indicator.

ment of copper switches that enabled any of 18 couples to be compared with the reference junction or one another. Under these conditions a deflexion of up to 18 mm per degree Centigrade was achieved, and ranges of less sensitivity up to 50° f.s.d. could be obtained by use of switched shunts. An apparatus of this type was more portable and stable than valve voltmeters or a.c. bridges.

Humidity close to the ground was measured with Edney paper hygrometers, checked occasionally against a wet and dry bulb instrument. Sea temperatures close to the shore were taken with a mercury thermometer of the standard meteorological pattern for the purpose.

In the absence of a solar radiation meter, an approximate index of sunlight intensity was given by an incident light exposure meter[1] calibrated roughly in kilolux by reference to a photocell light recorder belonging to Dr W. R. G. Atkins, F.R.S. The meter was pointed at the sun, and recorded both sunlight and general skylight from the same direction.

Screen air temperatures for Mount Batten, Plymouth, have been extracted from *The Daily Weather Report* (Air Ministry, 1953–4) and converted to the nearest 0·5°.

All but one series of measurements were made on the rocks below the Plymouth Laboratory, at a level just below M.H.W.N. The procedure was as follows: (i) galvanometer placed on level surface and reference junction plugged into flask of ice and water; (ii) hygrometer put down; (iii) two thermocouples placed inside two barnacles through holes pierced in the shells; two more placed into limpets in the same way; two inanimate bodies, consisting of dead barnacle shells filled with plaster of Paris and each containing a couple, set up close to live barnacles but not quite touching rock surface; (iv) one thermocouple set up 1 cm from rock surface in shade of galvanometer; other couples, if available, inserted into top-shells or tide-pools; (v) sea temperature and sunlight measured while couples reached equilibrium; (vi) finally each couple switched in turn to reference junction and reading noted. In all cases several readings were taken, while with the air close to the ground, the temperature of which fluctuated continually, the maximum and minimum during 1 min were noted and the average taken.

In one series of observations at Wembury the above procedure was followed, but readings were made at several tide levels.

RESULTS

The results of the observations of temperature, humidity and sunlight on the shore at or near Plymouth are presented in Table 4, where possible to the nearest 0·1°. Some earlier observations at Port Erin, under weather conditions that were not studied at Plymouth, are given in Table 5; in this case the pointer galvanometer used allowed readings to the nearest 0·5° only.

[1] Weston Master II, with Invercone attachment.

TABLE 4. OBSERVATIONS OF TEMPERATURES IN THE INTERTIDAL ZONE AT OR NEAR PLYMOUTH

(For further explanation refer to text.)

			Meteorology			Conditions on the shore										
									Temp. of inanimate body		Tissue temp. of barnacles		Tissue temp. of limpets		Temp. of tide-pools	
Date	Time, h G.M.T.	Hours after high water	Remarks on weather	Screen air temp., noon	Sea temp. inshore	Sunlight in kilolux	Percentage relative humidity	Shade air temp. close to rocks	Range	Average	Range	Average	Range	Average	Range	Average
22. x. 53	10	5	No sun: rocks wet	14·5	14·3	—	—	15·0	15·3–15·7	15·5	14·6–14·9	14·7	14·6–14·9	14·7	14·3–14·8	14·5
28. x. 53	16	7	Strong sun after warm day	12·0	14·0	10	—	15·0	—	16·7	—	22·4	—	17·4	—	—
28. x. 53	16	7	Hazy sun after warm day	12·0	14·0	5	—	14·2	—	15·1	—	18·2	—	17·9	—	—
4. xi. 53	10	6	Cool: a little sun at first	12·0	12·5	6	—	12·2	—	12·4	—	13·7	—	13·1	—	11·4
4. xi. 53	11	7	After an hour with full sun	12·0	12·5	35	—	13·6	17·2–20·6	18·6	—	19·6	—	18·5	—	11·7
18. xi. 53	10	7	Cool, with hazy sun	7·0	11·9	10	85	6·8	—	7·4	—	7·2	—	7·9	—	8·6
18. xi. 53	11	8	After an hour in hazy sun	7·0	11·9	15	93	7·5	9·4–9·9	9·6	8·8–9·3	9·0	—	9·6	—	—
8. xii. 53	12	6	Weak sun	12·0	12·8	10	88	13·6	13·4–13·8	13·6	13·0–13·3	13·1	—	13·2	12·3–13·2	12·8
18. xii. 53	10	7	Weak sun: thin cloud	10·0	12·2	15	93	11·4	—	12·5	—	12·4	—	12·4	—	11·4
18. xii. 53	11	8	After hour in stronger sun	10·0	12·2	30	—	12·4	—	16·2	—	15·5	—	15·5	—	12·2
2. ii. 54	11	7	Cold spell, sunny: ice on pools	−2·0	6·6	25	64	—	—	—	—	—	7·8–8·2	8·0	−0·5–4·0	2·8
2. ii. 54	11	7	As above, but animals in shade	—	—	—	—	—	—	—	—	—	1·2–2·6	1·9	—	—
4. ii. 54	13	7	Cold spell: sunny	0	6·3	30	57	3·9	9·2–11·2	10·2	9·8–11·2	10·5	5·6–9·4	7·5	4·4–5·3	4·9
4. ii. 54	13	7	As above but animals in shade	—	—	—	—	—	—	—	—	—	—	4·0	—	—
1. iii. 54	10	8	Light sun	4·5	7·6	7	—	6·1	—	7·7	6·6–7·5	7·0	—	6·4	—	4·6
1. iii. 54	10	8	Becoming cloudy	4·5	7·6	7	—	5·8	—	6·4	6·0–6·7	6·3	—	5·4	—	4·9
11. iii. 54	15	5	Sunny spring day	14·0	9·4	50	45	14·3	20·6–22·2	21·4	18·5–19·4	18·9	15·4–17·2	16·2	—	10·7
17. v. 54	12	7	Sunny	14·5	12·2	75	49	17·1	22·2–23·0	22·6	24·1–24·4	24·2	—	18·0	14·8–15·0	14·9
17. v. 54	12	7	Hazy cloud passing over	14·5	12·2	—	49	16·8	21·4–21·7	21·5	20·6–21·4	21·0	—	18·4	14·8–15·0	14·9
19. v. 54	12	6	Wembury: H.W.N.; sun and breeze	16·5	13·7	75	46	20·6	26·3–28·7	27·4	27·6–28·4	28·0	24·4–25·5	24·9	16·4–17·2	16·8
19. v. 54	13	7	Wembury: L.W.N.; stronger breeze	16·5	13·7	75	58	16·8	19·6–20·6	20·2	21·4–22·5	21·9	20·1–20·4	20·2	13·6–13·9	13·7
19. v. 54	15	9	Wembury: H.W.N.; sheltered from wind	16·5	13·7	75	47	20·7	25·2–27·4	26·7	28·4–31·4	29·9	—	—	—	—
19. v. 54	15	9	As above, animals in shade	—	—	—	—	—	—	—	—	—	19·6–22·2	20·9	—	—
14. viii. 54	10	5	Sunny	16·5	14·6	75	59	17·7	25·5–27·3	27·1	23·0–23·9	23·4	—	20·9	—	17·4
14. viii. 54	11	6	An hour's further exposure	16·5	14·6	75	59	17·8	27·9–28·9	28·4	26·5–27·1	26·8	24·7–24·9	24·8	—	17·4

General inferences

Some generalizations can be made, from Tables 4 and 5, regarding temperatures in the intertidal zone. Clearly, screen air temperature, even when read at a nearby site, may have very little reference to conditions on the shore. Thus in the colder months when the sea is usually warmer than the land, and in cloudy weather when the humidity is high, the air temperature close to the rocks and the body temperature of the animals may be higher than screen air temperature even 7–8 h after being uncovered by the sea. No doubt, heat storage by the rocks may assist in maintaining temperatures. Again in the colder months, but in sunny weather, when the humidity is less, evaporative cooling on exposure to the air is counterbalanced by radiant heating from the sun, and the air temperature close to the rocks and the body temperature of the animals are usually much higher than either screen air temperature or sea

TABLE 5. OBSERVATIONS OF TEMPERATURE IN THE INTERTIDAL ZONE AT OR NEAR PORT ERIN

(For further explanation refer to text.)

			Meteorology				Conditions on shore				
								Tissue temp. of barnacles		Tissue temp. of limpets	
Date	Time, h G.M.T.	Hours after high water	Remarks on weather	Screen air temp.	Sea temp. inshore	Percentage relative humidity	Shade air temp. close to rocks	Range	Mean	Range	Mean
23. ii. 50	10	7	Cloudy	—	8·0	87	9·0	8·0–8·5	8·0	8·0–8·5	8·0
22. xi. 50	16	6	Low cloud, rain at times	6·5	9·5	85	7·0	—	7·0	—	7·0
13. xii. 50	10	8	Cold spell, cloudy	0	9·0	67	1·5	—	2·0	—	3·5

temperature. No doubt at night in cold clear weather, evaporative cooling may produce temperatures below that of the sea; but although measurements have not been possible in the dark, it has been noticed that the rocks remain quite wet, hence evaporative cooling cannot be very marked.

In the warmer months of the year, and in sunny weather, temperatures on the shore after the tide has fallen are well above sea temperature and usually higher than screen air temperature. Again at night evaporative cooling may take place, but again the shore has been observed to remain wet. In cloudy weather in that part of the year when air temperatures exceed sea temperature during the day there may be closer agreement between screen air temperature, the air temperature close to the rocks and the temperatures of the animals themselves, but even the slightest amount of sunlight filtering through the clouds causes warming up of the shore.

To sum up, it seems that, in daylight, intertidal animals exposed to the air will often have higher body temperatures than would be expected from sea and air temperatures. This is clearly brought out if we compare the maximum, minimum and mean temperatures of the observation period (Table 6). The minimum temperatures of the animals may be below the minimum sea temperature, but both means and maxima are well above the corresponding values of sea and air. Compared with the temperature of the tide pools or

the air on the shore, the animals show an upward shift of the range of temperatures experienced. Some of this shift may be due to retention of the warmth of the sea, but most of it can be attributed to the warming effects of sunlight. These effects are not felt by animals that are always in the shade, and such animals, as far as can be ascertained from the few observations made, are subject to a lower annual range of temperature. Animals in the shade may

TABLE 6. ANALYSIS OF OBSERVATIONS SHOWN IN TABLE 4

(For the air on shore, the limpets, the inanimate body and the barnacles the figures refer to the average of the readings on each occasion. Figures for animals in the shade are shown in parentheses.)

Medium or animal	Temperature		
	Maximum	Mean	Minimum
Screen air	16·5	10·1	−2·0
Tide pools	17·4	10·8	2·8
Sea inshore	14·6	11·3	6·3
Air on shore	20·7	13·3	3·9
Limpets	24·9 (20·9)	14·2	5·4 (1·9)
Dry, inanimate body	28·4	17·0	6·4
Barnacles	29·9	17·3	6·3

follow shade air temperatures more closely than those on the open rock, but should never show temperatures as low as the extreme screen temperatures.

Effect of sunlight and wind

It has already been remarked that an upward shift in the range of temperatures experienced by animals on the open rock can be attributed in large part to warming by sunlight. Comparison of the temperatures of the living animals and of the inanimate bodies not in contact with the rock (the dead barnacle shells) shows how much of this warming up is direct or by conduction from the layer of warm air close to the ground (cf. Parry, 1951), and not by conduction from the heated rocks (Fig. 3). Only on days when there had been prolonged heating by the sun, and the rocks were thoroughly warmed up, were the living animals at a higher temperature than the inanimate bodies. With limpets, however, there was somewhat less agreement between their tissue temperatures and those of inanimate bodies above 20°. No doubt this difference reflects the larger size of the limpets and their correspondingly lower proportion of surface area.

The effect of sunlight is well illustrated by the records for 4 November and 18 December in Table 4. On the latter occasion an hour's exposure to a twofold increase in sunlight raised the shade temperature of the air close to the rocks by 1° only, while the animals rose 3–4°; on the earlier date the sunlight increased nearly sixfold, and the animals rose by 5° or 6° compared with an increase of only just over 1° in shade air temperature. Such heating up by sunlight can be very rapid. On one occasion after a light cloud had passed from in front of the sun a barnacle increased by 1° in 1 min. On another

occasion on the laboratory roof a top-shell practically doubled its temperature, from 17·6° to 31·4°, in 35 min. The results of the latter experiment are shown in Table 7, with readings of sunlight and the temperatures of an inanimate body (one of the dead barnacles filled with plaster of Paris).

In the above experiment there was little or no movement of the air. Parry (1951) has shown the importance of the layer of warm air close to the ground in the heating up of small bodies in sunlight. Clearly, the presence of wind will modify the heating-up process, and should permit cooling by convection. This is borne out by the data from Wembury on 19 May (Table 3). The difference between an absolute calm and a light breeze under otherwise similar conditions amounted to some 1–3° in body temperature of the barnacles.

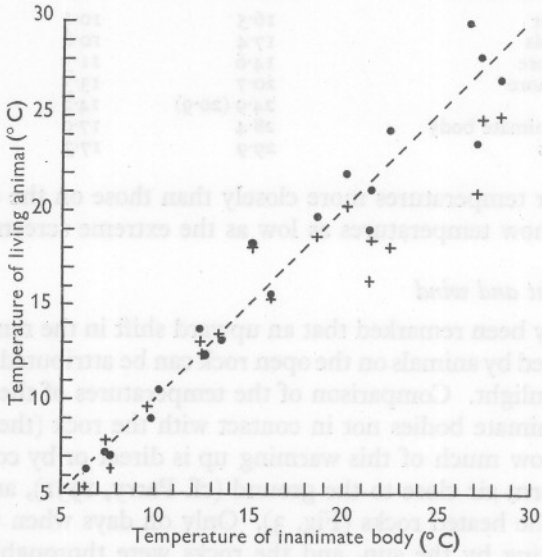

Fig. 3. The body temperatures of barnacles (dots) and limpets (crosses) in sunlight, plotted against the temperatures assumed by an inanimate body. The dotted line represents perfect agreement between the animate and inanimate bodies. For further details refer to text.

TABLE 7. TEMPERATURES OF A LIVING TOP-SHELL AND AN INANIMATE BODY* IN SUNLIGHT AT PLYMOUTH

(On roof of laboratory, no wind: taken directly from room temperature.)

	Illumination from sun and part of sky in approx. kilolux	Top-shell (° C)	Inanimate body (° C)
At start	30	17·6	16·3
10 min later	35	20·4	18·9
13 min later	40	23·9	20·8
30 min later	60	31·3	29·3
35 min later	60	31·4	30·0

* A dead barnacle shell filled with plaster of Paris.

DISCUSSION

The experiments and field measurements that have been described above were carried out in support of work on distribution. For this reason they were not continued when it became clear that actual temperatures experienced on the shore were, in most cases, well inside the tolerance limits of the animals. This might have been expected, for all the animals live successfully at the places where the temperatures were measured.

It must be remembered that more extreme conditions occasionally experienced, even if only for a short period, might have lethal effects. This is particularly so with the northern species, such as *Balanus balanoides*, which is near the limits of its tolerance of high temperature. On one occasion in the Isle of Man temperatures up to 38° were measured in barnacles (*Chthamalus*) above M.H.W.N., while Moore (1935) records a temperature of 36·5° in a barnacle at Plymouth. Temperatures of this order can produce coma in *Balanus balanoides*, and cause over 50% mortality if experienced for more than 45 min. Thus the detrimental effects of heating up in the sun may contribute to the geographical limit of *B. balanoides*, and may help to restrict it to low tide levels or shaded habitats near its southern boundary. The remaining species, however, appear capable of withstanding even the exceptional conditions, and there is no evidence for limitation of their habitat.

There is little possibility of directly lethal effects of occasional low-temperature extremes, even if we consider temperature conditions at a locality where the less tolerant species are absent. For example, from Table 2 it can be seen that exposure of the midlittoral species *B. perforatus*, *Monodonta lineata* and *Gibbula umbilicalis* to temperatures lower than $-5°$ might cause heavy mortality within one tidal period (6–12 h). During the course of the work there was a fairly long cold spell with consistently subzero temperatures; Table 8 shows the values of screen air temperature at 6-hourly intervals for 10 days of this cold spell at Plymouth and at a coastal station in the eastern Channel, where these animals are absent. Only on one occasion at Plymouth did temperatures fall below $-5°$, and although this low temperature was maintained for more than 6 h it coincided with a rising tide and the intertidal zone was exposed for only a few hours. At the other station temperatures fell below $-5°$ on five occasions, several times for more than 6 h and once for over 18 h (nearly two tidal cycles). The significance of this difference between the two stations is reduced if we consider the evidence of the field measurements of the animals' temperature. The species in question have body masses more comparable to limpets than to the barnacles (*Chthamalus*) used in the field work; nevertheless, even limpets in the shade had temperatures well above zero at Plymouth on the coldest day of the cold spell, while animals in the sun were as much as 8–10° warmer than the screen air at midday. There is thus little likelihood that lethal temperatures were experienced on the shore at Plymouth during this cold spell, though the

presence of ice on the tide pools shows that temperatures on the shore were sometimes below −2°. If the temperature differentials between the animals and the screen air temperature can be applied also to Lympne, the more easterly station, there is little possibility that directly lethal temperatures were experienced there either, even if allowance is made for the much lower values of air temperature recorded.

It is clear that during a cold spell the rocks and the animals on the shore retain the warmth of the sea while out of water long enough to maintain temperatures above zero during the period of exposure, while the smallest

TABLE 8. COMPARISON OF SCREEN AIR TEMPERATURES AT PLYMOUTH AND LYMPNE

(Readings at 6-hourly intervals during a cold spell in 1954. From Air Ministry, 1954. Converted from ° F to nearest 0·5° C.)

	Lympne					Plymouth				
	Temperature at hours:				Hours	Temperature at hours:				Hours
Date	0	6	12	18	sun	0	6	12	18	sun
28. i.	−5·5	−8·0	−4·0	−4·5	4·5	0·5	0	0	−1·0	0
29. i.	−2·0	−0·5	−1·5	−0·5	2·2	−3·0	−1·0	0·5	1·0	0·3
30. i.	0	−3·5	−1·0	−4·0	2·2	0·5	1·0	0	−0·5	7·2
31. i.	−6·0	−5·0	−6·0	−5·0	5·0	−1·5	−1·5	−1·0	−4·0	6·6
1. ii.	−4·5	−6·5	−4·5	−5·0	6·8	−4·0	−4·0	−1·5	−3·0	7·3
2. ii.	−5·0	−4·5	−3·0	−3·5	1·9	−5·5	−5·5	−2·0	−1·0	7·0
3. ii.	−3·5	−4·0	−1·5	−1·5	8·3	−3·5	−2·0	0·5	−1·0	6·9
4. ii.	−1·0	−3·0	−1·5	−3·0	6·6	−3·0	−1·5	0	−1·5	5·2
5. ii.	−4·5	−4·5	−1·5	−3·0	5·7	−3·0	−2·0	−2·0	−2·0	1·5
6. ii.	−5·0	−7·0	−3·0	−1·5	0·5	−2·0	−3·5	0	1·0	3·3
	Mean −3·56					Mean −1·55				

amount of sunshine should immediately warm them up. The only circumstances that can be envisaged as having directly lethal effects are those existing during the exceptional cold spells that occur in certain years, when the sea temperatures inshore may be below zero and there is little sunshine (see Orton, 1931). Such circumstances are not experienced very often, and are hardly regular environmental factors, even in eastern England; moreover, they can kill other animals apart from any strictly southern species.

In a more severe climate than that of Western Europe it is possible that direct lethal effects of low temperature may be a normal occurrence and may influence the distribution of the less hardy forms (see Kanwisher, 1955). In Western Europe, at any rate, evidence for a causal relationship between temperature and distribution must be sought in non-lethal terms such as debilitating effects at some stage in the life history. Dr D. J. Crisp informs me that some preliminary experiments he has made show that among the barnacles the southern forms may take longer to recover from chill coma than the northern species, and may thus lay themselves open to selective attack by predators. Other effects of temperature may be found in combination with

other factors or indirectly through competition between species (Southward & Crisp, 1956; Crisp and Southward, 1958).

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SUMMARY

In experiments on four species of barnacles and four species of top-shells, the barnacles were found to be more resistant to high or low temperatures than the top-shells. Among each group of animals the degree of tolerance was related to the geographical distribution of the species and their zonation on the shore. Thus most tolerance of high temperature was shown by species of southern distribution, especially by those occurring at the upper limit of the midlittoral zone, and most tolerance of low temperature by species of northern distribution. Least tolerance of high and low temperatures was shown by species found only in the infralittoral fringe or below low water.

Field measurements of the body temperatures of barnacles and limpets while exposed to the air were made with thermocouples. Under many weather conditions the body temperatures were higher than would be expected from local meteorological values of air temperature. This difference was due to retention of sea temperature by the animals and the rocks and to the heating effects of sunlight.

The results of the laboratory experiments and of the field measurements are discussed together in relation to weather and geographical distribution. It is clear that the temperatures experienced on the shore are well within the tolerance limits of most of the animals, and even exceptional extremes of temperature may have little direct influence on the distribution of adult intertidal animals. Evidence for a causal relation between temperature and distribution must be sought in non-lethal terms such as debilitating effects, or indirectly through competition between species, or in combination with other factors.

REFERENCES

AIR MINISTRY, 1953–4. *The Daily Weather Report*. London: Meteorological Office.

ANDREWARTHA, H. G. & BIRCH, L. C., 1954. *The Distribution and Abundance of Animals*. Chicago: University Press.

BROEKHUYSEN, C. J., 1940. A preliminary investigation of the importance of desiccation, temperature and salinity as factors controlling the vertical distribution of certain marine gastropods in False Bay, South Africa. *Trans. roy. Soc. S. Afr.*, Vol. 28, pp. 255–92.

CRISP, D. J. & SOUTHWARD, A. J., 1958. The distribution of intertidal organisms along the coasts of the English Channel. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 157–208.

EDNEY, E. B., 1953. The temperature of woodlice in the sun. *J. exp. Biol.*, Vol. 30, pp. 331–49.

EDWARDS, G. A., 1946. The influence of temperature upon the oxygen consumption of several arthropods. *J. cell. comp. Physiol.*, Vol. 27, pp. 53–64.

EVANS, R. G., 1948. The lethal temperatures of some common British littoral molluscs. *J. Anim. Ecol.*, Vol. 17, pp. 165–73.

GOWANLOCH, J. N. & HAYES, F. R., 1926. Contributions to the study of marine gastropods. I. The physical factors, behaviour and intertidal life of *Littorina*. *Contr. Canad. Biol.*, N.S., Vol. 3, pp. 133–66.

GURJANOVA, E., SACHS, I. & USCHAKOV, P., 1930. Das littoral des Kola Fjords. III. *Trav. Soc. Nat. Leningrad*, Vol. 60, pp. 17–107. (In Russian, German summary.)

HEILBRUNN, L. V., 1943. *An Outline of General Physiology*, 3rd ed. Philadelphia and London: Saunders.

HUNTSMAN, A. G. & SPARKS, M. I., 1924. Limiting factors for marine animals. III. Relative resistance to high temperatures. *Contr. Canad. Biol.*, N.S., Vol. 2, pp. 95–114.

HUTCHINS, L. W., 1947. The bases for temperature zonation in geographical distribution. *Ecol. Monogr.*, Vol. 17, pp. 325–35.

KANWISHER, J. W., 1955. Freezing in intertidal animals. *Biol. Bull., Woods Hole*, Vol. 109, pp. 56–63.

LOOSANOFF, V. L., MILLER, W. S. & SMITH, P. B., 1951. Growth and setting of larvae of *Venus mercenaria* in relation to temperature. *J. mar. Res.*, Vol. 10, pp. 59–81.

MOORE, H. B., 1935. The biology of *Balanus balanoides*. IV. Relation to environmental factors. *J. mar. biol. Ass. U.K.*, Vol. 20, pp. 279–307.

ORR, P. R., 1955. Heat death. I. Time-temperature relationships in marine animals. *Physiol. Zoöl.*, Vol. 28, pp. 290–4.

ORTON, J. H., 1920. Sea temperatures, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 339–66.

——, assisted by LEWIS, H. M., 1931. On the effect of the severe winter of 1928–1929 on the oyster drills of the Blackwater Estuary. *J. mar. biol. Ass. U.K.*, Vol. 17, pp. 301–13.

PARRY, D. A., 1951. Factors determining the temperature of terrestrial arthropods in sunlight. *J. exp. Biol.*, Vol. 28, pp. 445–62.

RUNNSTRÖM, S., 1929. Weitere Studien über die Temperaturanpassung der Fortpflanzung und Entwicklung mariner Tiere. *Bergens Mus. Aarb.*, 1929, No. 10, 33 pp.

SOUTHWARD, A. J., 1950. Occurrence of *Chthamalus stellatus* in the Isle of Man. *Nature, Lond.*, Vol. 165, p. 408.

—— 1955. On the behaviour of barnacles. I. The relation of cirral and other activities to temperature. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 403–22.

—— 1957. On the behaviour of barnacles. III. Further observations on the influence of temperature and age on cirral activity. *J. mar. biol. Ass. U.K.*, Vol. 36, 323–34.

SOUTHWARD, A. J. & CRISP, D. J., 1954. The distribution of certain intertidal animals around the Irish coast. *Proc. R. Irish Acad.*, Vol. 57 (B), No. 1, 29 pp.

—— —— 1956. Fluctuations in the distribution and abundance of intertidal barnacles. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 211–29.

VERNON, H. M., 1899. The death temperature of certain marine organisms. *J. Physiol.*, Vol. 25, pp. 131–6.

J. mar. biol. Ass. U.K. (1958) 37, 67–79
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THE ADHESIVE MECHANISMS OF MONOGENETIC TREMATODES: THE ATTACHMENT OF SPECIES OF THE DICLIDOPHORIDAE TO THE GILLS OF GADOID FISHES

By J. LLEWELLYN

Department of Zoology and Comparative Physiology,
University of Birmingham

(Plate I and Text-figs. 1–3)

An account of the adhesive organs of *Diclidophora denticulata* and of the attachment of this parasite to the gills of *Gadus virens* was given by Cerfontaine (1896, 1898) in what has come to be regarded (Dawes, 1947) as a classic piece of description. The adhesive organs of other species of *Diclidophora* have been described by Gallien (1937) and Brinkmann (1942), while Price (1943), Sproston (1945, 1946), Dawes (1946, 1947), Chauhan (1953), and Hargis (1955) have made extensive use of the structure of adhesive organs in systematic surveys of the Diclidophoroidea. However, a consideration of all these accounts, in order to compare the structure of the adhesive organs of the Diclidophoridae with those of *Plectanocotyle gurnardi* and *Kuhnia scombri* which I have described previously (Llewellyn, 1956*a*, 1957*a*), showed that some of the more recent accounts were in very considerable conflict with Cerfontaine's description of *Diclidophora denticulata*, but that no actual comparisons had been made. In the present paper a comparative study is made, from actual specimens, of what are probably all of the valid species reported so far as belonging to the genus *Diclidophora*.

Specimens were obtained from the sources indicated in Table 1, and have been named as in Sproston (1946, pp. 469–85). In addition to the species of *Diclidophora* listed by Sproston, *D. gadi* (Reichenbach-Klinke, 1951), syn. *Dactylocotyle gadi* Reichenbach-Klinke, 1951, has been studied from whole-mount preparations. It has not been possible for me to examine *Diclidophora maccallumi* (Price, 1943) Sproston, 1946, but from Price's diagram (1943, fig. 5) of the adhesive organ, this species seems more likely to belong to the Cyclocotylinae than to the Diclidophorinae. With regard to *Diclidophora morrhuae* (van Beneden & Hesse, 1863) Sproston, 1946, it seems probable that no such animal exists: Dawes (1947, p. 107) stated that the identity of the species was very doubtful; Gallien (1937) searched for, but did not find any monogenean on cod; and I have examined about fifty specimens of *Gadus morrhua* without finding a trematode gill parasite; a comparatively

great amount of fisheries work has been done on cod, and it is unlikely that a gill trematode would have escaped attention.

The histological techniques used have been those described previously (Llewellyn, 1956*a*, 1957*a*).

TABLE 1. SOURCES OF *DICLIDOPHORA* MATERIAL

Parasite	Host	Locality	Collector
D. merlangi	*Gadus merlangus*	Aberystwyth Plymouth	Rees & Llewellyn (1941) Llewellyn (1956*b*)
D. denticulata	*G. virens*	Irish Atlantic Slope St Andrews	Rees & Llewellyn (1941) Frankland (1955)
D. luscae	*G. luscus*	Plymouth	Llewellyn (1956*b*)
D. macruri	*Macruris rupestris*	Skager-Rak	Brinkmann (1942)
D. minor	*Gadus poutassou*	Skager-Rak and North Sea	Brinkmann (1942)
D. palmata	*Molva molva*	Irish Atlantic Slope Iceland (64° 20′ N., 14° 19′ W.)	Rees & Llewellyn (1941) King Edward's School Five Ways Birmingham Marine Expedition, 1956
D. phycidis	*Urophycis blennoides*	Irish Atlantic Slope	Rees & Llewellyn (1941)
D. pollachii	*Gadus pollachius*	Plymouth	Llewellyn (1956*b*)
D. gadi	*G. aeglefinus*	North Sea	Reichenbach-Klinke (1951)

THE ADHESIVE MECHANISM OF *DICLIDOPHORA*

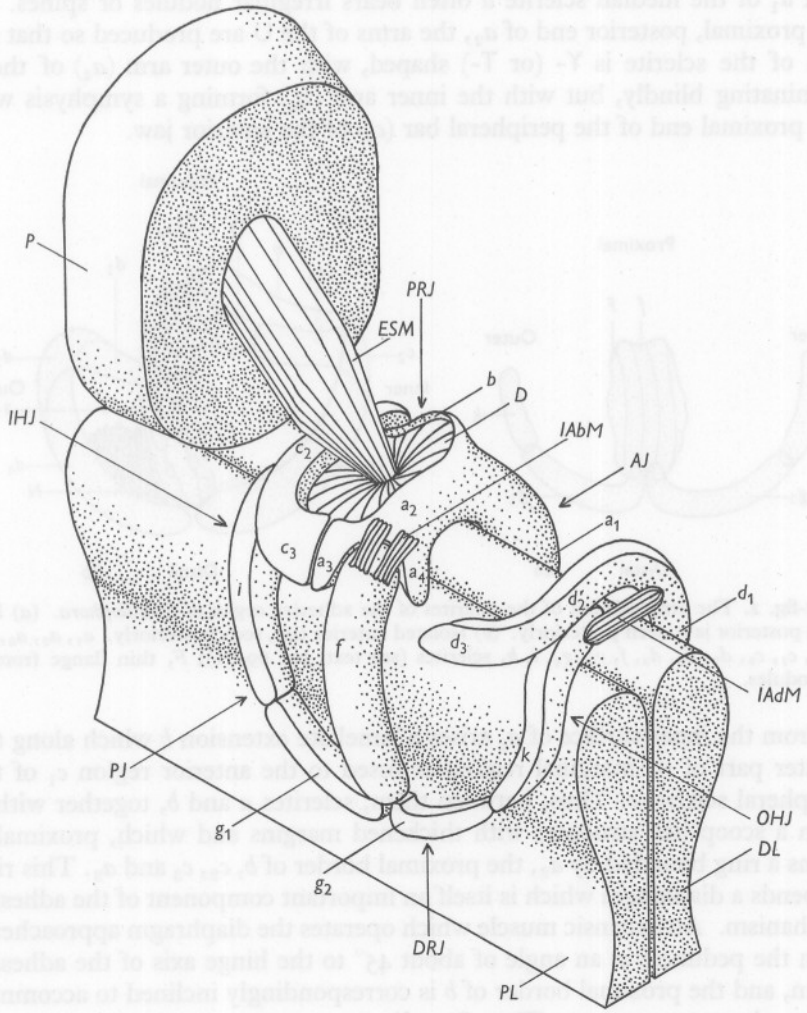
A comparative account of the adhesive attitudes of several species of *Diclidophora* has been given previously (Llewellyn, 1956*b*), and is further illustrated here for *D. denticulata* in Pl. I, fig. 1. In all species, each of the eight adhesive organs grasps secondary gill lamellae in exactly the same manner, and, moreover, the general structure of all of the adhesive organs in all species is very similar, and is illustrated in Text-figs. 1 and 2. There are minor specific differences, and these will be described later (pp. 72–73). As far as possible, Cerfontaine's notation for the identification of various structures has been adopted, but some modifications and extensions to his scheme have been found necessary.

The structure of the adhesive organs

Each adhesive organ consists of a pair of dissimilar opposable hinged jaws that grasps two or three secondary gill lamellae so that the anterior jaw lies distally with respect to the origin of the primary lamella from the gill arch of the host fish, and the posterior jaw proximally (Pl. I, fig. 1). The anterior jaw is deeply hollowed and is relatively fixed, while the posterior jaw is shallow and is movable about its hinged attachment to the anterior jaw, the movement being effected by both intrinsic and extrinsic muscles. The walls of the adhesive organ are supported by sclerites (= skeletal bars, plates, nodules and teeth), the arrangement of which is asymmetrical, making it possible to

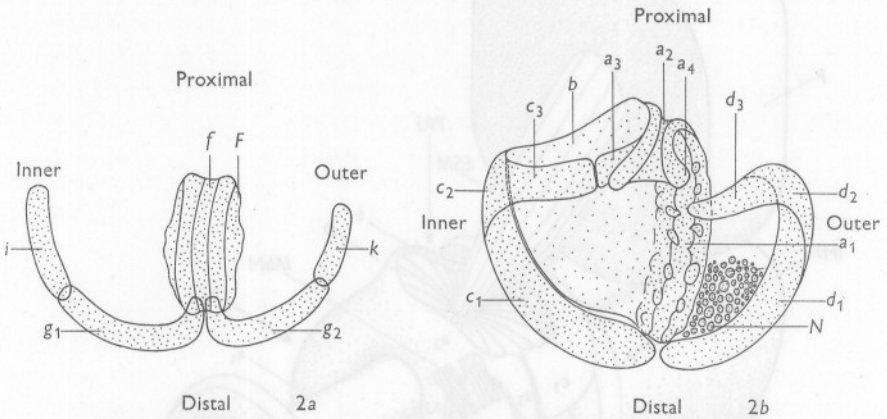
recognize an 'inner' (more median) and an 'outer' (more lateral) half to each organ (Text-figs. 1 and 2).

The inner half of the anterior jaw is supported by two fused sclerites, one, a ($=a_1a_2a_3a_4$), in the median region of the jaw, and the other, c ($=c_1c_2c_3$), a peripheral bar. Bar a is a curved rod passing (a_1) from a median distal



Text-fig. 1. Stereogram of an adhesive organ of *Diclidophora* grasping secondary gill lamellae of host. a_1, a_2, a_3, a_4, b, c_2, c_3, d_1, d_2, d_3, f, g_1, g_2, i, k, sclerites (see text, pp. 69–71); *AJ*, anterior jaw; *D*, diaphragm; *DL*, distal lamella; *DRJ*, distal region of jaw; *ESM*, extrinsic suctorial muscle; *IAbM*, intrinsic abductor muscle; *IAdM*, intrinsic adductor muscle; *IHJ*, inner half of jaw; *OHJ*, outer half of jaw; *P*, peduncle; *PJ*, posterior jaw; *PL*, proximal lamella; *PRJ*, proximal region of jaw.

position in the anterior jaw to a median proximal position, where it curves posteriorly (a_2) and articulates with the corresponding median bar f of the posterior jaw. For most of its length sclerite a is hollow, but, especially in its proximal region a_2, it may be U- or V-shaped in section, with the opening of the U directed away from the cavity of the adhesive organ, i.e. towards the peduncle bearing the adhesive organ. The anterior surface(s) of the anterior part a_1 of the median sclerite a often bears irregular nodules or spines. At the proximal, posterior end of a_2, the arms of the U are produced so that the end of the sclerite is Y- (or T-) shaped, with the outer arm (a_4) of the Y terminating blindly, but with the inner arm (a_3) forming a symphysis with the proximal end of the peripheral bar (c_3) of the anterior jaw.



Text-fig. 2. The arrangement of the sclerites of the adhesive organ of *Diclidophora*. (*a*) Isolated posterior jaw, seen posteriorly. (*b*) Isolated anterior jaw, seen posteriorly. a_1, a_2, a_3, a_4, b, c_1, c_2, c_3, d_1, d_2, d_3, f, g_1, g_2, i, k, sclerites (see text, pp. 69–71); F, thin flange from f; N, nodules.

From the inner surface of a_1 arises a lamellate extension b which along the greater part of its marginal regions is fused to the anterior region c_1 of the peripheral sclerite c. Thus, between them, sclerites a and b, together with c, form a scoop-like structure with thickened margins and which, proximally, forms a ring bounded by a_2, the proximal border of b, c_2, c_3 and a_3. This ring suspends a diaphragm which is itself an important component of the adhesive mechanism. An extrinsic muscle which operates the diaphragm approaches it from the peduncle at an angle of about 45° to the hinge axis of the adhesive organ, and the proximal border of b is correspondingly inclined to accommodate such an arrangement (Text-fig. 2*b*).

The outer half of the anterior jaw is supported by a single solid peripheral sclerite d ($=d_1d_2d_3$) which curves in three different planes: first it borders the outer margin of the anterior jaw (d_1), secondly it curves into the proximal region of the adhesive organ (d_2), and thirdly it curves again, obliquely, into

the plane of the posterior jaw (d_3) and ends blindly at some distance distal to the outer spur a_4 from a, i.e. there is no fusion between d and a.

On the anterior surface of the outer half of the anterior jaw (i.e. on the outside of the adhesive organ), in the region bounded by a_1 and d_1, there is present, in some species, a number of small sclerites, e.g. the 'teeth' of *Diclidophora denticulata* and the 'nodules' of *D. luscae*, but some species, e.g. *D. palmata*, are without corresponding structures.

The posterior jaw is supported by a median hollow rod f hinged to sclerite a of the anterior jaw, and by two pairs of solid peripheral sclerites, one pair i and k proximally with i in the inner half of the jaw and k in the outer, and one pair g_1 and g_2 distally with g_1 in the inner half of the jaw, and g_2 in the outer. The proximal ends of i and k are hinged on to the proximal arched portions c_2 and d_2 respectively of the peripheral sclerites of the anterior jaw, while the distal ends of sclerites g_1 and g_2 are immovably jointed to the distal end of the median sclerite f of the posterior jaw. In most species i and g_1 (and k and g_2) are contiguous, but in *D. denticulata* i is separated from g_1 (and k from g_2) by a considerable gap.

The inner walls of the clamp are lined with slender rib-like sclerites similar to those illustrated previously for *Plectanocotyle* (Llewellyn, 1956*a*, Pl. I, figs. 5, 6, 8).

The mode of action of the adhesive organs

Each posterior adhesive organ of *Diclidophora* is operated by two distinct but co-ordinated mechanisms. First there is a set of intrinsic muscles consisting of three adductors connecting i, f, and k of the posterior jaw with c_2, a_2 and d_2 respectively of the anterior jaw, and a median abductor, itself composed of two distinct muscle bundles, connecting f with a_2 (Text-fig. 1; Pl. I, figs. 3, 6). By use of these antagonistic intrinsic muscles the jaws may be opened and closed, and in living specimens of *D. merlangi*, forcibly detached from the host tissue, I have seen the peduncle repeatedly extended with the jaws opening, and then the jaws rapidly snapped together and the peduncle withdrawn.

The second component of the adhesive mechanism consists of a suctorial device comprising the hinged jaws with their enclosed cavity bordered by soft lips, and a fibrous diaphragm suspended in the ring formed by the proximal regions of sclerites $a_2bc_2c_3a_3$, and actuated by the powerful extrinsic muscle. The extrinsic muscles of the adhesive organs are arranged in the same manner as I have illustrated previously for *Plectanocotyle gurnardi* (Llewellyn, 1956*a*; Text-fig. 2). Other relatively small extrinsic muscles are associated with the adhesive organs, but these serve for orientating the organs, and play no part in the actual adhesive action.

The diaphragm consists of part of the fibrous wall of the adhesive organ, normally a structure consisting of fibres arranged perpendicularly between

inner and outer basement membranes, in which the extrinsic muscle has perforated the outer or proximal (with respect to the peduncle) basement membrane to become inserted on to the central region of the inner basement membrane (Pl. I, fig. 3). At this region of insertion there may be sclerotization, the hardened tissue taking the form of irregular small nodules, or of a single disc, or of a ring or a pair of semicircular arcs. The degree of sclerotization of the central region of the diaphragm varies, not only among the different species of *Diclidophora*, but in different individuals and even between different adhesive organs of the same individual. Cerfontaine stated that there were such sclerotizations in all individuals of all the five species of *Diclidophora* that he had examined, and while this may well be so, it is perhaps worth while pointing out that sometimes it is only in sections that they may be identified.

The mode of action of the adhesive organs is the same in all species of *Diclidophora*. First the jaws are opened by the abductor muscle, then they are applied to two or three secondary gill lamellae, and a preliminary grasp is taken by the closing of the jaws by the intrinsic adductor muscles. Next follows the second and more important adhesive action: the powerful extrinsic muscle contracts, the central part of the diaphragm is withdrawn into the peduncle, the marginal lips of the jaws act as valves to seal off the internal cavity of the adhesive organ, and a suction pressure is set up in the cavity of the adhesive organ. The two jaws being freely hinged (the abductor muscle being relaxed), this pressure is converted into a clamping action which is proportional to the pull of the extrinsic muscle. The change in internal volume occasioned by the withdrawal of the diaphragm would be small, but the sea water in the clamp cavity would act as the agent for the very efficient hydraulic transmission of a suctorial action into a clamping action.

Variations in the structure of the adhesive organs in the different species of Diclidophora

Variations in the sizes and shapes of whole organs and of their component parts are illustrated in Text-fig. 3.

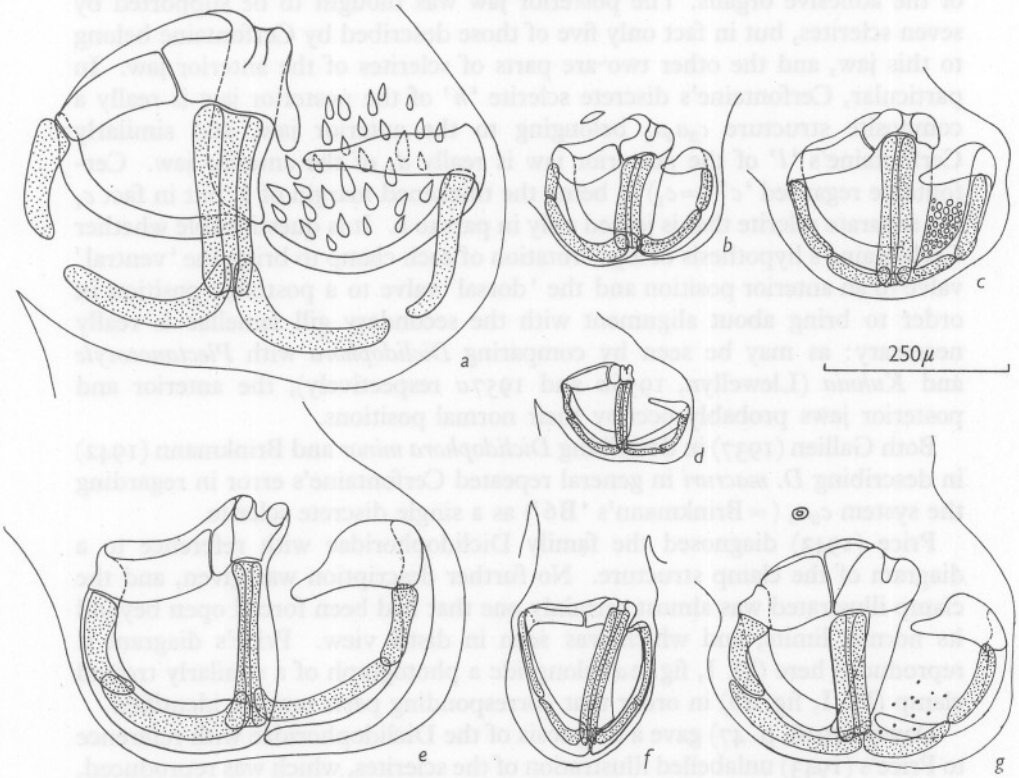
D. merlangi (Text-fig. 3*g*). About 5–15 minute tubercles are present on the distal region of the outer surface of the outer half of the anterior jaw. The diaphragm sclerotization consists of a ring or of a pair of semicircular arcs.

D. denticulata (Text-fig. 3*a*). About 25–35 conical teeth are present on the outer surface of the outer half of the anterior jaw. The proximal and distal peripheral sclerites of the posterior jaw are separated from each other by a considerable gap. The diaphragm sclerotization is feeble or absent.

D. luscae (Text-fig. 3*c*). About 50–100 well-developed tubercles are present on the outer surface of the outer half of the anterior jaw. The diaphragm sclerotization is feeble or absent.

D. macruri (Text-fig. 3*f*). The width of the adhesive organ (=length of the hinge axis) is less than the length of the median sclerite *f* of the posterior

jaw, i.e. the clamp is narrower than its own length, whereas the clamps of all other species are wider than their own length. This difference is accompanied by a reduction of the posterior proximal region d_3 of the outer peripheral sclerite of the anterior jaw, and a corresponding increase in the length of c_3 in the inner half of the jaw, i.e. a greater proportion of the proximal surface of each clamp is involved in the suctorial mechanism.



Text-fig. 3. Variations in size and shape of the sclerites of the adhesive organs of various species of *Diclidophora*, all drawn to the same scale. (*a*) *D. denticulata*, (*b*) *D. phycidis*, (*c*) *D. luscae* and *D. pollachii*, (*d*) *D. minor*, (*e*) *D. palmata*, (*f*) *D. macruri*, (*g*) *D. merlangi* and *D. gadi*.

D. minor (Text-fig. 3*d*). The sclerotization of the diaphragm is feeble or absent. No special features are present.

D. palmata (Text-fig. 3*e*). The sclerotization of the diaphragm is feeble or absent. No special features are present.

D. phycidis (Text-fig. 3*b*). The sclerotization of the diaphragm consists of a single well-developed disc.

D. pollachii (Text-fig. 3*c*). The adhesive organ resembles exactly that of *D. luscae*.

D. gadi (Text-fig. 3*g*). The adhesive organ resembles exactly that of *D. merlangi*.

Comparison with previous accounts of the adhesive organs of Diclidophora

Cerfontaine (1896, 1898) gave a generally accurate account of the adhesive mechanism of *Diclidophora*, but was mistaken in some aspects of the structure of the adhesive organs. The posterior jaw was thought to be supported by seven sclerites, but in fact only five of those described by Cerfontaine belong to this jaw, and the other two are parts of sclerites of the anterior jaw. In particular, Cerfontaine's discrete sclerite '*h*' of the posterior jaw is really a composite structure $c_3a_3a_4$ belonging to the anterior jaw, and similarly Cerfontaine's '*l*' of the posterior jaw is really d_3 of the anterior jaw. Cerfontaine regarded '*c*' ($=c_1$) as being the thickened margin of *b*, but in fact c_1 is a separate sclerite that is joined only in part to *b*. It is questionable whether Cerfontaine's hypothesis of a 90° rotation of each clamp to bring the 'ventral' valve to an anterior position and the 'dorsal' valve to a posterior position in order to bring about alignment with the secondary gill lamellae is really necessary: as may be seen by comparing *Diclidophora* with *Plectanocotyle* and *Kuhnia* (Llewellyn, 1956*a* and 1957*a* respectively), the anterior and posterior jaws probably occupy their normal positions.

Both Gallien (1937) in describing *Diclidophora minor* and Brinkmann (1942) in describing *D. macruri* in general repeated Cerfontaine's error in regarding the system c_3a_3 (=Brinkmann's 'B6') as a single discrete sclerite.

Price (1943) diagnosed the family Diclidophoridae with reference to a diagram of the clamp structure. No further description was given, and the clamp illustrated was almost certainly one that had been forced open beyond its normal limits, and which was seen in distal view. Price's diagram is reproduced here (Pl. I, fig. 2*a*) alongside a photograph of a similarly treated clamp (Pl. I, fig. 2*b*) in order that corresponding parts may be identified.

Dawes (1946, p. 47) gave a diagnosis of the Diclidophoridae with reference to Price's (1943) unlabelled illustration of the sclerites, which was reproduced, and qualified the diagram with a description of the arrangement of the sclerites. Later, Dawes (1947) repeated this description, and gave a translation of Cerfontaine's account of the adhesive organs of *D. denticulata* accompanied by a reproduction of Cerfontaine's diagrams, and also included original diagrams, but without descriptions, of the adhesive organs of *D. merlangi*, *D. palmata* and *D. denticulata*. According to Dawes's description of Price's diagram, there are eight sclerites in all:

(i) 'Four pieces bordering the opening of the sucker ventrally.'

According to my interpretation, Price's diagram shows two or at most three such sclerites, and in fact there are two (c_1 and d_1).

(ii) 'Two others bent at right angles, one part of each bordering the opening dorsally, the other being directed towards its centre.'

These I take to be g_1 and g_2 bordering the opening, with f directed towards the centre; such interpretation would impute a double structure to f when it is in fact a single hollow bar (as was illustrated in Dawes, 1947, fig. 18*c*, in a reproduction of Cerfontaine's figure) that appears double as seen in optical section as shown in Pl. I, fig. 2*b*, and would make no provision for i and k, which are shown clearly in Price's diagram.

(iii) 'Two others reinforcing the dorsal wall, one T-shaped, the other continuing the stem of the T above its limbs (thus †).'

In the 1947 publication (p. 94) the description of these last two sclerites (i.e. (iii) above) was altered somewhat, but still referred to them as occurring in the dorsal wall and forming a cruciform arrangement, with the stem of the T-piece having a lamellar extension and with its fellow supporting the concavity of the organ.

It seems to me that Dawes's 'T-shaped piece with lamellar extension' can only be the bar extending from the centre of Price's diagram directly to the bottom and then curving to the left of the figure, and equivalent to a_1a_2. However, both in Price's illustration and in fact sclerite a_1a_2 is in the 'ventral' (=anterior) valve. This interpretation presents a further difficulty in that the continuation of the stem above the limbs of the T would be equivalent to f, which, in my interpretation of Dawes's description, is already occupied by the right-angled centrally directed bends from g_1 and g_2.

It may be concluded then that there are some rather fundamental inconsistencies in Dawes's descriptions of the adhesive organs of *Diclidophora*.

In two accounts, that in certain respects were conflicting, Sproston (1945, p. 193; 1946, pp. 471 and 472) described the structure of the clamps of the Diclidophoridae and of the Diclidophorinae (which contains the single genus *Diclidophora*) in terms of what were thought to be the homologies of the various sclerites throughout the superfamily Diclidophoroidea. According to this scheme, the most generalized pattern of sclerites survived in the Mazocraeidae, and the diclidophorid pattern was relatively advanced. It has been shown elsewhere (Llewellyn, 1957*a*) that Sproston's interpretation of the clamp of the Mazocraeidae was inaccurate, and the present study shows that the descriptions of the clamp of *Diclidophora* are also erroneous. Among the chief mistakes are the following:

(i) The lateral walls of the single hollow median sclerite f of the posterior jaw were seen in optical section as two separate parallel bars that were then assumed to be derived from elongations of the 'middle loop' (=peripheral sclerites g_1 and g_2) so that the 'cuticularized tendon' or 'spring' (=median sclerites f and a_1a_2 in my description) were correspondingly reduced and were represented by an irregular ring in the middle of the 'anterior' (=proximal) edge of the capsule. In fact, f and a_1a_2 are the largest components of the sclerite system of *Diclidophora*, and far from being a 'spring', are articulated with each other in a hinge joint provided with adductor and abductor muscles

as had been described by Cerfontaine, and as are illustrated here in Pl. I, figs. 2*b*, 6.

(ii) No reference was made to the lamellate extension *b* from sclerite *a*.

(iii) More than half the main muscles were thought to be attached directly on to the inner moiety of the 'dorsal loop' ($=c_3a_3$). In fact the whole of the main muscle (= the extrinsic muscle) is attached to the centre of the diaphragm, which is itself in part suspended by c_3a_3.

Sproston thought the adhesive organs of the Diclidophoroidea (excepting the Choricotylinae and Hexostomatidae) functioned as clamps (pincers) in which certain curved portions of the clamp skeleton were under strain when the main retractor muscles contracted, and straightened out and opened the mouth of the clamp when these muscles relaxed. (A similar arrangement had been described by Goto (1895, p. 148) in a general account of the adhesive organs of various polyopisthocotylineans, but in this case to explain a suctorial and not a clamping action). The main muscle bundle in the Diclidophoridae was described by Sproston as being attached to the 'inner remnant of the dorsal loop', and though an incipient sucker was thought to be present in the Diclidophorinae, the whole organ was thought to maintain the usual function of a clamp. Since the 'spring' ($=af$) was thought to be reduced, and the 'sucker' to be only incipient, and since no explanation was offered as to how a muscle attached to the 'inner remnant of the dorsal loop' could operate the clamp, Sproston's accounts are without any feasible explanation of a clamping mechanism. In fact, the main muscle does not itself form an incipient sucker, but motivates a suctorial device which brings about a clamping action. Moreover, there is absolutely no evidence for a 'spring mechanism', since except for the limitations imposed by the muscular attachments, the posterior jaw of the clamp is freely hinged at its three regions of attachment to the anterior jaw.

Chauhan (1953), in a key to the families of the Diclidophoroidea, used Sproston's terms for diclidophoroidean sclerites, but accepted Price's characters in diagnosing the Diclidophoridae.

Hargis (1955), in the course of a review of the families of monogenean parasites from fishes of the Gulf of Mexico, stated that he agreed with Sproston's establishment of the homology of the clamp sclerites of the diclidophorid subfamilies Diclidophorinae and Choricotylinae with those of less modified clamps, and it is therefore unnecessary to repeat the comparisons already made above.

DISCUSSION

The present investigation of the adhesive organs of *Diclidophora* has confirmed Cerfontaine's conception of a hinged clamp operated by both intrinsic muscles and a suctorial device, and thus disagrees completely with Sproston's theory of a 'sprung' clamp operated by extrinsic muscles attached to the skeletal framework.

The comparative survey has shown that there is a basic arrangement of sclerites that is common to all species of *Diclidophora*, and this common pattern is compared in Table 2 with the pattern of sclerites in two diclidophoroideans that I have studied previously, viz. *Plectanocotyle gurnardi* and *Kuhnia scombri*. It is considered that no useful purpose would be served by speculating further upon the homologies of the various sclerites until representatives of other polyopisthocotylinean families have been examined in detail.

TABLE 2. THE CORRESPONDENCE OF SCLERITES IN THREE DICLIDOPHOROIDEAN GENERA

	Diclidophora (Present paper)	*Plectanocotyle* (Llewellyn, 1956*a*)	*Kuhnia* (Llewellyn, 1957*a*)
Peripheral sclerites			
Anterior jaw	$c_1c_2c_3$, $d_1d_2d_3$	$b_1b_2b_3$	*a*
Posterior jaw	ig_1g_2k	*cdc*	*e*
Median sclerites			
Anterior jaw	$a_1a_2a_3a_4$	*a*	*cd* (+*b*?)
Posterior jaw	*f*		

A comparison of the variations in the structure of the clamps in the different species of *Diclidophora* yields information that may be of taxonomic importance. The adhesive organ of *D. pollachii* is quite indistinguishable from that of *D. luscae*, and this observation has prompted an examination of the specific diagnoses of these two parasites. In a sample of sixteen specimens of *D. luscae* and of two specimens of *D. pollachii*, which I collected from hosts of which the identification was confirmed by an experienced student of fishes, I found the characters listed by Cerfontaine (1898) to be insufficient to separate the parasites, and my identification of the parasites rests solely upon host specificity. Under these circumstances it would be highly desirable (but very difficult) to carry out experiments on host specificity in *D. pollachii* and *D. luscae*.

The adhesive organ of *D. gadi* was found to resemble exactly that of *D. merlangi*, in size, shape, the presence of minute tubercles on the anterior jaw, and in the presence of a ring-shaped sclerotization of the diaphragm. In fact, excepting for Reichenbach-Klinke's (1951) diagnostic feature concerning the hook shape of the anterior polar filament of the egg capsule (actually it is the posterior, or ab-opercular polar filament which was illustrated as being hook-shaped), the two specimens of *D. gadi* I have examined are quite indistinguishable from *D. merlangi*. In the course of studies on larval monogeneans (Llewellyn, 1957*b*) I have observed that egg capsule formation may continue in moribund parasites, with the formation, often, of abnormally shaped capsules. It is possible, therefore, that '*Diclidophora gadi* Reichenbach-Klinke, 1951' is really *D. merlangi* from an unusual host, *Gadus aeglefinus*.

Finally, it is suggested that the development of tubercles or teeth on the anterior jaws of the adhesive organs is an indication of the closer affinity

between the species of *Diclidophora* parasitizing the host genus *Gadus* (*Diclidophora minor* on *Gadus poutassou* representing the survival of the ancestral condition without tubercles) than between these species and the diclidophoran parasites on the host genera *Molva*, *Urophycis* and *Macruris*.

It is a pleasure to acknowledge the great help given to me by the Director and Staff of the Plymouth Laboratory. I am also pleased to express my gratitude to Prof. A. Brinkmann of Bergen and to Prof. O. Nybelin of Gothenburg for the loan of whole-mount preparations and serial sections of *Diclidophora minor* and *D. macruri*, to Dr H. H. Reichenbach-Klinke of Brunswick for the loan of specimens of *D. gadi*, to Dr H. M. T. Frankland for the gift of specimens of *D. denticulata* from St Andrews, and to Mr J. E. Saunders and members of the Sixth Form at King Edward's School, Five Ways, Birmingham, for collecting fresh specimens of *D. palmata* from ling off the coast of Iceland.

SUMMARY

An investigation of the adhesive mechanisms in all nine species of the genus *Diclidophora* has shown that there is a common structure consisting of a pair of hinged jaws operated by intrinsic muscles and also by a more powerful extrinsic muscle which acts on a diaphragm to produce a suction pressure that is converted into a clamping action. This investigation has revealed errors in Cerfontaine's and other descriptions of the anatomy of *Diclidophora* adhesive organs, but substantiates Cerfontaine's account of the adhesive mechanism, and therefore disagrees completely with the more recent account given by Sproston.

REFERENCES

BRINKMANN, A., 1942. On some new and little known *Dactylocotyle* species, with a discussion on the relations between the genus *Dactylocotyle* and the 'Family' Diclidophoridae. *Göteborgs VetenskSam. Handl.*, Följ. VI, Ser. B, Bd. I, No. 13, 32 pp.

CERFONTAINE, P., 1896. Contribution à l'étude des Octocotylidés. I–III. *Arch. Biol., Paris*, T. 14, pp. 497–560.

—— 1898. Contribution à l'étude des Octocotylidés. IV. *Arch. Biol., Paris*, T. 15, pp. 301–28.

CHAUHAN, B. S., 1953. Studies on the trematode fauna of India. Part I. Subclass Monogenea. *Rec. Indian Mus.*, Vol. 51, pp. 113–208.

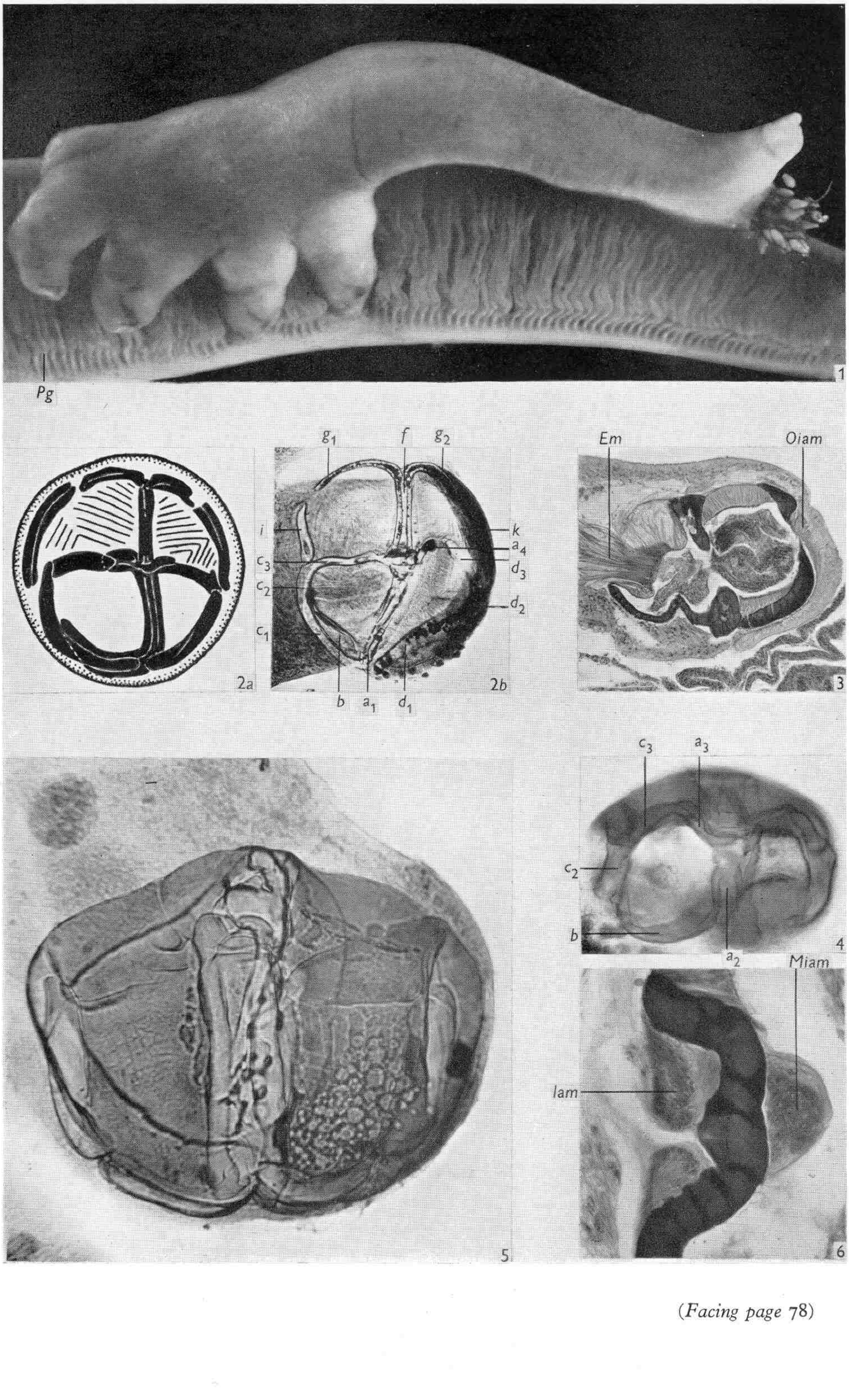
DAWES, B., 1946. *The Trematoda*. Cambridge University Press.

—— 1947. *The Trematoda of British Fishes*. London: The Ray Society.

FRANKLAND, H. M. T., 1955. The life history and bionomics of *Diclidophora denticulata* (Trematoda: Monogenea). *Parasitology*, Vol. 45, pp. 313–51.

GALLIEN, L., 1937. Recherches sur quelques trématodes monogénèses nouveaux ou peu connus. *Ann. Parasit. hum. comp.*, T. 15, pp. 146–54.

GOTO, S., 1895. Studies on the ectoparasitic trematodes of Japan. *J. Coll. Sci. Tokyo*, Vol. 8, pp. 1–273.



(*Facing page* 78)

HARGIS, W. J., 1955. Monogenetic trematodes of Gulf of Mexico fishes. Part IX. The family Diclidophoridae Fuhrmann, 1928. *Trans. Amer. micr. Soc.*, Vol. 74, pp. 377–88.

LLEWELLYN, J., 1956*a*. The adhesive mechanisms of monogenetic trematodes: the attachment of *Plectanocotyle gurnardi* (v. Ben. & Hesse) to the gills of *Trigla*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 507–14.

—— 1956*b*. The host-specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 113–27.

—— 1957*a*. The mechanism of the attachment of *Kuhnia scombri* (Kuhn, 1829) (Trematoda: Monogenea) to the gills of its host *Scomber scombrus* L., including a note on the taxonomy of the parasite. *Parasitology*, Vol. 47, pp. 30–9.

—— 1957*b*. The larvae of some monogenetic trematode parasites of Plymouth fishes. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 243–59.

PRICE, E. W., 1943. North American monogenetic trematodes: VI. The family Diclidophoridae (Diclidophoroidea). *J. Wash. Acad. Sci.*, Vol. 33, pp. 44–54.

REES, G. & LLEWELLYN, J., 1941. A record of the trematode and cestode parasites of fishes from the Porcupine Bank, Irish Atlantic Slope, and Irish Sea. *Parasitology*, Vol. 33, pp. 390–6.

REICHENBACH-KLINKE, H.-H., 1951. Eine neue art der trematodengattung *Dactylocotyle* am schellfisch (*Gadus aeglefinus* L.). *Z. Parasit.*, Bd. 15, pp. 4–7.

SPROSTON, N. G., 1945. A note on the comparative anatomy of the clamps in the superfamily Diclidophoroidea (Trematoda: Monogenea). *Parasitology*, Vol. 36, pp. 191–4.

—— 1946. A synopsis of the monogenetic trematodes. *Trans. zool. Soc. Lond.*, Vol. 25, pp. 185–600.

EXPLANATION OF PLATE I

The adhesive mechanism of *Diclidophora*

Fig. 1. *Diclidophora denticulata* attached to gills of *Gadus virens*.

Fig. 2. Clamp structure in the Diclidophoridae: fig. 2*a*, after Price (1943); fig. 2*b*, clamp of *D. denticulata* forced open and seen distally for comparison with Price's diagram.

Fig. 3. Vertical section of adhesive organ of *D. palmata* parallel to the longitudinal axis of the extrinsic muscle, and obliquely through the remainder of the clamp.

Fig. 4. Adhesive organ of *D. luscae* in proximal view to show the ring which supports the diaphragm.

Fig. 5. Adhesive organ of *D. luscae*, from a flattened specimen in which sclerites of both anterior and posterior valves are seen in transparency. For interpretation see Text-figs. 1–2.

Fig. 6. Transverse section of sclerite a_2 showing the median intrinsic abductor and adductor muscles.

Abbreviations: a_1, a_2, a_3, a_4, b, c_1, c_2, c_3, d_1, d_2, d_3, f, g_1, g_2, i, k, sclerites (see text, pp. 69–71); *Em*, extrinsic muscle; *Iam*, intrinsic abductor muscles; *Miam*, median intrinsic adductor muscle; *Oiam*, outer intrinsic adductor muscle; *Pg*, proximal end of gill.

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NOTES ON THE MEDUSA *AMPHINEMA KRAMPI* RUSSELL

By F. S. RUSSELL, F.R.S.
The Plymouth Laboratory

(Text-figs. 1–3)

In a recent paper (Russell, 1956) I described a new medusa *Amphinema krampi* from a single specimen. I have now found three more specimens in collections made with a 2 m stramin ring trawl by R.V. *Sarsia*. Two of these were caught on 13 June 1956 at 48° 29′ N., 9° 05′ W. with 450 fathoms of wire out; one was a female 7 mm high and the other a male about 5 or 6 mm in height. The third specimen, a male 6·5 mm high, was taken on 4 July 1956 at 47° 03′ N., 5° 47′ W. with 880 fathoms of wire out. Of the three specimens the female being the best preserved was kept intact; the two males were used for sectioning.

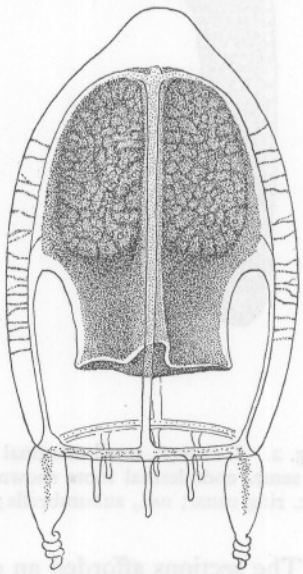

Fig. 1. *Amphinema krampi*, female, 7 mm high.

It is now possible to add to my previous description of the species. As in the specimen already described, the umbrellas are crumpled and have their margins turned inwards. In the female, however, it is certain that there is a small apical projection. The radial canals leave the stomach below the lower limits of the gonads so that there are fairly long 'mesenteries'. The marginal tentaculae are six in number in each specimen, two perradial and four interradial. It is possible therefore that the eight which I recorded in my first specimen may have been an abnormal number. The interradial female gonads are irregularly folded and much corrugated. All specimens have exactly the same coloration as that given in my previous description. A drawing of the female medusa is given in Fig. 1. The medusa bears a superficial resemblance to *Merga rubra* described by Kramp (1957, p. 14) from the Discovery Collections.

I was unable to see any discharged nematocysts so could not be certain of their type. Those on the marginal tentacles and small tentaculae are 7–9 μ

in length and appear to be micro-basic euryteles. The nematocysts along the margin of the mouth are longer, being about 13 μ in length, and may also be micro-basic euryteles.

The two males were sectioned, one longitudinally and the other transversely. Sections show that the marginal tentacles are hollow. The small marginal tentaculae are filled with endoderm cells, which are not arranged in a single row, and have endodermal roots which clasp the ring canal above and below (Fig. 2 *a–c*).

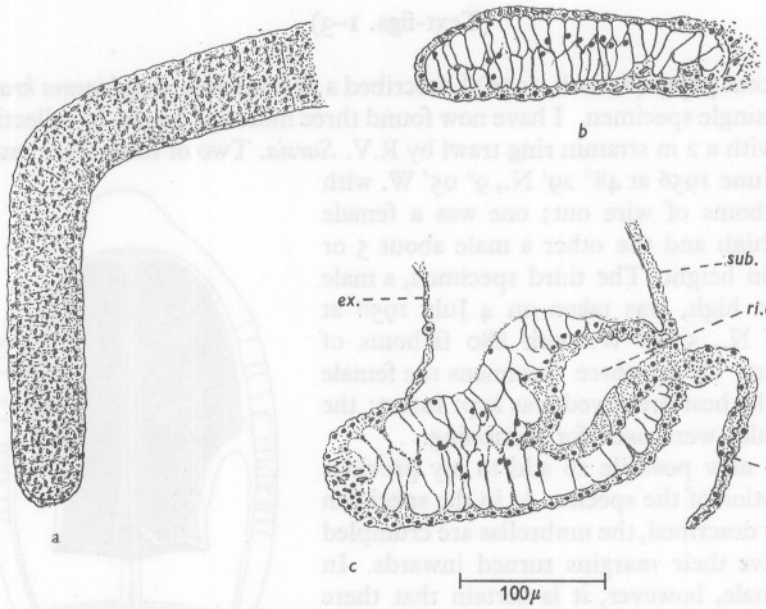

Fig. 2. *A. krampi*, small marginal tentacula. *a*, appearance of whole tentacula; *b*, *c*, sections of same, endodermal roots shown in *c*. The scale refers only to *b* and *c*. *ex*, exumbrella; *ri.c.* ring canal; *sub.*, subumbrella; *v.*, velum.

The sections afforded an opportunity to make a closer examination of the peculiar strands of tissue which run from the radial canals to the surface of the exumbrella. It should be emphasized that these medusae, which had been preserved in formalin and sea water in which they had remained for several months, were not in a good state for histological observations. Nevertheless, it was possible to observe a number of details.

There may be as many as seventeen strands in any one radius. They are distributed at intervals from about the level of the mouth well up beyond the middle of the stomach, where they occur along the lines of attachment of the stomach to the subumbrella. The strands may run through the mesogloea in rather a tortuous manner; some of them branch towards the exumbrellar

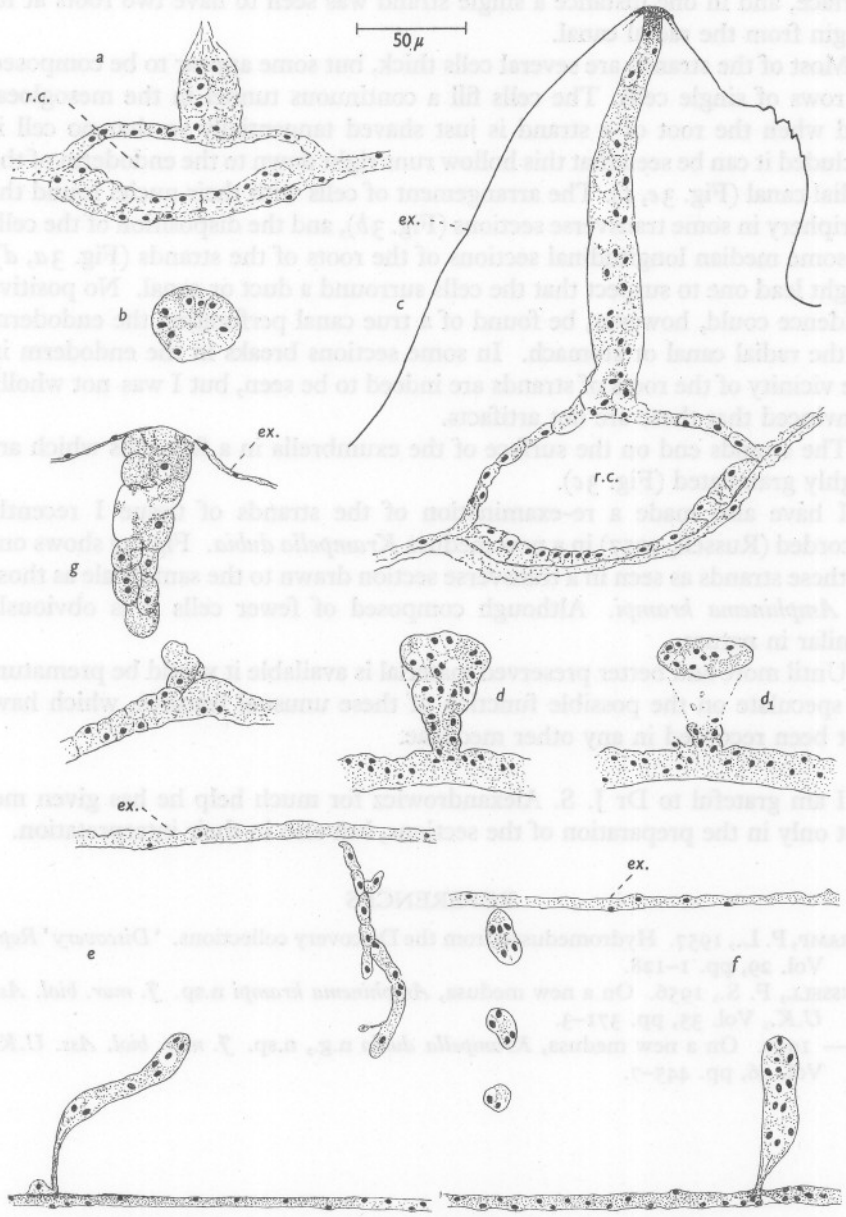

Fig. 3. Appearance of sections of cellular strands in mesogloea. *a–f*, *Amphinema krampi*; *g*, *Krampella dubia*. *a*, *b* and *c* are from transverse sections of medusae; *d*, *e* and *f* are from longitudinal sections; *d* and d_1 are consecutive sections. *ex.*, exumbrella; *r.c.*, radial canal. Camera lucida drawings, all to same scale as shown.

surface, and in one instance a single strand was seen to have two roots at its origin from the radial canal.

Most of the strands are several cells thick, but some appear to be composed of rows of single cells. The cells fill a continuous tunnel in the mesogloea, and when the root of a strand is just shaved tangentially so that no cell is included it can be seen that this hollow runs right down to the endoderm of the radial canal (Fig. 3*e*, *f*). The arrangement of cells with their nuclei round the periphery in some transverse sections (Fig. 3*b*), and the disposition of the cells in some median longitudinal sections of the roots of the strands (Fig. 3*a*, *d*), might lead one to suspect that the cells surround a duct or canal. No positive evidence could, however, be found of a true canal perforating the endoderm of the radial canal or stomach. In some sections breaks in the endoderm in the vicinity of the roots of strands are indeed to be seen, but I was not wholly convinced that these are not artifacts.

The strands end on the surface of the exumbrella in a few cells which are highly granulated (Fig. 3*c*).

I have also made a re-examination of the strands of tissue I recently recorded (Russell, 1957) in a new medusa *Krampella dubia*. Fig. 3*g* shows one of these strands as seen in a transverse section drawn to the same scale as those of *Amphinema krampi*. Although composed of fewer cells it is obviously similar in nature.

Until more and better preserved material is available it would be premature to speculate on the possible function of these unusual features, which have not been recorded in any other medusae.

I am grateful to Dr J. S. Alexandrowicz for much help he has given me, not only in the preparation of the sections, but also in their interpretation.

REFERENCES

KRAMP, P. L., 1957. Hydromedusae from the Discovery collections. '*Discovery*' *Rep.*, Vol. 29, pp. 1–128.

RUSSELL, F. S., 1956. On a new medusa, *Amphinema krampi* n.sp. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 371–3.

—— 1957. On a new medusa, *Krampella dubia* n.g., n.sp. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 445–7.

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THE MODES OF ACTION OF TOXIC AGENTS
III. MERCURIC CHLORIDE AND *N*-AMYLMERCURIC CHLORIDE ON CRUSTACEANS

By E. D. S. CORNER
International Paints Research Fellow, the Plymouth Laboratory,
and F. H. RIGLER
Zoology Dept., University of Toronto

(Text-figs. 1–3)

In a recent study (Corner & Sparrow, 1957) it was found that differences between the toxicities of various mercury compounds to certain Crustacea were related to the resistances of the test animals to mercury poisoning, the differences being greater when a more resistant species was used. In addition, the toxicities of these poisons to a highly resistant animal were found to be closely related to the corresponding lipoid solubilities. These results were consistent with the view that Hg poisons act by penetrating the test animals, the enhanced toxicity of a highly lipoid-soluble poison such as *n*-amylmercuric chloride being the result of this compound's ability to penetrate rapidly. In the present work, tracer isotopes have been used in experiments with larvae of the brine shrimp *Artemia salina* (L.), larvae of the barnacle *Elminius modestus* Darwin and adults of the prawn *Leander serratus* (Pennant), in order to investigate further the modes of action of mercuric chloride ($HgCl_2$) and *n*-amylmercuric chloride (n-$C_5H_{11}HgCl$) as poisons to crustaceans. The results have provided evidence more direct than that obtained previously in favour of the view that penetration of the test animal is an important factor in mercury poisoning.

GENERAL METHODS

^{203}Hg-*labelled mercuric chloride*. Radioactive mercuric oxide was obtained from A.E.R.E., Harwell, and a stock solution containing 10 mg Hg/ml. was prepared by dissolving 1 g of this material in 93 ml. 50% HCl. Appropriate quantities of this solution were then added to sea water and the pH values of the solutions were adjusted to 8·1 with 2N-NaOH. To estimate the amounts of radioactivity in these sea-water solutions, samples (0·05–0·10 ml.) were first mixed with an excess of sodium sulphide to prevent volatilization of the Hg, and then evaporated to dryness on planchettes.

^{203}Hg-*labelled* n-*amylmercuric chloride*. A pure sample of this material (m.p. 121–122° C) was obtained from the Radiochemical Centre, Amersham, and a stock solution containing 0·5 mg Hg/ml. was prepared in absolute ethanol. Measured amounts of this solution were added to sea water and estimates of the radioactivity in these sea-water solutions were made on samples (0·05–0·10 ml.) slowly evaporated to dryness after treatment with an excess of reduced glutathione to prevent volatilization of the Hg compound.

Animals. For experiments in which *Artemia* and *Elminius* larvae were used the animals were obtained by methods described earlier (Corner & Sparrow, 1956). Studies were also made with the prawn *Leander serratus*, and the specimens used in these experiments were from a stock maintained at the Plymouth Laboratory.

EXPERIMENTS

Uptakes of mercuric chloride and *N*-amylmercuric chloride by *Elminius* and *Artemia*

All the experiments were carried out at 18° C. Solutions of the poisons in sea water were prepared immediately before the experiments were started because an earlier study (Corner & Rigler, 1957) had shown that significant amounts of Hg are lost from sea-water solutions of mercuric chloride on standing. *Elminius* larvae (200–300 animals) were added to samples (50 ml.) of sea water containing 5·0, 1·0 and 0·2 mg Hg/l. as ^{203}Hg-labelled $HgCl_2$, and a second series of samples containing 0·25, 0·05 and 0·01 mg Hg/l. as ^{203}Hg-labelled n-$C_5H_{11}HgCl$. At suitable time intervals the animals were removed from the solutions by filtration on a disc of bolting silk (200 m.p.i.), suspended in plain sea water (5 ml.) and removed from the washing medium by filtration on a new disc of bolting silk. The animals were then dried by gentle suction, the sample, together with the silk, was transferred to a planchette, and its radioactivity was estimated. Control experiments showed that the bolting silk used in the first filtration was always contaminated with mercury and that this contamination was considerable on silks used to collect animals during the later stages of the experiments. It had to be taken into account in calculations. However, it was found that if the animals were washed quickly with sea water immediately after they had been removed from the toxic media they did not lose any of the Hg which they had taken up, and the disc of bolting silk used for the second filtration bore no trace of radioactivity. Accordingly, this washing procedure was used in all the experiments. After the animals in each sample had been examined for radioactivity, they were transferred to acidified sea water containing a trace of sodium azide and, as soon as all were motionless, they were counted and the quantity of Hg which they had taken up was determined as μg Hg/animal. At the end of the experiment, which was usually continued until approximately 75% of the test animals had died, the animals from all the samples were collected on a

previously weighed disc of bolting silk and were dried to constant weight at 105° C. The dry weight of the animals so determined was then used to calculate the amount of Hg taken up as mg Hg/g dry wt. of larvae.

Uptake experiments were also carried out with *Artemia* larvae immersed in sea-water solutions containing 1 g and 0·5 g Hg/l., as $HgCl_2$ (10% of the mercury as ^{203}Hg) and 1·0 and 0·5 mg Hg/l. as ^{203}Hg-labelled *n*-$C_5H_{11}HgCl$. The method used in these experiments with *Artemia* was identical with that just described, except that after they had been removed from the toxic media the animals were washed twice with sea water because it was found that a single washing (as used in the procedure with *Elminius*) did not remove all traces of radioactivity from the bolting silk.

Reduced glutathione

Corner & Sparrow (1957) have shown that certain thiol compounds greatly reduce the toxicities of mercury poisons to *Elminius* and *Artemia*, and have reported that very marked effects are observed when reduced glutathione is used to protect *Elminius* from poisoning by $HgCl_2$. These workers have also demonstrated that reduced glutathione lowers the death rate of *Elminius* returned to plain sea water after a preliminary immersion in either $HgCl_2$ or *n*-$C_5H_{11}HgCl$.

Protection experiments. In the present work, by means of the methods described earlier, the rates of uptake of Hg by *Elminius* from sea water containing ^{203}Hg-labelled $HgCl_2$ (1 mg Hg/l.) and *n*-$C_5H_{11}HgCl$ (0·05 mg Hg/l.) were compared with those from similar solutions to which a tenfold excess of reduced glutathione had been added.

Recovery experiments. A study was made of the effect of reduced glutathione on the rates at which mercury was lost from poisoned *Elminius* and *Artemia* when the animals were returned to plain sea water. The procedure used was as follows. A large number of *Elminius* larvae were immersed for 20 min in sea water containing 1 mg Hg/l., and similarly *Artemia* larvae in sea water with 1 g Hg/l. Representative samples were then removed and examined immediately for radioactivity in the usual way. Simultaneously, a second series of samples were transferred to plain sea water and a third series to sea water containing reduced glutathione (10 mg/l.). The radioactivity of these animals was then estimated after they had been immersed in the two media for 1 and 5 h (*Elminius*) and 2 h (*Artemia*). Attempts were also made to repeat these experiments using ^{203}Hg-labelled *n*-$C_5H_{11}HgCl$, but these were unsuccessful because the mercury compound was insufficiently radioactive.

Experiments with *Leander serratus*

Studies were made with prawns to determine the distribution of radioactivity on and inside the test animals after they had been poisoned with ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$.

Preliminary experiments showed that prawns immersed in sea water containing 50 mg Hg/l. as $HgCl_2$ and 1 mg Hg/l. as n-$C_5H_{11}HgCl$ died in approximately the same time (3 h). The animals were, therefore, immersed in the equitoxic media for 2 h, after which time they were washed with plain sea water and various tissues, both internal (e.g. the hepatopancreas) and external (e.g. body chitin) were removed by dissection and examined for radioactivity. With chitin, pieces (*ca.* 1 cm^2) were cut from various parts of the body, dried and immediately placed under the Geiger counter. Soft body tissues, however, were first worked into a paste with ethanol and small quantities of the mixture (*ca.* 2 mg) were smeared on planchettes. The samples were then dried by slow heating and their radioactivity was estimated. As fairly large amounts of tissue were used and as ^{203}Hg is a weak emitter of β radiation it is probable that these determinations of radioactivity gave low results because of errors introduced by self-absorption. However, as exactly the same procedure was used to examine animals which had been treated either with $HgCl_2$ or with n-$C_5H_{11}HgCl$, an adequate comparison of the distributions of the two poisons was possible. After the radioactivity had been estimated the samples were dried to constant weight at 105° C and the mercury content was expressed as mg Hg/g dry wt. of tissue.

Because these experiments showed high concentrations of Hg on the gills and on the tissue under the branchiostegites of prawns poisoned with the two Hg compounds, tests were made to show whether the poison was merely attached to the surfaces of these tissues or had penetrated into them. In these experiments prawns were immersed for 2 h in sea water containing 50 mg ^{203}Hg/l. as $HgCl_2$. The animals were then removed from the solution, quickly washed with plain sea water and dissected, and the gills and branchiostegites of one of them were then examined at once for radioactivity. Gills and branchiostegites taken from one side of each of the remaining animals were immersed for 30 min in *Homarus* Ringer solution (Cole, 1941), and those dissected from the other side were immersed for the same time in *Homarus* Ringer solution containing 100 mg reduced glutathione/l. After this washing, the tissues were examined for radioactivity in the usual way.

Further experiments were carried out with prawns to show whether injecting the animals with a small quantity of a mercury poison led to a distribution of radioactivity throughout the tissues similar to that observed after the animals had been immersed in a toxic solution of the same poison. ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$ were used in these experiments, and care was taken to see that the animals received the same dose of each com-

pound. The fact that the n-$C_5H_{11}HgCl$ had to be injected in ethanolic solution restricted the size of the dose which could be used, for in control experiments it was found that the test animals (approximately 10 g body wt.) showed signs of distress when injected with more than 0·02 ml. of ethanol. Accordingly, the quantity of each Hg compound injected was equivalent to 10 μg Hg. The needle was inserted laterally between the second and third abdominal segments and the solution was injected dorsally into the lacuna at the first segment. Immediately after the injection had been made the animals were placed in fresh sea water from which specimens were then removed at suitable intervals for dissection followed by examination of various tissues for radioactivity in the usual way.

RESULTS

Experiments with *Elminius* and *Artemia*

Corner & Sparrow (1957) have shown that n-$C_5H_{11}HgCl$ is 20 times as toxic as $HgCl_2$ to *Elminius* and 1000 times as toxic as this compound to *Artemia*. Interest therefore attaches to our experiments, which have shown that the rates at which *Elminius* removes mercury from sea water containing various concentrations of n-$C_5H_{11}HgCl$ are roughly the same as those at which the

TABLE 1. QUANTITIES OF MERCURY AS $HgCl_2$ AND AS n-$C_5H_{11}HgCl$ TAKEN UP BY *ELMINIUS* AND *ARTEMIA* FROM EQUITOXIC CONCENTRATIONS OF THE POISONS IN SEA WATER

(Concentrations of poisons gave TD_{50} of approximately 3 h with each species.)

	$HgCl_2$		n-$C_5H_{11}HgCl$	
Animal	Concn. in toxic medium (mg Hg/l)	Concn. taken up by animals (mg Hg/g dry wt.)	Concn. in toxic medium (mg Hg/l)	Concn. taken up by animals (mg Hg/g dry wt.)
Elminius	0·2	0·92	0·01	0·70
Artemia	1000	0·47	1·0	0·28

TABLE 2. INFLUENCE OF REDUCED GLUTATHIONE ON THE LOSS OF MERCURY BY *ELMINIUS* AND *ARTEMIA* POISONED WITH $HgCl_2$

(*Elminius* immersed in sea-water solution of $HgCl_2$ (1 mg Hg/l.) for 20 min. *Artemia* immersed in sea-water solution of $HgCl_2$ (1 g Hg/l.) for 90 min. GSH = reduced glutathione (10 mg/l.).)

	Washing medium			
	Sea water. Concn. of Hg in animals		GSH in sea water. Concn. of Hg in animals	
Time (h)	mg Hg/g dry wt.	%	mg Hg/g dry wt.	%
	Elminius			
0	0·54	100	0·54	100
1	0·35	65	0·35	65
5	0·16	29	0·18	33
	Artemia			
0	0·52	100	0·52	100
2	0·33	63	0·27	52

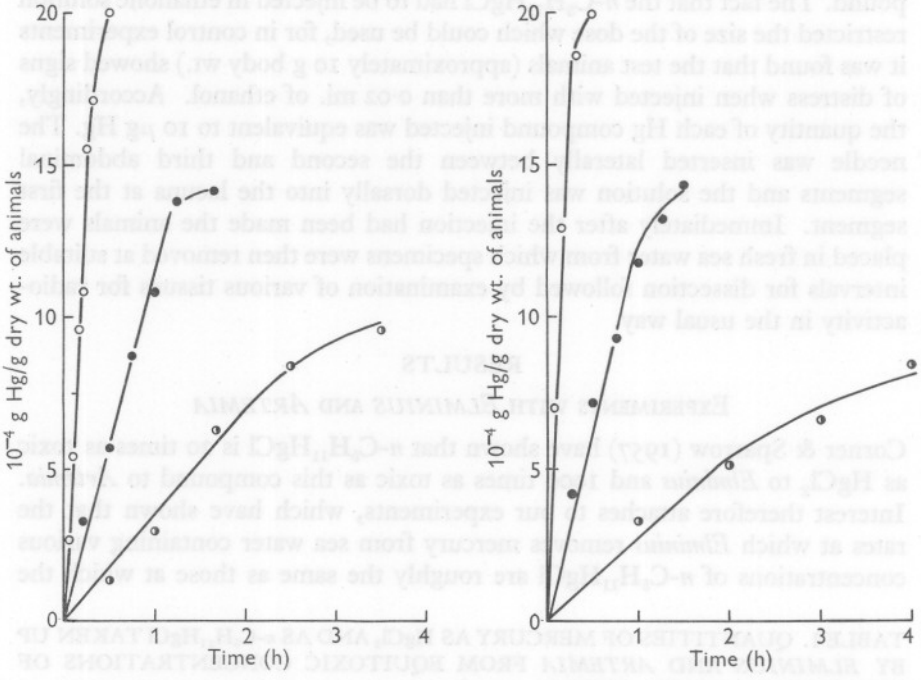

Fig. 1. Left: uptake of mercury as mercuric chloride by *Elminius* larvae. ○—○, 5 mg Hg/l. (TD_{50} = 0·55 h); ●—●, 1 mg Hg/l. (TD_{50} = 1·1 h); ◑—◑, 0·2 mg Hg/l. (TD_{50} = 2·9 h). Right: uptake of mercury as *n*-amylmercuric chloride. ○—○, 0·25 mg Hg/l. (TD_{50} = 0·6 h); ●—●, 0·05 mg Hg/l. (TD_{50} = 0·9 h); ◑—◑, 0·01 mg Hg/l. (TD_{50} = 3·0 h).

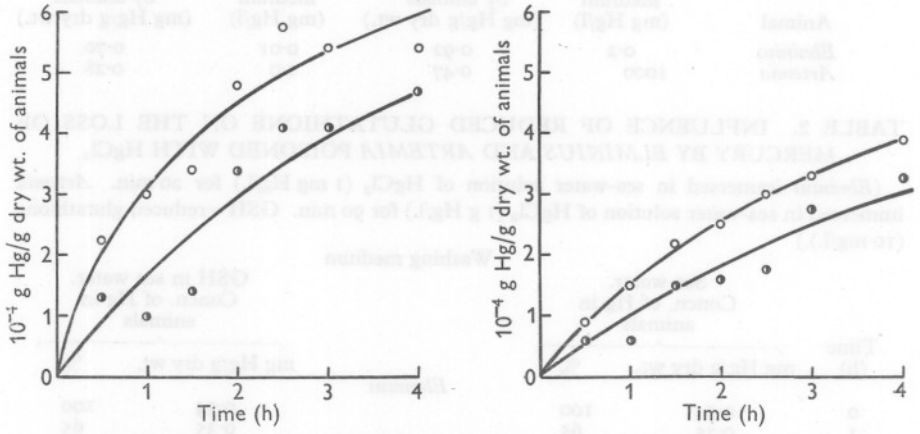

Fig. 2. Left: uptake of mercury as mercuric chloride by *Artemia* larvae. ○—○, 1 g Hg/l. (TD_{50} = 2·3 h); ◑—◑, 0·5 g Hg/l. (TD_{50} = 3·6 h). Right: uptake of mercury as *n*-$C_5H_{11}HgCl$. ○—○, 1 mg Hg/l. (TD_{50} = 2·5 h); ◑—◑, 0·5 mg Hg/l. (TD_{50} = 3·5 h).

animal takes up Hg from sea water containing 20 times each concentration as $HgCl_2$ (Fig. 1). Similar experiments with *Artemia* (see Fig. 2) have shown that the rates at which this animal concentrates mercury as n-$C_5H_{11}HgCl$ are approximately the same as those at which it takes up the poison from sea water containing 500 times each concentration as $HgCl_2$. The correlation between toxicity and uptake data is, therefore, less exact for *Artemia*. Nevertheless, it may be concluded that differences between the toxicities of the two poisons reflect to a considerable extent differences between the rates at which the compounds are taken up by both species of test animal.

The results shown in Table 1 draw attention to the vast difference between the concentrations of each Hg compound which must be added to the surrounding medium in order to kill *Artemia* and *Elminius* at the same rate.

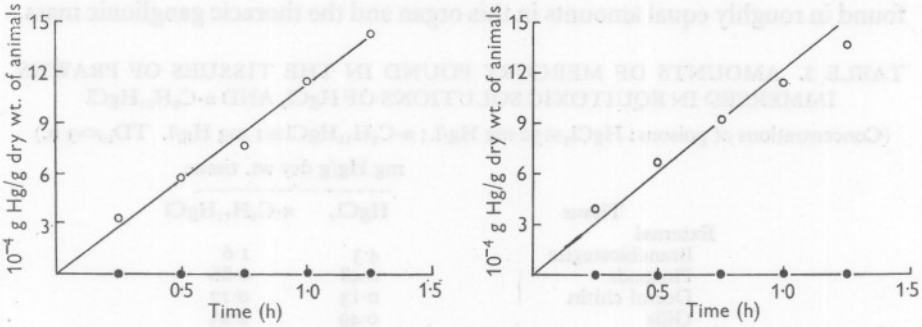

Fig. 3. Left: uptake by *Elminius* larvae of mercuric chloride (1 mg Hg/l.), alone (○—○) and in the presence of a tenfold excess of reduced glutathione (●—●). Right: uptake of n-$C_5H_{11}HgCl$ (0·05 mg Hg/l.) alone (○—○) and in the presence of a tenfold excess of reduced glutathione (●—●).

Thus, the concentrations of $HgCl_2$ and n-$C_5H_{11}HgCl$ which are equitoxic to *Artemia* and *Elminius* are in the ratio 5000:1 and 100:1 respectively. Of greater interest, however, is the finding that although much higher external concentrations are needed to kill *Artemia*, the actual quantities of mercury which these animals accumulate by the time 50% have died are significantly less than the corresponding amounts taken up by *Elminius*.

The results of experiments in which reduced glutathione was used to protect *Elminius* from the toxic effects of $HgCl_2$ and n-$C_5H_{11}HgCl$ (see Fig. 3) showed that the addition of a tenfold excess of the thiol compound to the toxic media completely prevented any uptake of mercury by the test animals during the time in which more than 75% of the unprotected animals died (3 h). When further experiments were carried out to examine the influence of reduced glutathione on the rates of loss of mercury by *Elminius* and *Artemia* previously poisoned with $HgCl_2$ and n-$C_5H_{11}HgCl$ it was found that the thiol compound had little or no effect on these very slow processes (see Table 2).

Experiments with *Leander*

Findings made in experiments with prawns were of considerable interest because they demonstrated that animals killed by immersion in toxic solutions of $HgCl_2$ and n-$C_5H_{11}HgCl$ contained significant amounts of Hg in several internal organs (see Table 3). Moreover, after the animals had been killed by immersion for 2 h in equitoxic solutions of the two poisons the patterns of distribution of the Hg throughout the animals were markedly different. Thus, when tissues in contact with the surrounding medium were examined it was found that whereas $HgCl_2$ concentrated in the tissue beneath the branchiostegites, n-$C_5H_{11}HgCl$ was detected primarily in the gills. The distributions of the poisons inside the test animals also differed in that whereas $HgCl_2$ concentrated almost exclusively in the antennary gland, n-$C_5H_{11}HgCl$ was found in roughly equal amounts in this organ and the thoracic ganglionic mass.

TABLE 3. AMOUNTS OF MERCURY FOUND IN THE TISSUES OF PRAWNS IMMERSED IN EQUITOXIC SOLUTIONS OF $HgCl_2$ AND n-$C_5H_{11}HgCl$

(Concentrations of poisons: $HgCl_2 \equiv 50$ mg Hg/l.; n-$C_5H_{11}HgCl \equiv 1$ mg Hg/l. $TD_{50} \simeq 3$ h.)

	mg Hg/g dry wt. tissue	
Tissue	$HgCl_2$	n-$C_5H_{11}HgCl$
External		
Branchiostegite	4·3	1·6
Pleopods	0·48	0·86
Dorsal chitin	0·13	0·17
Gills	0·49	2·23
Internal		
Antennary gland	0·32	0·46
Hepatopancreas	0·02	0·05
Central nervous system	0·04	0·38
Muscle	0·00	0·04

By contrast, it was found that after the animals had been injected with sublethal doses of $HgCl_2$ and n-$C_5H_{11}HgCl$ the patterns of distribution of the two poisons were similar in that both compounds were found to concentrate in the gills and antennary glands of the test animals (see Table 4).

Further experiments using reduced glutathione gave results consistent with those obtained in similar studies with *Elminius* and *Artemia*. Thus, no significant amount of mercury was removed when the gills of prawns previously treated with mercuric chloride were washed either with *Homarus* Ringer alone or fortified with reduced glutathione. In addition, the branchiostegites of these animals were found to lose less than half their Hg content when washed with either medium (see Table 5). Because these tissues are in continuous contact with the surrounding medium and were found to accumulate far more Hg than any other tissue, it seemed reasonable to expect that if Hg acted by simply becoming attached to the surfaces of the test animals these would be the most likely sites of attachment. However, it appeared

from these findings that all the mercury accumulated by the gills and most of that taken up by the tissue under the branchiostegite was not attached to the surfaces of these tissues, but had penetrated into them.

TABLE 4. AMOUNTS OF MERCURY DETECTED IN THE TISSUES OF PRAWNS INJECTED WITH MERCURIC CHLORIDE AND *n*-AMYLMERCURIC CHLORIDE

(Concentrations used: 10 μg Hg/l animal injected in 0·01 ml. sea water ($HgCl_2$) and 0·02 ml. ethanol (n-$C_5H_{11}HgCl$).)

Tissue	μg Hg/g dry wt. tissue	
	$HgCl_2$	n-$C_5H_{11}HgCl$
External		
Branchiostegite	13·0	4·3
Pleopods	2·2	1·0
Dorsal chitin	3·4	0·2
Gills	29·3	22·0
Internal		
Antennary gland	13·3	6·7
Hepatopancreas	4·4	4·2
Central nervous system	3·5	3·0
Muscle	2·7	0·0

TABLE 5. THE REMOVAL OF MERCURIC CHLORIDE FROM THE GILLS AND BRANCHIOSTEGITES OF PRAWNS POISONED WITH THIS COMPOUND

Washing medium	Counts/min/mg dry wt. of tissue	
	Gills	Branchiostegite
None	100	1795
Homarus Ringer	118	1160
Homarus Ringer (plus 100 mg reduced glutathione/l.)	102	1150

DISCUSSION

In earlier studies, Corner & Sparrow (1956, 1957) showed that great differences are found between the susceptibilities of *Artemia* and *Elminius* to mercury poisons; that differences between the toxicities of certain organomercury poisons and that of mercuric chloride are far greater when a highly resistant test animal like *Artemia* is used; that, in general, poisons which are very toxic are also highly lipoid-soluble; and that no correlation exists between the toxicities of these compounds and their abilities to inactivate enzymes. Corner & Sparrow considered these observations to be consistent with the view that Hg poisons act by penetrating the test animal and assumed that the extreme resistance of *Artemia* was a direct result of this animal's impermeability. Their findings did not, however, exclude the possibility that the large differences between the susceptibilities of *Artemia* and *Elminius* to Hg poisons were not because *Artemia* accumulated the compounds at a much slower rate, but because this animal was able to tolerate a much higher concentration of mercury in its tissues. This possibility has therefore been examined in the present work, and it has been found that both $HgCl_2$ and n-$C_5H_{11}HgCl$ are, in

fact, accumulated much more rapidly by *Elminius* than by *Artemia*. Moreover, the amount of Hg, as either compound, taken up by *Artemia* at a time when 50% of the test animals die, is significantly less than the corresponding amount accumulated by *Elminius*. It therefore seems clear that *Artemia* is very resistant to Hg compounds, not because the tissues of this animal are able to withstand large concentrations of mercury, but because this animal accumulates the poisons at a very slow rate.

Further results of the present work were that both *Artemia* and *Elminius* take up *n*-$C_5H_{11}HgCl$, which is very toxic and very lipoid-soluble, at a rate much faster than that at which they accumulate $HgCl_2$; and that the relative rates of uptake of the two poisons are related to their relative toxicities. However, although these findings emphasized that high toxicity attends a high rate of accumulation by the test animal, they did not demonstrate how this accumulation took place, and the possibility could not be ignored that the organomercury compound might be taken up more rapidly than $HgCl_2$ because of preferential adsorption on the animal's surface. It is true that Corner & Sparrow (1957) have provided evidence in favour of the view that as far as interaction with proteins present on the surface of the test animal is concerned, *n*-$C_5H_{11}HgCl$ is likely to be less effective than $HgCl_2$, but in the present work it was thought desirable to seek more direct evidence that penetration of the test animal took place. Accordingly, semi-quantitative studies were made with *Leander*, an animal large enough to permit excision of various internal organs which could be examined for their Hg content. In these experiments it has been found that when the animals are immersed in equitoxic solutions of the two poisons, significant amounts of Hg can, in fact, be detected in their internal tissues. Moreover, these amounts are slightly greater for *n*-$C_5H_{11}HgCl$, although the concentration of this poison used in the external medium is equivalent to only one-fiftieth of the quantity of Hg used as $HgCl_2$. These experiments, therefore, provide direct evidence that Hg compounds can penetrate a crustacean and that an organomercury poison enters the test animal at a rate much faster than that of $HgCl_2$. The poisons have also been found in considerable quantity on external sites such as the gills and branchiostegites, on which they might be in a position to exert at least part of their toxic action. Further experiments, however, have provided evidence in favour of the view that attachment to the surface is not of primary importance for the toxic action of either poison. Thus, if the compounds act simply by becoming attached to these surfaces it seems likely that their toxic effects would be readily reversed by substances such as reduced glutathione, because the marked affinities of thiol compounds for heavy metals (cf. Gurd, 1954) have often been exploited as a means of removing these poisons from biological surfaces. During toxicity studies with *Elminius* and *Artemia*, Corner & Sparrow (1957) found that thiol compounds effectively protect the test animals against mercury poisons, and in the present work it has been

found that reduced glutathione can prevent *Elminius* from accumulating mercury either as $HgCl_2$ or as n-$C_5H_{11}HgCl$. These results support the view that the affinity of the thiol compound is greater than that of the surface of the test animal for either poison. Because of this it might be expected that reduced glutathione would readily remove these poisons from the surface of *Elminius*, and some evidence of this was obtained from further experiments by Corner & Sparrow in which it was found that reduced glutathione lowers the death rates of *Elminius* transferred to plain sea water after preliminary treatments with mercury poisons. However, these experiments lasted a long time and it is possible that the thiol compound did not remove the poisons from the surfaces of the test animals but penetrated into them and inactivated a proportion of the mercury poison after it had reached the tissues. The results of shorter experiments carried out in the present work have shown that reduced glutathione has little or no influence on the very slow rates of loss of mercury by either *Elminius* or *Artemia* previously poisoned with mercuric chloride, nor does it influence the loss of mercury from the surface tissues (e.g. gills and branchiostegites) of prawns poisoned with this compound. These findings therefore lend support to the view that the Hg compound was not simply attached to the surfaces of the test animals, but penetrated into them.

Examination of the patterns of distribution of n-$C_5H_{11}HgCl$ and $HgCl_2$ injected into prawns has given further information concerning the origin of the mercury detected on the gills and branchiostegites, for it has been found that these tissues also contain large quantities of Hg after the animals have been injected with either poison. This finding suggests that the Hg detected on these surfaces after the animals have been immersed in toxic solutions of the poisons might not arise exclusively as the result of direct accumulation from the surrounding medium, but might to some extent represent an attempt by the animal to excrete through these tissues some of the assimilated Hg. On this point it is interesting that Hg poisons have been found to concentrate in the antennary gland of the prawn after the animals are immersed in toxic solutions, and after they have been given a sublethal dose of each compound by injection, for the decapod antennary gland is known to be associated with excretory mechanisms. A further point of interest is that $HgCl_2$ has been found to affect osmoregulation in *Daphnia magna* (Holm-Jensen, 1948).

There are obvious dangers in attempting to base a theory of Hg poisoning on the results of studies made with several different test species, but from the findings just discussed it appears that penetration of the animal and subsequent interference with excretory mechanisms is a useful working hypothesis on which to base future studies of the modes of action of Hg compounds as poisons to crustaceans.

During this work one of us (F. H. R.) was supported by a Research Fellowship from the National Research Council of Canada. The other is indebted to International Paints Ltd. for a Research Fellowship, and both gratefully acknowledge the assistance of this firm in the purchase of radioactive materials. The research facilities provided by the Plymouth Laboratory of the Marine Biological Association are also gratefully acknowledged.

SUMMARY

Experiments have been carried out with ^{203}Hg-labelled *n*-amylmercuric chloride (n-$C_5H_{11}HgCl$) and mercuric chloride ($HgCl_2$) to study the modes of action of these compounds as poisons to some crustaceans.

Differences between the susceptibilities of *Artemia salina* and *Elminius modestus* to the poisons do not reflect differences between the quantities of these compounds which the animals can tolerate in their tissues, but are directly related to the rates at which the poisons are accumulated. Thus, experiments with either species have shown that Hg is taken up at approximately the same rate from equitoxic solutions of the two poisons; and the rates at which Hg is accumulated by the two species from equitoxic solutions of either poison are of the same order.

Experiments with reduced glutathione have given results consistent with the view that most of the Hg taken up by either species penetrates into the tissues of the test animals and does not act simply by becoming attached to their surfaces.

Direct evidence of the penetration of Hg compounds into a crustacean has been obtained from experiments with *Leander serratus*. Considerable amounts of Hg have been detected in the antennary glands.

REFERENCES

COLE, W. H., 1941. A perfusing solution for the lobster (*Homarus*) heart and the effects of its constituent ions on the heart. *J. gen. Physiol.*, Vol. 25, pp. 1–6.

CORNER, E. D. S. & SPARROW, B. W., 1956. The modes of action of toxic agents. I. Observations on the poisoning of certain crustaceans by copper and mercury. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 531–48.

—— —— 1957. The modes of action of toxic agents. II. Factors influencing the toxicities of mercury compounds to certain crustacea. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 459–472.

CORNER, E. D. S. & RIGLER, F. H., 1957. The loss of mercury from stored sea-water solutions of mercuric chloride. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 449–458.

GURD, F. R. N., 1954. The specificity of metal-protein interactions. *In* Clarke, H. T., 1954, *Ion Transport Across Membranes*, 246 pp. New York: Academic Press Inc.

HOLM-JENSEN, I., 1948. Osmotic regulation in *Daphnia magna* under physiological conditions and in the presence of heavy metals. *Biol. Medd., Kbh.*, Bd. 20, pp. 5–64.

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OBSERVATIONS ON *COCHLODESMA PRAETENUE* (PULTENEY) [EULAMELLIBRANCHIA]

By J. A. ALLEN

Dove Marine Laboratory, Cullercoats

(Text-figs. 1–12)

Cochlodesma praetenue is the sole British species of the family Laternulidae included in the suborder Anomalodesmata (Thiele, 1935). Shell characters clearly distinguish this suborder from the remainder of the Eulamellibranchia. These characters include a thin, fragile, white shell that is usually inequivalve, hinge teeth that are weak or absent and a ligament that is well developed, complex and may include a lithodesma. Further, Yonge (1952) and Allen (1954) showed that the shell valves may be united dorsally by the periostracum and that some members of the group incorporate sand grains in the periostracum. Work by Ridewood (1903) and Atkins (1937*a*, *b*) and more recently by Yonge (1952) and Allen (1954) on the anatomy of members of the Anomalodesmata also shows other distinguishing characters, notably in the form of the ctenidia and of the stomach.

Knowledge of the functional morphology of the suborder is confined to the families Lyonsiidae, Pandoridae and Thraciidae. When *C. praetenue* was found to be obtainable in numbers from the Northumberland coast, advantage was taken to examine a member of another family of the Anomalodesmata and to compare it with related forms.

HABITAT

Cochlodesma praetenue is distributed from Iceland and the southern part of Norway to the Mediterranean (Forbes & Hanley, 1853). It is recorded from all British waters except the southern North Sea. It occurs in fine gravel, sand and muddy sands from extreme low-water mark of spring tides to a depth of 60 fathoms. It is most commonly found in sand and sandy-gravel in sheltered conditions just below the level of low water of spring tides. Specimens for this present study were taken in such a habitat from a beach at Low Newton on the Northumberland coast.

The common species of the associated infauna were: *Nephthys hombergi* Lam., *Lanice conchilega* (Pallas), *Ampelisca brevicornis* (A. Costa), *Tellina fabula* Gmelin, *Venus striatula* da Costa, *Phacoides borealis* (Linné), *Ensis siliqua* (Linné) and *Echinocardium cordatum* (Pennant). *Cochlodesma* is buried

to a depth of 7 cm below the surface of the substratum and usually lies in a horizontal plane. Allowed to burrow in its normal substratum in aquarium tanks the animal comes to lie horizontally on either valve, slight preference being shown for the left side to be uppermost.

MORPHOLOGY AND FUNCTIONS

Mantle/shell

The thin shell valves are white and slightly inequivalve. The right valve is more convex than the left and overlaps it slightly. The margins of the valves are sinuate (Fig. 1). Except for an area posterior to the umbonal ridge the shell is smooth. Posterior to the umbonal ridge the shell is roughened by numerous small pustules. Fine sand grains adhere to the roughened surface and make the shells conspicuous in sievings of sand samples. Unlike *Lyonsia* and *Pandora* the grains are not incorporated within the periostracum but lie on the surface and are kept in position by the roughness alone.

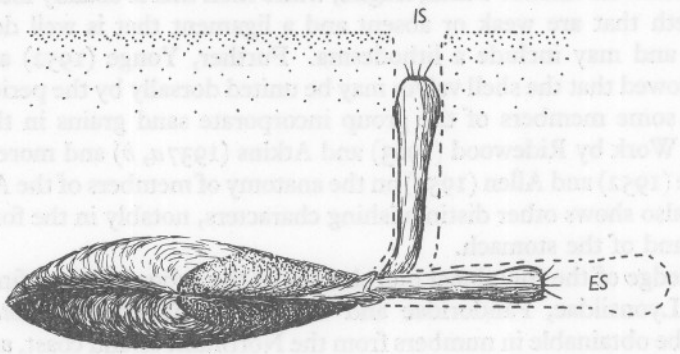

Fig. 1. *Cochlodesma praetenue* in its normal position with the siphons extended. *ES*, exhalant siphon; *IS*, inhalant siphon.

Hinge teeth are not present. The ligament is complex and unusual, and is composed of an internal and an external portion. The internal portion of the ligament is held between a pair of spoon-shaped extensions of the shell which are carried at the end of a pronounced internal ridge that runs forward from the anterior edge of the posterior adductor muscle scar (Fig. 2). The internal portion of the ligament is wedge-shaped and is composed of three layers. The latter probably correspond with the outer, inner and fusion layers of Owen, Trueman & Yonge (1953) and are referred to as such in the following account. The tip of the wedge connects with the external part of the ligament which, apart from a pair of small wing-like extensions of the inner layer, is composed entirely of fusion layer and its covering of periostracum (Fig. 3A, B). This external part extends for some distance anterior and posterior to the umbones and at the junction of the internal and external portions there is a lateral extension of the fusion layer that follows the line of the umbonal ridge

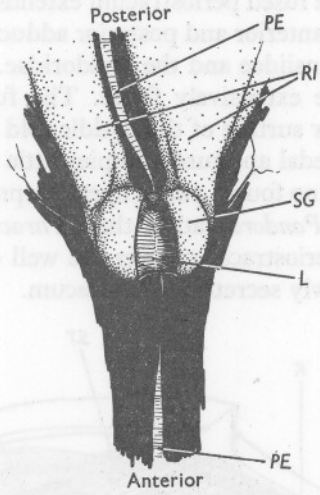

Fig. 2. The hinge mechanism as seen from the inside of the valves showing the terminal spoon-shaped extensions (*SG*) of the ridge (*RI*) holding the ligament (*L*). The valves are united dorsally by the periostracum (*PE*).

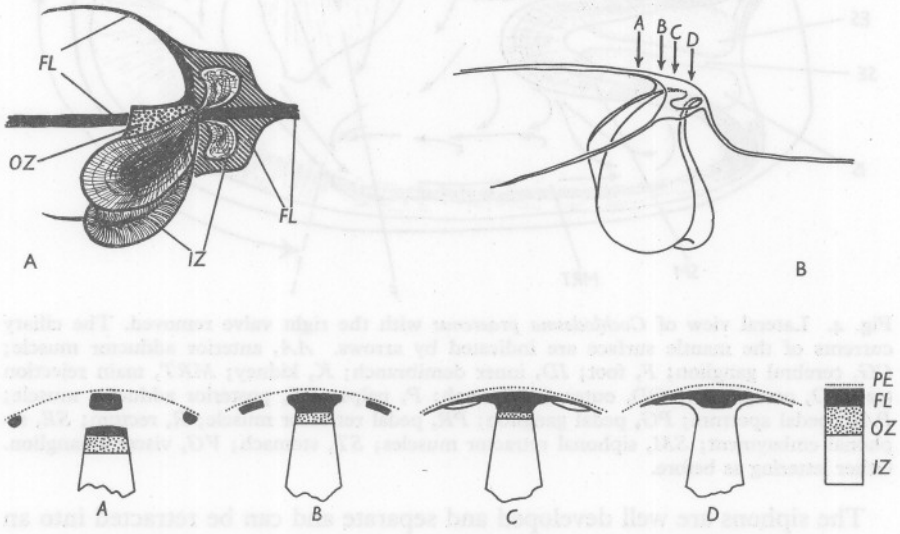

Fig. 3. A. The ligament as seen from the ventral side. The inner portion has been inclined to the side. *FL*, fusion layer; *IZ*, inner layer; *OZ*, outer layer. B. Outline drawing of the ligament in side view with arrows indicating the position of four transverse sections, *A*, *B*, *C* and *D*. The latter are drawn diagrammatically. Lettering as before.

(Fig. 3A, B). Dorsally the fused periostracum extends beyond the limits of the fusion layer as far as the anterior and posterior adductor muscles and parallels the condition in the Lyonsiidae and the Pandoridae.

The mantle edges are extensively fused. The fusion involves the inner mantle fold and the inner surface of the middle fold in the manner described by Yonge (1957). The pedal aperture occupies little more than a third of the ventral margin. There is no fourth pallial aperture present, and in this respect *Cochlodesma* resembles *Pandora* rather than *Thracia* and *Lyonsia*. Unlike those of *Pandora*, the periostracal grooves are well defined, and sand grains do not adhere to the newly secreted periostracum.

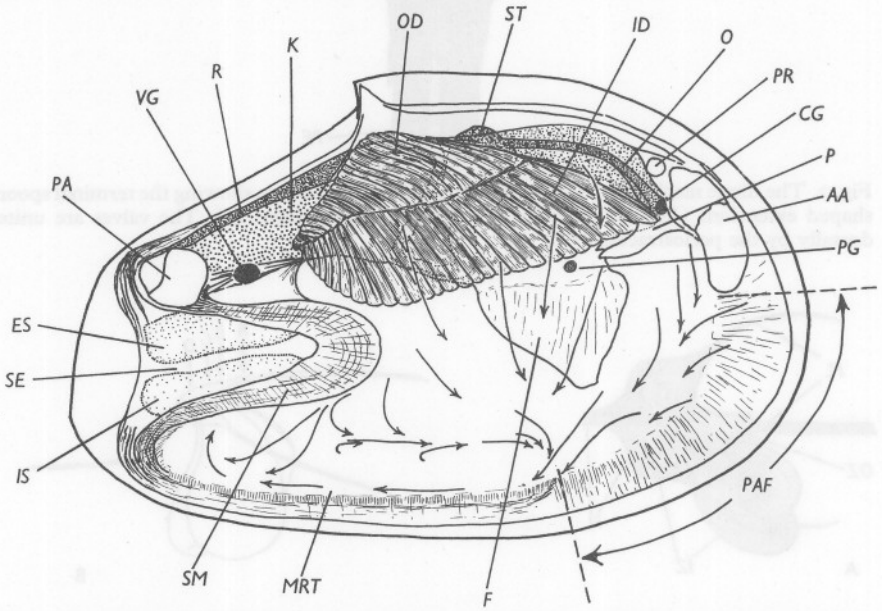

Fig. 4. Lateral view of *Cochlodesma praetenue* with the right valve removed. The ciliary currents of the mantle surface are indicated by arrows. *AA*, anterior adductor muscle; *CG*, cerebral ganglion; *F*, foot; *ID*, inner demibranch; *K*, kidney; *MRT*, main rejection tract; *O*, oesophagus; *OD*, outer demibranch; *P*, palps; *PA*, posterior adductor muscle; *PAF*, pedal aperture; *PG*, pedal ganglion; *PR*, pedal retractor muscle; *R*, rectum; *SE*, siphonal embayment; *SM*, siphonal retractor muscles; *ST*, stomach; *VG*, visceral ganglion. Other lettering as before.

The siphons are well developed and separate and can be retracted into an extensive siphonal embayment (Figs. 1, 4). They are formed from the inner fold of the mantle and can expand to more than twice the length of the shell. Each siphon can expand and contract independently of the other. Separate mucus-lined tubes are formed by the siphons. The tubes are similar to those of *Thracia* (Yonge, 1937), but whereas in *Thracia* both tubes open to the surface only the inhalant tube does so in *Cochlodesma*. The exhalant tube

extends horizontally into the substratum for a distance approximately equal to the depth the shell is buried. The mucous linings are less robust than those of *Thracia* and a complete tube was never separated from the substratum. Inflation of the distal ends of the siphons is not as marked as in the case of *Thracia*. Some inflation occurs for the purpose of consolidating the mucous secretion. Observations on tubes built against the sides of glass containers show that on completion of the tube the siphons do not completely retract, but remain extended for a few millimetres beyond the valve margins. No further extension of the siphon occurs except for the repair of the tube. Under experimental conditions *Cochlodesma* does not remain in the same position for

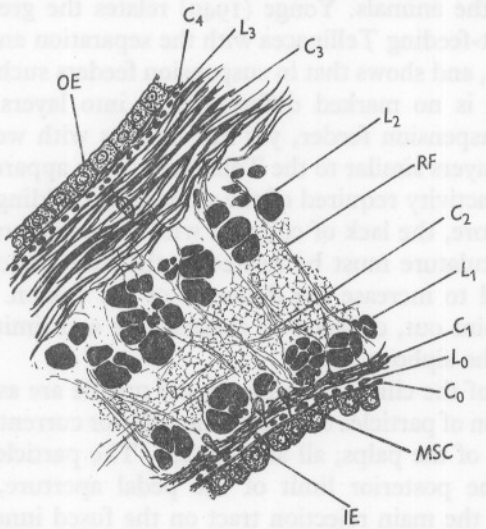

Fig. 5. Transverse section through a siphon to show the arrangement of the various layers. C_0–C_4, circular muscle layers; *IE*, inner epithelium; L_0–L_3, longitudinal muscle layers; *MSC*, mucus-secreting cells; *OE*, outer epithelium; *RF*, radial muscle fibres.

long periods of time, new tubes being built every 12–72 h. This differs from the Lucinacea (Allen, 1953) which also build a mucus-lined tube yet remain in the same position for considerable periods of time. Unlike *Thracia* (Yonge, 1937), there is no concentration of mucus-secreting cells at the distal end of the siphons, secretion taking place along their whole length in *Cochlodesma*.

The siphons elongate smoothly and expand and contract when the terminal aperture is either open or closed. No definite pattern of movement was observed. It appears that expansion is not necessarily a result of pressure on the water in the mantle cavity, a method described for *Mya* (Chapman & Newell, 1956). The histology of the siphons of *Cochlodesma* is similar to that of the Tellinacea (Yonge, 1949; Chapman & Newell, 1956) (Fig. 5), and they expand in a way similar to that described by Chapman & Newell for *Scrobicularia plana*. Trichrome staining shows that there is much more circular

muscle and less collagen in *Cochlodesma* than in the latter species and no lattice work of collagen was demonstrated. In Fig. 5 the nomenclature of the various muscle layers corresponds with that given by Yonge (1949) for the Tellinacea, the only differences being the presence of additional layers L_0 and C_0. C_2 is not well defined and a parenchymatous packing tissue fills the space between layers L_1 and L_2. Thus, the arrangement of the muscle layers is the same on either side of the central packing tissue. Well-defined nerve strands, four in the inhalant and six in the exhalant siphon, run through the central tissue, and supply four to eight simple tentacles at the aperture edge.

It is possible to correlate siphon structure in lamellibranchs with differences in the habits of the animals. Yonge (1949) relates the great activity of the siphons in deposit-feeding Tellinacea with the separation and arrangement of the various layers, and shows that in suspension feeders such as the Veneracea (and *Mya*) there is no marked differentiation into layers. *Cochlodesma* is undoubtedly a suspension feeder, yet has siphons with well developed and intricate muscle layers similar to the Tellinacea. This apparent anomaly must be related to the activity required of the siphons in building the mucus-lined tubes. Furthermore, the lack of collagen with the corresponding increase in the circular musculature must be related to the tube-building function and the periodic need to increase the diameter of the siphon. As Chapman & Newell (1956) point out, collagen is inextensible and limits any increase in the diameter of the siphons.

The direction of the ciliary currents on the mantle are as shown in Fig. 4. With the exception of particles carried in the anterior currents, which may pass on to the surface of the palps, all are rejected. The particles are carried to a point close to the posterior limit of the pedal aperture, and from there posteriorly along the main rejection tract on the fused inner mantle folds to a point ventral to the inhalant aperture. Unlike *Pandora*, there is no mantle fold forming a roof over the main rejection tract. Apart from scattered mucus-secreting cells, the only concentration of glandular tissue in the mantle is close to the posterior apertures over the area of insertion of the siphonal retractor muscles. The elongate cells are modified epidermal cells and it is probable that their secretion is used in the formation of the pseudo-faeces.

Viscera

The ciliary currents over the surface of the body are concerned with the rejection of particles. There is a main tract from the palps to the heel, and particles from the remainder of the body surface move posteriorly towards this tract. Apart from a narrow strip at the ventral edge, the foot is not ciliated (Fig. 6).

The viscera can be seen through the body wall. Gonads and digestive diverticula are compact and, as in most members of the Anomalodesmata,

Cochlodesma is hermaphrodite. The ovaries lie posterior to the long axis through the style sac and stomach, the digestive diverticula being anterior to this line. The testes are placed between the digestive diverticula and the musculature of the foot. The course of the alimentary canal is also seen through the body wall as shown in Fig. 6. The kidney, although elongate, is similar to that of other Eulamellibranchia. The large visceral ganglion, instead of lying close to the posterior adductor muscle, is some distance anterior to the muscle below the kidney. This is probably correlated with the considerable depth of the siphonal embayment, the siphonal nerve having a short and direct route between the ganglion in this position and the base of the siphon.

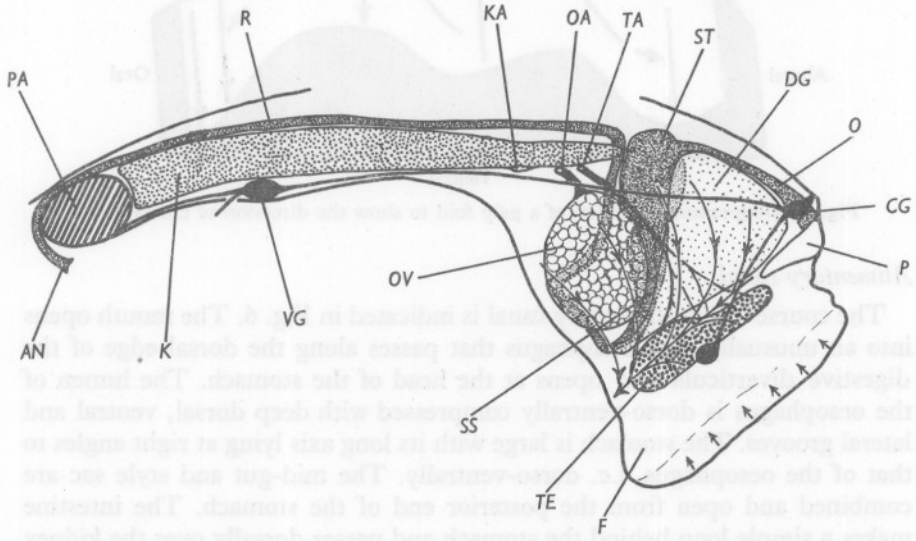

Fig. 6. View of the right side of the body to show the arrangement of the viscera and the direction of the ciliary currents on the body surface. *AN*, anus; *DG*, digestive diverticula; *KA*, renal aperture; *OA*, female genital pore; *OV*, ovary; *SS*, style sac; *TA*, male genital aperture; *TE*, testis. Other lettering as before.

Gills

The gills are typical of the Anomalodesmata with the outer demibranch reduced and reflected dorsally. They are deeply plicate (9 filaments/plica). Occasional specimens have the first 15–20 filaments non-plicate. The ciliary sorting mechanisms are of the *Pinna* type (Atkins, 1937*a*). All particles reaching the gill surface are carried to the marginal groove of the inner demibranch. Atkins (1937*a*, p. 349) figures the margin of the inner demibranch of *Cochlodesma praetenue* and the present work confirms her observations. The terminal frontal cilia are not modified to form a sorting device of the type described in *Pandora* (Allen, 1954). The gills are short and terminate anterior to the posterior adductor muscle (Fig. 4).

Palps

The palps are strap-shaped and small. The sorting folds of the inner surface are relatively broad because of the unusual breadth of the shelf on the aboral side of the fold (Fig. 7). The ciliation shows no significant difference from the basic eulamellibranch type and the palps function in the typical manner.

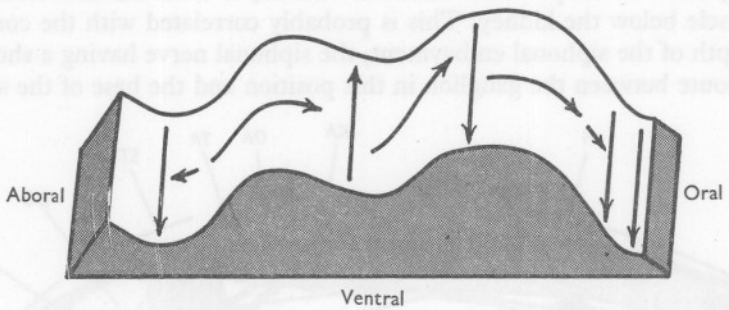

Fig. 7. Diagrammatic section of a palp fold to show the direction of ciliary beat.

Alimentary canal

The course of the alimentary canal is indicated in Fig. 6. The mouth opens into an unusually long oesophagus that passes along the dorsal edge of the digestive diverticula and opens at the head of the stomach. The lumen of the oesophagus is dorso-ventrally compressed with deep dorsal, ventral and lateral grooves. The stomach is large with its long axis lying at right angles to that of the oesophagus, i.e. dorso-ventrally. The mid-gut and style sac are combined and open from the posterior end of the stomach. The intestine makes a simple loop behind the stomach and passes dorsally over the kidney and the posterior adductor muscle to open at the anus.

The stomach was studied in some detail as earlier work on the related genus *Pandora* (Allen, 1954) showed differences from the stomachs of other eulamellibranchs. By dissection and serial sections the morphology and ciliary currents of the stomach were investigated. The nomenclature of Owen (1953, 1955) has been followed.

The stomach of *Cochlodesma* is similar to that of *Pandora* (Figs. 8, 9. Distortion in Fig. 8 has been confined largely to the gastric shield). Accepted particles entangled in a string of mucus are pulled into the stomach by the capstan-like action of the style. The average time taken for one revolution of the style was recorded for twenty-one specimens. In three (at 16·5° C) the time for one revolution was 3·2 sec. In the remaining eighteen specimens (at 17° C) the time varied from 2·6 to 4·9 sec/rev., eleven records lying between 3·2 and 3·8 sec/rev. The style rotates in a clockwise direction when viewed from the oesophageal end of the stomach. It is prevented from

entering the dorsal hood by the tooth of the gastric shield (Fig. 10). Typically the shield is held in position by flanges penetrating the left pouch and the dorsal hood. The morphology and ciliary currents are basically similar to those of *Pandora* (Allen, 1954). In *Cochlodesma* the dorsal hood is well developed. The acceptance tract, terminating in a pad of tissue anterior to the end of the minor typhlosole, is considerably wider than that of *Pandora*. The posterior extension of the posterior sorting area is developed as a blind

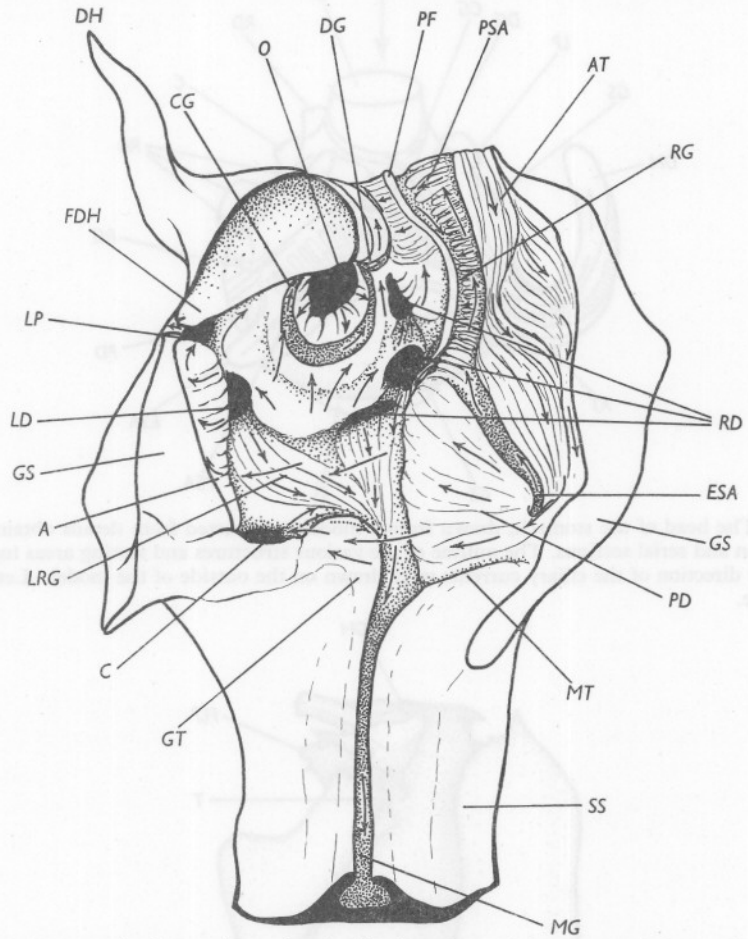

Fig. 8. The stomach dissected by a longitudinal cut through the centre of the gastric shield. The dorsal hood has not been opened. The direction of the ciliary currents are shown. *A*, collecting area; *AT*, acceptance tract; *C*, caecum; *CG*, circular groove; *DG*, dorsal groove; *DH*, dorsal hood; *ESA*, extension of the posterior sorting area; *FDH*, dorsal hood flange of the gastric shield; *GS*, gastric shield; *GT*, major typhlosole; *LD*, isolated left aperture; *LP*, left pouch; *LRG*, lateral rejection grooves; *MG*, mid-gut; *MT*, minor typhlosole; *PD*, pad; *PF*, posterior fold; *PSA*, posterior sorting area; *RD*, isolated right ducts; *RG*, rejection groove. Other lettering as before.

pocket. The walls of this extension are without ridges and all particles on them are carried forward to the posterior sorting area. The extension is adjacent to a constriction in the acceptance tract where the latter fans out as a pad of tissue, described above. It is probable that this constriction controls the size of the particles passing on to the pad, and so to the apertures of the digestive diverticula. Particles wider than the constriction contact the strongly beating

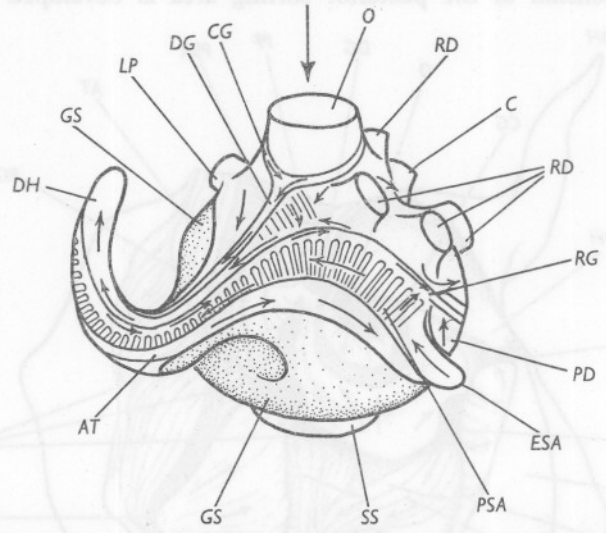

Fig. 9. The head of the stomach, drawn from a model constructed from details obtained by dissection and serial sections. The outline of the various structures and sorting areas together with the direction of the ciliary currents were drawn on the outside of the model. Lettering as before.

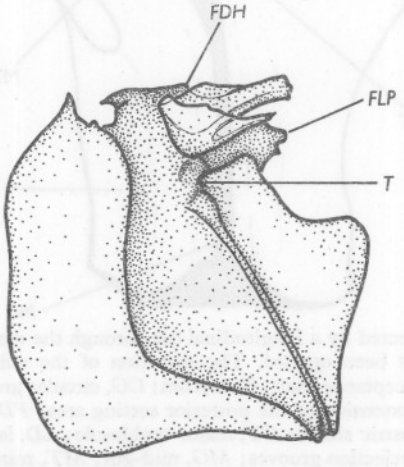

Fig. 10. Drawing of the gastric shield removed from the stomach. *FLP*, left pouch flange of the gastric shield; *T*, tooth. Other lettering as before.

cilia of the extension and are diverted to the posterior sorting area. The replacement of an intestinal groove by a series of grooves (lateral rejection grooves) as was first described in *Pandora*, also occurs in *Cochlodesma*. The lateral rejection grooves extend from the apertures of the digestive diverticula to the mid-gut opening. In the depths of the grooves rejected material from the diverticula is carried to the mid-gut, while accepted material circulating in the stomach is carried across the grooves by the action of the cilia of the crests between the grooves.

The extent of the major typhlosole in *Cochlodesma* is also similar to that in *Pandora*. The typhlosole terminates in the entrance of what is probably the right caecum. In the description of *Pandora* (Allen, 1954) the possibility that the opening immediately posterior to the left pouch (present in *Cochlodesma*) is homologous to the left caecum is discussed. While the arguments still hold in the case of *Cochlodesma*, Purchon, in a personal communication, has suggested that there is possibly only the one caecum and that the opening on the left side, posterior to the left pouch, is comparable to the isolated ducts of the right side—even though several ducts open at this one aperture.

Apart from these divergences the basic pattern of circulation of particles in the stomach is as described by Owen (1953, 1955) for other Eulamellibranchia.

Digestive diverticula

The organization of the digestive diverticula is similar to that of other lamellibranchs (Yonge, 1926; Owen, 1955). They form a compact organ anterior to the stomach (Fig. 6) composed of main and secondary ducts and tubules. The main ducts are short and similar in cross-section to those of *Zirphaea crispata* (Owen, 1955) (Fig. 11 A). The lumen is deeply grooved, the number of grooves varying from four to seven. A single ciliated tract is present. Main and secondary ducts do not differ histologically from those of other Eulamellibranchia (Owen, 1955).

The digestive tubules, clustered at the distal end of each secondary duct, are elongate (Fig. 11 B). They are unusual in that there are a number of minor branches along the length of each tubule. Each minor branch terminates at a single, large cell filled with a clear fluid. Non-ciliated crypt cells are situated close to this large cell and the remainder of the tubule is lined with typical vacuolated cells. Similar large cells have been reported in *Lasaea rubra* by Oldfield (1955) and Morton (1956) and in the Lucinacea (Allen, in the press). It is possible that, if the suggestion (Owen, 1955) that fluid is drawn into the tubule by the absorptive function of the cells is correct, these large cells carry out the major part of the absorption. Their terminal position and large size, almost entirely filled with a single vacuole containing a fluid and no particulate matter, suggests that this may well be the case, digestive function being confined to the vacuolated cells.

SIZE AND AGE GROUPS

On three occasions *Cochlodesma* was obtained in sufficient numbers to give information on the age and size grouping of the Newton population. Shell markings give no indication of age and annual growth rate. The sieve used (aperture size 3 mm²) did not retain shells less than 5 mm long, and examination of material not retained by it showed that one size-group had passed through in each of the samples. The approximate average length of this group

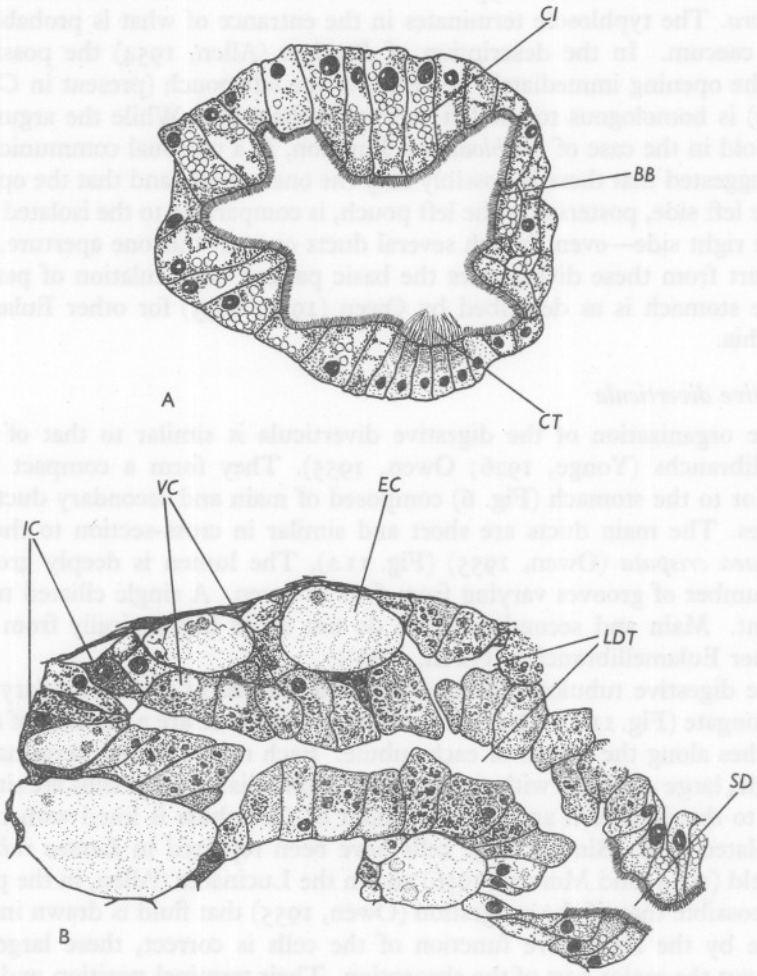

Fig. 11. A. Transverse section of a main duct of the digestive diverticula. *BB*, brush border; *CI*, refractive cell inclusions; *CT*, ciliated tract. B. Longitudinal section of a tubule to show the minor branching. *EC*, large terminal cell; *IC*, non-ciliated crypt cells; *LDT*, lumen of the digestive tubule; *SD*, secondary duct; *VC*, vacuolated cell.

is indicated by arrows in Fig. 12. The figure shows length/number plots of the three samples. The Newton population comprises four or possibly five size groups. Regular sampling showed that mature sperm and eggs are present only during early November. Groups one and two are immature. Thus, the size groups are almost certainly year groups, *Cochlodesma* not living more than 5 years—the majority for only 4—on this beach. There is a fairly steady increase in length of about 4 mm per year during the life of the animal.

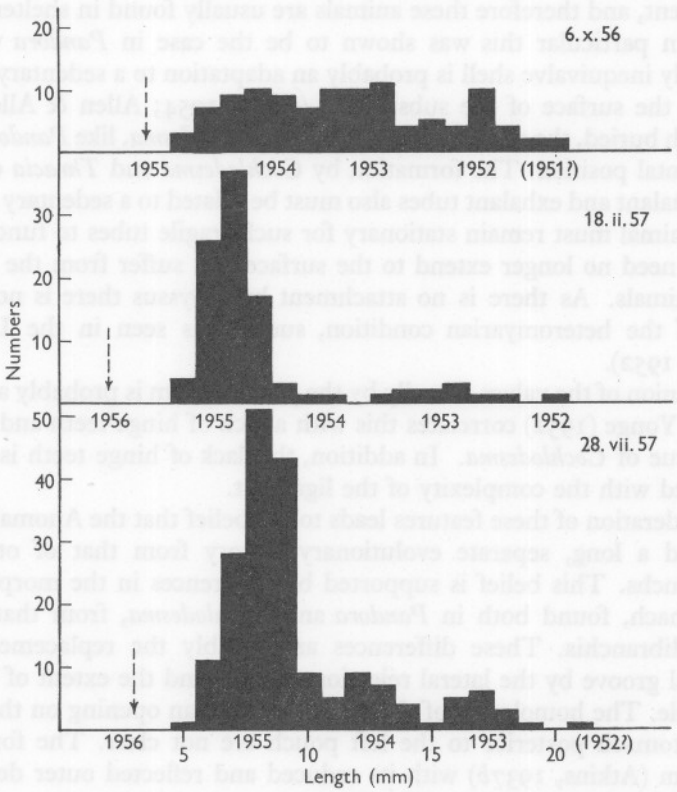

Fig. 12. Histograms of the length/number relationship of samples from the Newton population of *Cochlodesma praetenue*.

The longest shell recorded was 21 mm. This is by no means large in comparison with shells from the west coast of Scotland. Prof. A. D. Hobson has kindly given me a number of specimens which he collected from the Island of Muck which are between 30 and 32 mm in length. Forbes & Hanley (1853) record even larger specimens from deep water off Aberdeen. The specimens from the Island of Muck give no indication whether their growth rate is faster, or whether they live longer than the Northumberland specimens.

DISCUSSION

The Anomalodesmata are rarely found in great numbers and must be regarded as being amongst the least successful of Lamellibranchia inhabiting sands and muds. Yonge (1952) emphasized this in his studies on the Lyonsiidae, and showed that in this family there was adaptation for sedentary life. A similar, but less marked tendency toward sedentary life is seen in the related families, Thraciidae, Pandoridae and Laternulidae. There is no byssal attachment, and therefore these animals are usually found in sheltered conditions. In particular this was shown to be the case in *Pandora* where the extremely inequivalve shell is probably an adaptation to a sedentary life on or close to the surface of the substratum (Allen, 1954; Allen & Allen, 1955). Although buried, the somewhat inequivalve *Cochlodesma*, like *Pandora*, adopts a horizontal position. The formation by *Cochlodesma* and *Thracia* of mucus-lined inhalant and exhalant tubes also must be related to a sedentary existence, as the animal must remain stationary for such fragile tubes to function. The siphons need no longer extend to the surface and suffer from the attacks of other animals. As there is no attachment by a byssus there is no development of the heteromyarian condition, such as is seen in the Lyonsiidae (Yonge, 1952).

The union of the valves dorsally by the periostracum is probably a primitive feature. Yonge (1952) correlates this with a lack of hinge teeth and this is no doubt true of *Cochlodesma*. In addition, this lack of hinge teeth is probably correlated with the complexity of the ligament.

Consideration of these features leads to the belief that the Anomalodesmata have had a long, separate evolutionary history from that of other eulamellibranchs. This belief is supported by differences in the morphology of the stomach, found both in *Pandora* and *Cochlodesma*, from that in other Eulamellibranchia. These differences are notably the replacement of the intestinal groove by the lateral rejection grooves and the extent of the major typhlosole. The homologies of the caecum and of an opening on the left side of the stomach posterior to the left pouch are not clear. The form of the ctenidium (Atkins, 1937*b*) with its reduced and reflected outer demibranch again is characteristic of the suborder.

Cochlodesma is highly specialized for a sedentary life in sheltered conditions at low-water mark and below. Although it is closely related to the Thraciidae, Pandoridae and Lyonsiidae it possesses characteristics that clearly distinguish it from these related forms.

SUMMARY

Cochlodesma praetenue is well adapted for a sedentary life in sheltered conditions in soft substrata at low-water mark to a depth of 60 fathoms.

The habits, morphology and ciliary feeding and cleansing mechanisms are

described and compared with those of members of the related families Thraciidae, Pandoridae and Lyonsiidae.

The complex ligament is described. The mantle edges are extensively fused. The detailed structure of the siphons is described and related to their function of laying down mucus-lined inhalant and exhalant tubes.

The morphology and ciliary currents of the stomach show modifications similar to those previously described for *Pandora*. These modifications involve the extent of the major typhlosole and the replacement of an intestinal groove by lateral rejection grooves.

Analysis of three large samples shows that *Cochlodesma* from the Northumberland coast live for a maximum of 5 years. These animals become mature in their third year, spawning in early November.

REFERENCES

ALLEN, J. A., 1953. Function of the foot in the Lucinacea (Eulamellibranchia). *Nature, Lond.*, Vol. 171, p. 1117.

—— 1954. On the structure and adaptations of *Pandora inaequivalvis* and *P. pinna*. *Quart. J. micr. Sci.*, Vol. 95, pp. 473–82.

—— (in the Press). On the basic form and adaptations to habitat in the Lucinacea.

ALLEN, M. F. & ALLEN, J. A., 1955. On the habits of *Pandora inaequivalvis* (Linné). *Proc. malac. Soc. Lond.*, Vol. 31, pp. 175–85.

ATKINS, D., 1937*a*. On the ciliary mechanisms and interrelationships of lamellibranchs. Part II. *Quart. J. micr. Sci.*, Vol. 79, pp. 339–73.

—— 1937*b*. On the ciliary mechanisms and interrelationships of lamellibranchs. Part III. *Quart. J. micr. Sci.*, Vol. 79, pp. 375–421.

CHAPMAN, G. & NEWELL, G. E., 1956. The rôle of the body fluid in the movement of soft-bodied invertebrates. II. The extension of the siphons of *Mya arenaria* L. and *Scrobicularia plana* (da Costa). *Proc. roy. Soc.* B, Vol. 145, pp. 564–80.

FORBES, E. & HANLEY, S., 1853. *A History of the British Marine Mollusca, and their Shells*. London.

MORTON, J. E., 1956. The tidal rhythm and action of the digestive system of the lamellibranch *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 563–86.

OLDFIELD, E., 1955. Observations on the anatomy and mode of life of *Lasaea rubra* (Montagu) and *Turtonia minuta* (Fabricius). *Proc. malac. Soc. Lond.*, Vol. 31, pp. 226–47.

OWEN, G., 1953. On the biology of *Glossus humanus* (L.) (*Isocardia cor* Lam.). *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 85–106.

—— 1955. Observations on the stomach and digestive diverticula of the Lamellibranchia. I. The Anisomyaria and Eulamellibranchia. *Quart. J. micr. Sci.*, Vol. 96, pp. 517–37.

OWEN, G., TRUEMAN, E. R. & YONGE, C. M., 1953. The ligament in the Lamellibranchia. *Nature, Lond.*, Vol. 171, p. 73.

RIDEWOOD, W. G., 1903. On the structure of the gills of the Lamellibranchia. *Phil. Trans.* B, Vol. 195, pp. 147–248.

THIELE, J., 1935. Classis Bivalvia. *Handbuch der Systematischen Weichtierkunde*, Tl. 3. Jena.

YONGE, C. M., 1926. The digestive diverticula in lamellibranchs. *Trans. roy. Soc. Edinb.*, Vol. 54, pp. 703–18.

—— 1937. The formation of siphonal openings by *Thracia pubescens*. *Proc. malac. Soc. Lond.*, Vol. 22, pp. 337–8.

—— 1949. On the structure and adaptations of the Tellinacea, deposit-feeding Eulamellibranchia. *Phil. Trans.* B, Vol. 234, pp. 29–76.

—— 1952. Studies on Pacific coast mollusks. IV–VI. Structure and adaptation in *Entodesma saxicola* (Baird) and *Mytilimeria nuttallii* Conrad, with a discussion on the evolution of the family Lyonsiidae (Eulamellibranchia). *Univ. Calif. Publ. Zool.*, Vol. 55, pp. 439–50.

—— 1957. Mantle fusion in the Lamellibranchia. *Pubbl. Staz. zool. Napoli*, Vol. 29, pp. 151–71.

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EUDACTYLINA RACHELAE N.SP., A COPEPOD PARASITIC ON THE ELECTRIC RAY, *TORPEDO NOBILIANA* BONAPARTE

By J. GREEN
Bedford College, University of London

(Text-fig. 1)

Five species of the genus *Eudactylina* are known from British waters (Scott & Scott, 1913), and of these only one, *E. acuta* Van Beneden, is recorded in the *Plymouth Marine Fauna* (Marine Biological Association, 1957, p. 181). The species described below was found among the gill filaments of a large specimen of *Torpedo nobiliana* Bonaparte (det. P. G. Corbin) taken in trawl by R.V. *Sula* off Plymouth in July 1957. There were five females.

Eudactylina rachelae n.sp.

Description of the female

The body is elongated, maggot-shaped. The length, excluding the egg strings, is 2·3–2·5 mm. When fresh the colour is cream, with the gut showing through brown. The first pedigerous segment is fused with the head, the whole being covered by a single dorsal shield which is one and a third times as long as wide, and somewhat narrowed anteriorly. The second and third pedigerous segments have dorsal shields which are similar in length, while that of the fourth pedigerous segment is longer. The dorsal shield of the fifth pedigerous segment is smaller than those of the second and third. The genital segment is narrower than the preceding segments. The urosome (excluding the genital segment) has two segments; the first being roughly twice as long as the second. The dorsal surface of the body bears numerous crescent-shaped lamellar processes, which look like minute spinules when viewed from the side.

The antennules (Fig. 1B) are stout, of five podomeres, the last indistinctly separated from the fourth. The armature is similar to that of *E. acuta* and *E. similis*. The two principal spines lack the fringe of minute spinules along the upper edge, which is found in *E. similis*. The large spine on the second podomere has a series of minute crescent-shaped depressions, each of which appears to contain a spinule lying almost parallel to the border of the spine. The large spine on the third podomere has three shallow oblique teeth on each side (Fig. 1C).

The antennae (Fig. 1D) have four podomeres. The second podomere has a strong spine-like process on the inner side, while the third has two such processes and a seta. The third podomere also bears numerous crescent-shaped processes similar to those on the dorsal surface of the body. The

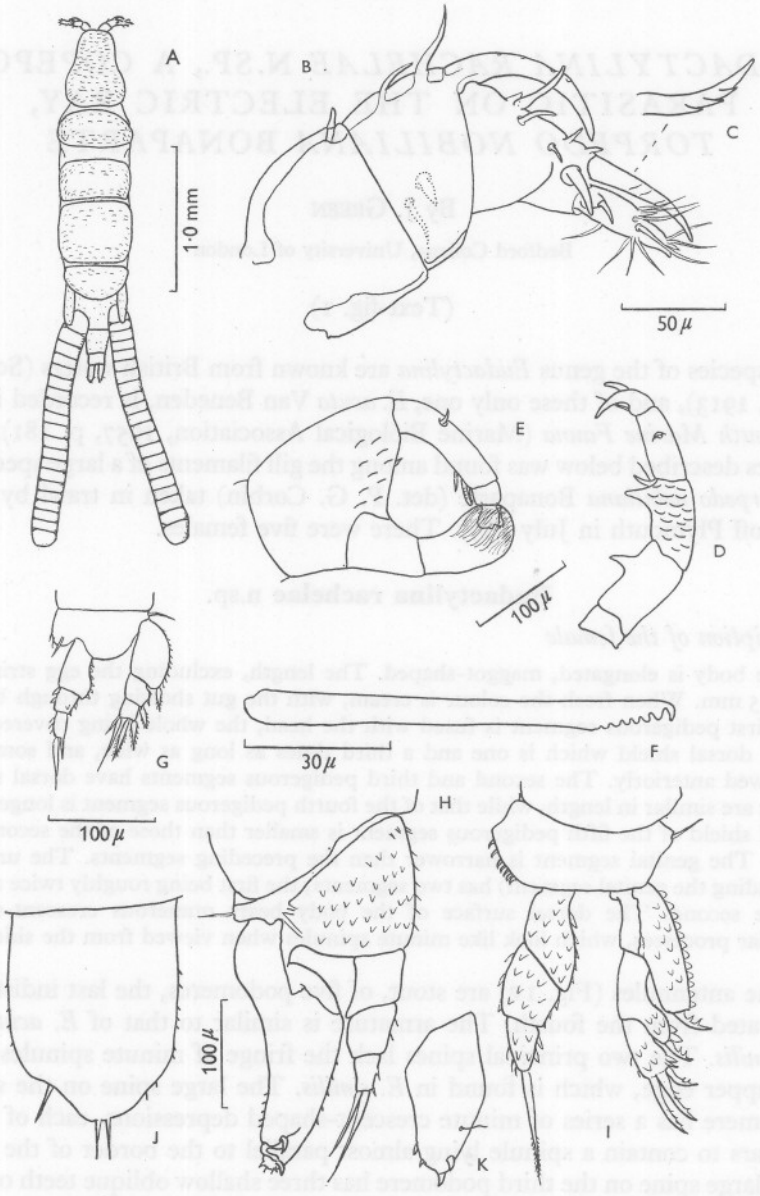

Fig. 1. A–K, *Eudactylina rachelae* n.sp., adult female. A, dorsal view; B, antennule; C, spine on third podomere of antennule; D, antenna; E, second maxilliped; F, mandible; G, first leg; H, second leg; I, third leg; J, fifth leg; K, ventral view of left caudal ramus. G–K are all drawn to the same scale.

fourth podomere bears what appears to be a sensory pit. The antenna ends in two stout spines and a seta.

The mandible (Fig. 1F) is long and straight, with a terminal hook and seven or eight short blunt teeth.

The maxilla, like that of *E. similis*, has two setae on the principal lobe and one on the secondary lobe.

The first maxilliped has three podomeres and terminates in a stout spine with two tufts of setae at its base.

The second maxillipeds (Fig. 1E) are large and chelate. The movable branch of the chela ends in an expanded cup-like structure which fits inside the thin, expanded and minutely fluted end of the immovable branch.

The first pair of legs is much smaller than the second to fourth pairs. The second basal podomere has one moderate and two small spines on its inner border. The endopod and exopod each have three podomeres.

The second legs have strongly modified exopods (Fig. 1H), while the endopod has three normal podomeres.

The third and fourth legs are similar in structure (Fig. 1I). The surface of the podomeres is drawn out into flattened spine-like processes which appear as short triangular lamellae when viewed from the side. The strong spines on the exopods are distinctly toothed.

The fifth leg consists of a single plate-like podomere, with three terminal setae and a few spinules along the lateral borders.

The caudal rami are one and a half times as long as the anal segment. Each ramus has two short terminal spines, two weaker inner spines and one feeble outer seta.

Remarks

This species is closely related to *E. similis* T. Scott, but can be separated by the differences given in Table 1.

TABLE 1

E. similis T. Scott	*E. rachelae* n.sp.
Large spine on third podomere of antennule with minute spinules along upper border	Minute spinules not present on this spine, but three oblique teeth present on each side
Mandible curved	Mandible straight
Movable branch of second maxilliped ends in a spine	Movable branch of second maxilliped ends in an expanded cup
First legs not much smaller than others. The inner border of the second basal podomere with two stout spines	First legs much smaller than others. Inner border of the second basal podomere with one moderate and two small spines
Spines on third and fourth legs not toothed	Spines on third and fourth legs toothed
Caudal rami without two inner spines	Caudal rami with two inner spines

Type material. The holotype and one paratype will be deposited at the British Museum. Three paratypes will be retained in my collection.

These specimens were collected while working at the Plymouth Marine Laboratory; my thanks are due to the Director and Staff for the facilities provided. My thanks are also due to Dr J. Llewellyn for bringing these copepods to my notice.

SUMMARY

Eudactylina rachelae n.sp. is described and figured. This copepod was found among the gill filaments of *Torpedo nobiliana* Bonaparte caught off Plymouth in July 1957.

REFERENCES

MARINE BIOLOGICAL ASSOCIATION, 1957. *Plymouth Marine Fauna*, 3rd ed.

SCOTT, T. & SCOTT, A., 1913. *The British Parasitic Copepoda*, Vol. 1, 252 pp.; Vol. 2, Plates. London: Ray Society.

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THE LOCALIZATION OF ORGANICALLY BOUND IODINE IN THE ENDOSTYLE OF *AMPHIOXUS*

By E. J. W. Barrington, D.Sc.
Department of Zoology, The University, Nottingham

(Plate I and Text-fig. 1)

Recent work has done much to strengthen, from a functional aspect, the classical view that the thyroid gland of vertebrates is homologous with the endostyle of the protochordates (Barrington & Franchi, 1956*a*), for the presence of organically bound iodine has been detected in the latter organ, both in *Ciona* and in other ascidians (Barrington, 1957 and unpublished work) and in *Amphioxus* (Thomas, 1956). The occurrence of iodine binding in the endostyle of the ammocoete larva of the lamprey (Gorbman & Creaser, 1942) is now known to be associated with a secretion which in certain properties resembles thyroid colloid, and may well be homologous with it (Barrington & Franchi, 1956*b*). The presence of a similar secretion in the *Ciona* endostyle (Barrington, 1957) implies that a specialized thyroidal biosynthesis is also operative there, and that the iodine binding is functionally significant for the organism and is not a biochemical 'accident' as the binding which takes place in the scleroproteins of many invertebrates has been thought to be (Gorbman, 1955).

It is thus a matter of importance to determine the cytological basis for the iodination which occurs in the endostyle of *Amphioxus*. According to Thomas (1956) the dorsal glandular tract is associated with this process, a situation quite different from that found either in the ammocoete larva or *Ciona*, and it is the purpose of the present work to examine this matter in detail as a basis for a comparison of the functional organization of the three types of endostyle.

MATERIAL AND METHODS

The work was carried out in part at the Plymouth Laboratory of the Marine Biological Association, and in part at Nottingham, the methods being substantially similar to those used in previous work on the ammocoete and *Ciona* referred to above. For radioactive studies the animals were immersed at 10° C in sea water containing 200 μc of ^{131}I per litre; this is one-tenth of the concentration used by Thomas (1956), who also immersed the animals for 15–17 h, whereas at Nottingham a period of 48 h has been adopted as a standard in all such experiments. Specimens were mostly fixed in Susa and in

Bouin's fluid in sea water, and autoradiographs were prepared by the stripping-film technique of Pelc (Pearse, 1954).

The endostyle is commonly described as a mucus-secreting organ, but the term 'mucus' is used so loosely in much zoological literature, to mean simply a viscous secretion, that it has been essential in the present work to select staining and histochemical methods which would give a more precise definition to it. The PAS reaction (counterstained with Harris's haematoxylin, followed either by metanil yellow or by tartrazine) has therefore been used (controlled by acetylation) to demonstrate the presence of 1:2-glycol groups, and toluidine blue metachromasia and the alcian blue method of Steedman (1950) to show whether or not the reacting secretion contains acid mucopolysaccharides, the importance of the latter being that they appear to be the main components of epithelial mucins (Pearse, 1954). The selectivity of the alcian blue was found to be much improved by the addition of an equal volume of 1% acetic acid to the 1% solution of the dye, as suggested by Lison (1954). The classical mucicarmine technique of Mayer, using Southgate's method of preparing the staining solution, has also been valuable, as has the Azan procedure.

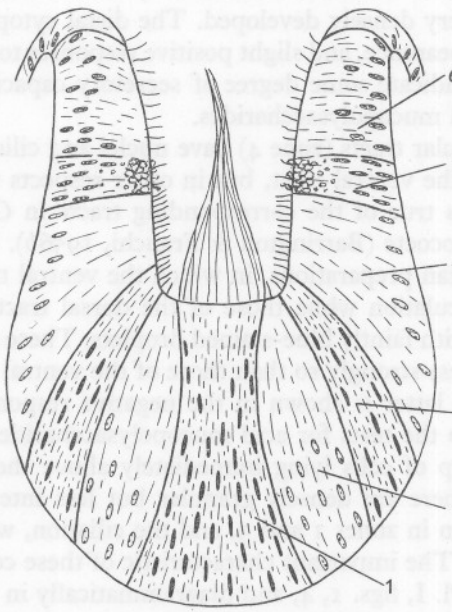
There has been no serious difficulty in interpreting the results of these tests, although there has been a good deal of quantitative variation; animals, for example, which have been fixed and processed side by side, may show considerable differences in the intensity of their response to mucicarmine, even in such clearly defined cell types as the secretory cells of the gill bars. It seems probable that although on superficial inspection *Amphioxus* seems to survive well under laboratory conditions, there is, in fact, much variation in the physiological condition of different specimens by the time that they are fixed. In confirmation of this, it is of interest to note that Možejko (1913) concluded that variation in the extent to which the tissues of living *Amphioxus* were coloured by ingested carmine reflected differences in their physiological condition. It is also clear that another source of variation arises from the fact that the secretory contents of the cells, and sometimes even cytoplasm and nuclei, may be discharged from the endostylar epithelium under the stress of fixation. The present account has therefore been based on examination of a number of specimens fixed at different times under different conditions, some at Plymouth and some at Nottingham, and there is no reason to suppose that the conclusions are in any way affected by such variations.

OBSERVATIONS

Organization of the endostyle

Previous workers have entirely overlooked what, from the point of view of the interpretation of the iodination process, is the most important feature of the endostyle, and it is therefore necessary to give a brief new description of its organization (Text-fig. 1; Pl. I, fig. 1).

At the base of the endostylar groove is a group of cells, here termed 'zone 1', which closely resemble the correspondingly placed zone 1 cells of *Ciona* (Barrington, 1957). They possess slender and densely staining nuclei, long cilia, and a conspicuously striated cell border. This ciliation and border are characteristic of the whole of the endostylar epithelium, although their degrees of development vary greatly from zone to zone. Secretory droplets are present in zone 1, and are strongly PAS-positive, the general cytoplasm also giving a



Text-fig. 1. Diagrammatic transverse section across the endostylar epithelium of *Amphioxus* to show the distribution of the several zones, which are indicated by corresponding numbers. For further explanation see text.

positive reaction. These droplets stain with mucicarmine, and the fact that they stain selectively with alcian blue, and that toluidine blue gives a definite gamma (red) metachromasia in the cells, indicates the presence of acid mucopolysaccharides. It may thus be concluded that these cells are producing an epithelial mucin, in the sense in which the term has been used above; this secretion is particularly dense at the lateral edges of the zone (Pl. I, fig. 4), from which it is discharged over the surface of the mass of long cilia, a situation which is in line with Orton's (1913) suggestion that the main function of these cilia is to deflect the endostylar secretion so that it passes out on to the lateral walls of the pharynx.

On either side of zone 1 lie the ventral glandular tracts (zone 2). These bear only a few cilia, while the nuclei are less densely stained than those of zone 1

and have a prominent nucleolus. PAS-positive droplets are present, and these react positively also with mucicarmine and alcian blue, while the cells show gamma metachromasia. The reactions are thus similar to those of zone 1, but they are usually less intense; it seems likely, then, that the secretion of these tracts contains acid mucopolysaccharides but that it may well differ from the secretion produced by zone 1.

Zone 3 is a narrow zone separating the ventral glandular tracts from the dorsal ones. The nuclei are much as in zone 1, but the cilia are considerably shorter, although very densely developed. The distal cytoplasm has a somewhat vacuolated appearance, and slight positive responses to the various histochemical reagents indicate some degree of secretory capacity and, probably, the presence of acid mucopolysaccharides.

The dorsal glandular tracts (zone 4) have nuclei and ciliation substantially similar to those of the ventral tract, but in other respects the two tracts are quite different, as is true of the corresponding tracts in *Ciona* (Barrington, 1957) and the ammocoete (Barrington & Franchi, 1956*b*). This difference is apparent even in Azan preparations, in which the ventral tract cells show an indefinite blue flocculation while those of the dorsal tract have an orange-stained cytoplasm with faintly blue-stained droplets. These droplets are PAS-positive, although less strongly so than those of the ventral tracts, but a clear difference from the latter is shown in the negative response of the cells to mucicarmine and to the tests for acid mucopolysaccharides (Pl. I, fig. 4).

Zone 5 is a group of cells lying immediately above the dorsal glandular tracts. The nuclei here are densely granular but less intensely stained and less compressed than in zones 1 and 3, and the ciliation, while detectable, is exceedingly sparse. The important characteristic of these cells is their clearly defined secretion (Pl. I, figs. 1, 4, and diagrammatically in Text-fig. 1). This can be seen as very fine droplets in the cytoplasm, sometimes extending down to the base of the cell, but is particularly conspicuous as an accumulation in the more distal parts, where it extends through the cell border (the striations of which are less clearly defined than elsewhere in the endostyle) and on to the occasional cilia. The extrusion of fine threads of this secretion is often evident, and there is much variation from animal to animal in the amount of secretion visible in the cell, but this is probably influenced by the factors mentioned earlier (p. 118). Such variation may, however, account for this particular region not having been previously described, for in well-fixed specimens the accumulation of secretion in these cells is impossible to overlook and, indeed, it provides the most conspicuous stainable feature of the whole endostyle. This secretion, which stains well with aniline blue in the Azan procedure, contrasts sharply with the product of the adjacent dorsal glandular tracts, for it is very strongly PAS-positive, and also reacts positively with mucicarmine and alcian blue, and shows gamma metachromasia with toluidine blue. The only other region of the endostyle which is comparable

with it in the clarity of these reactions is the lateral edge of zone 1, and even so the reactions of zone 5 tend to be more brilliant.

Zone 6 differs from zone 5 in the much greater density of ciliation and the greater chromophilia of the nuclei. Very small PAS-positive droplets may be visible distally and may indicate, as in zone 3, a rudimentary secretory capacity. Zone 5, in fact, has the appearance of having been differentiated out of zone 6 by a development of its secretory capacity and a reduction of its ciliation.

The above observations lead to the conclusion that zones 1 and 5 are the parts of the endostyle which are most obviously mucin-secreting in the sense of producing a secretion which is rich in acid mucopolysaccharides. The latter appear also to be present in the ventral glandular tracts, and probably in zone 3, but are certainly absent from the secretion of the dorsal glandular tracts. Here, then, as in *Ciona* and the ammocoete, it is evident that the endostyle is far from being a simple 'mucus-secreting' organ, and that much remains to be learned as to the nature and functions of its secretions.

Autoradiographs

Transverse sections (Pl. I, fig. 2) clearly show that bound iodine is restricted in the endostyle to two areas, lying one on each side immediately above the dorsal ciliated tracts, and that these areas correspond precisely to the groups of cells described above as zone 5.

Each area extends over the upper ends of the cells and also into the endostylar lumen (Pl. I, figs. 3, 5). One must assume that random scatter contributes to the peripheral part of the image, but the central point of the latter can be taken as the centre of distribution of the iodine, and this appears to be located, as far as one can judge, at about the level of the surface of the epithelium (Pl. I, figs. 4, 5, and also fig. 3). In other words, it lies somewhat distal to the centre of the area which is commonly occupied by the stored secretory contents of these cells. If, then, one assumes, as seems reasonable, that it is these contents which become iodinated, it seems likely that the iodination process takes place at or very near to the cell border. The autograph image may thus represent an iodinated secretion which, at least in part, has probably been discharged from the cells and which is bound to their surface and, perhaps, to their cilia. Some secretion in process of being discharged is often visible in sections, but it is not always present, and by no means corresponds exactly in amount or shape to the iodine image. Such material, in fact, need not necessarily be iodinated, for, as mentioned above, its discharge is probably in part a consequence of contraction during fixation, and the image may well be produced largely by a substance which is not actually visible in stained sections. A precisely similar situation obtains in the endostyle of the ammocoete larva (Barrington & Franchi, 1956*b*), where the secretion is difficult to identify once it has left the cells, and where a clearly defined iodine image

overlies cilia which have little, if any, visible secretion associated with them.

It follows from the above that the iodinated secretion is normally discharged into the lumen of the pharynx, and this is confirmed by the existence of radio-iodine images in the lumen adjacent to the endostylar cells. Such are also to be found in the food cord in the dorsal groove, into which, as is well known, material secreted by the endostyle and gill bars is swept in consequence of the action of the pharyngeal ciliation, while the food cords in the mid-gut and intestine also give strong images. It is always to be remembered that food organisms themselves may bind iodine and contribute to such images (Barrington, 1957), but it seems wholly improbable that this could account for all of the latter; I agree, therefore, with Thomas (1956), who noted similar images in his own preparations, that the iodinated secretion of the endostyle is ultimately incorporated into the food cords.

Thomas noted that the amount of iodine taken up by *Amphioxus* increased to reach a maximum in about 7–10 days after they were caught. My own preparations were obtained from animals of which some were fixed at Plymouth within 5 days of being caught, while others were fixed at Nottingham, 3–4 weeks after being brought into the Plymouth Laboratory. All gave essentially similar autoradiographs. In none of them was there any certain sign of organically-bound iodine in any other part of the endostyle, such slight spots as were visible being indistinguishable from random scatter and background fogging. The absence of iodine from the lateral edges of zone 1, where there are usually distinctive masses of secretion which are histochemically similar to those of the thyroidal cells, is particularly striking (Pl. I, figs. 3, 5). One is driven to conclude, then, that iodine binding in this endostyle, as in that of *Ciona*, is not a generalized or random process, but is a specialized property of a specific group of cells.

DISCUSSION

The facts described here bring the iodine-binding activities of the endostyle of *Amphioxus* into closer line with those of *Ciona* and the ammocoete than was apparent from Thomas's account. He described the images as being 'situated near the periphery of and slightly lateral to the lateral series of mucous glands', with which I would agree, although I should not myself use the term 'mucous' (see below). Further on, however, he says that 'the most obvious centre of radioactivity in ^{131}I-treated animals is in the mucus of the lateral mucous glands'. Of this my own preparations give no evidence. On the contrary, the present account defines the iodine binding as a property of a specialized group of mucus-secreting cells lying immediately above (lateral to) the lateral glandular tracts, and it is important to bear in mind that these tracts, the lateral mucous glands of Thomas's description, do not secrete mucus at all in the sense of a substance rich in acid mucopolysaccharides.

This localization of function agrees very well with the situation in *Ciona*; in that animal there is, as compared with *Amphioxus*, an additional glandular tract on each side, but the region where iodination occurs lies immediately above the glandular tracts in a position which essentially corresponds with that of the iodination centre in *Amphioxus*. It is doubtful whether any significant iodination occurs in the tracts of *Ciona*, and it is certain that none does so in those of the ammocoete. Iodination extends over a much wider area of the endostylar epithelium of the latter than it does in the protochordates, but the regions chiefly concerned bear essentially the same relationship to the glandular tracts as do the iodination centres in *Amphioxus* and *Ciona*, allowing, of course, for the much greater complexity of the organ in the ammocoete.

In view of this fundamental morphological similarity, it is all the more interesting to find from the present work that there is a significant difference in certain histochemical properties of the iodinated material. Evidence has already been given that in the ammocoete larva (Barrington & Franchi, 1956*b*) this material resembles thyroid colloid in showing, as far as these histochemical procedures go, the characteristic properties of a glycoprotein (or mucoprotein, since the two are not distinguishable by these methods). Renewed tests, in conjunction with the present work, have confirmed that this ammocoete secretion, like mammalian thyroid colloid, is negative to mucicarmine and alcian blue, and that it does not exhibit gamma metachromasia, and it may be added that the same appears to be true of *Ciona*, although the ascidians need much more extended study before this statement can be regarded as sufficiently broadly grounded. In *Amphioxus*, on the contrary, the secretion includes an acid mucopolysaccharide component, and in this respect resembles typical epithelial mucins, including those of certain other parts of the endostyle and of parts of the gill bars and dorsal groove of the pharynx. This appears, in fact, to be the first reported case of the association of iodine binding with an epithelial mucin, characterized as such by its localization and its histochemical properties.

In this connexion Thomas (1956) has suggested from his own work on *Amphioxus* that thyroid colloid 'is the direct evolutionary successor to the endostylar mucus of a protochordate ancestor'. The present results confirm and clarify this suggestion, but it is very important to appreciate that they show that iodine-binding in *Amphioxus* is not a generalized binding to all endostylar mucus. None is associated with the secretion of zone 1, the acid mucopolysaccharide properties of which are indistinguishable from those of the thyroidal region of zone 5, and this means that in *Amphioxus*, just as in *Ciona* (Barrington, 1957), iodine-binding is a specialized biochemical property of a particular group of cells. This conclusion carries the implication that the product is not the result of random iodination, but that it is of biochemical significance to the organism. As Thomas (1956) points out, the work of

Sembrat (1953) gives some reason for supposing that this product can be regarded as resembling thyroid hormone. It seems quite possible, then, from the situation in *Amphioxus*, as it does from that in *Ciona* (Barrington, 1957), that the production of thyroid hormone may already be established at the protochordate level of chordate evolution but that it is passed into the lumen of the pharynx instead of being secreted directly into the blood stream. It may well be, as suggested by Thomas's work, that it is then taken up into the body through the epithelium of the alimentary canal as part of the normal process of digestion and absorption of food, but it is hoped to report further on this aspect in a later publication.

I am much indebted to the Director and Staff of the Plymouth Laboratory for the trouble taken to provide me with the animals and facilities needed for this work, to Mr T. Berbank for taking the photomicrographs, and to Miss J. Plumtree for technical assistance.

SUMMARY

An account is given of some features of the organization of the endostyle of *Amphioxus*, with particular reference to the distribution of sites of mucus secretion as indicated by positive responses to tests for acid mucopolysaccharides. Autoradiography shows that the centre of distribution of organically-bound iodine is associated with the tips of the mucus-secreting cells of zone 5, and not with the glandular tracts. Mucus secretion also occurs elsewhere in the endostyle (although not in the dorsal glandular tracts), but as it is not associated with any accumulation of bound iodine it is concluded that the binding in zone 5 is a specialized property of the cells of that particular zone. The results are discussed in the light of recent work on the protochordates and the ammocoete larva, and it is concluded that in *Amphioxus*, as in the Tunicata, there is reason for supposing that the iodination process is a biochemical specialization and that its product must therefore be of some physiological significance to the organism. Attention is also drawn to the evolutionary interest of the association of iodine-binding with a mucin rather than with a glycoprotein.

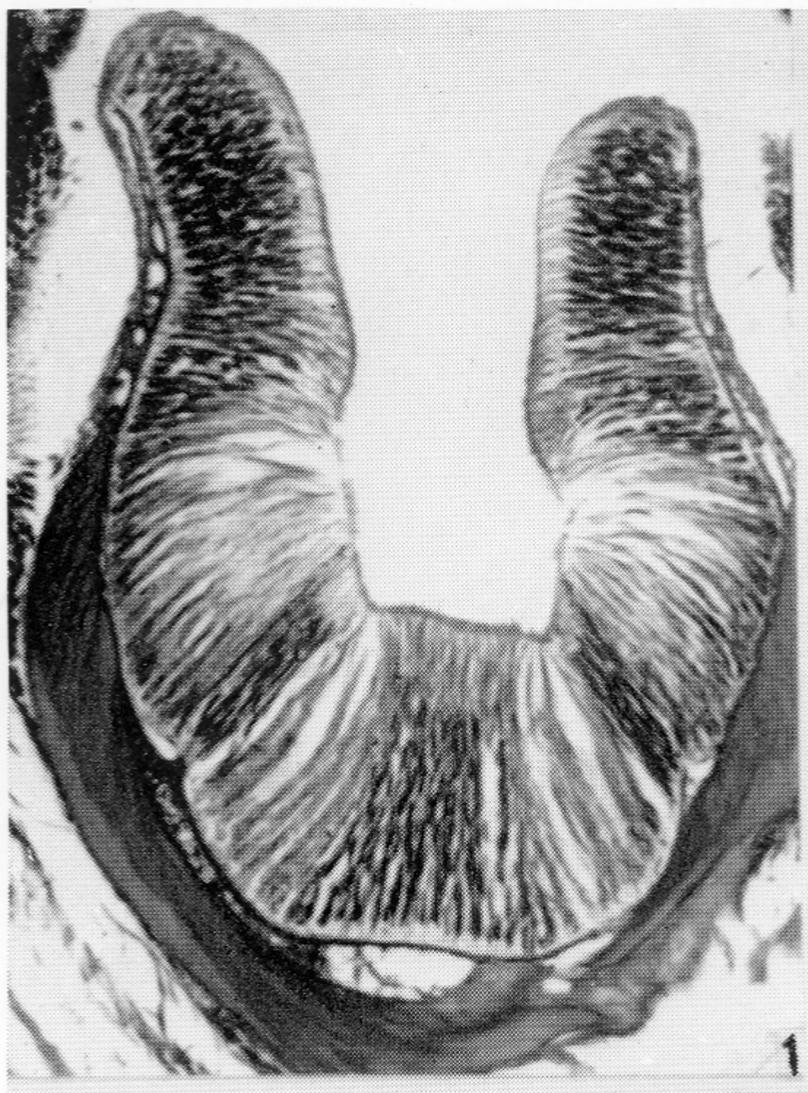
REFERENCES

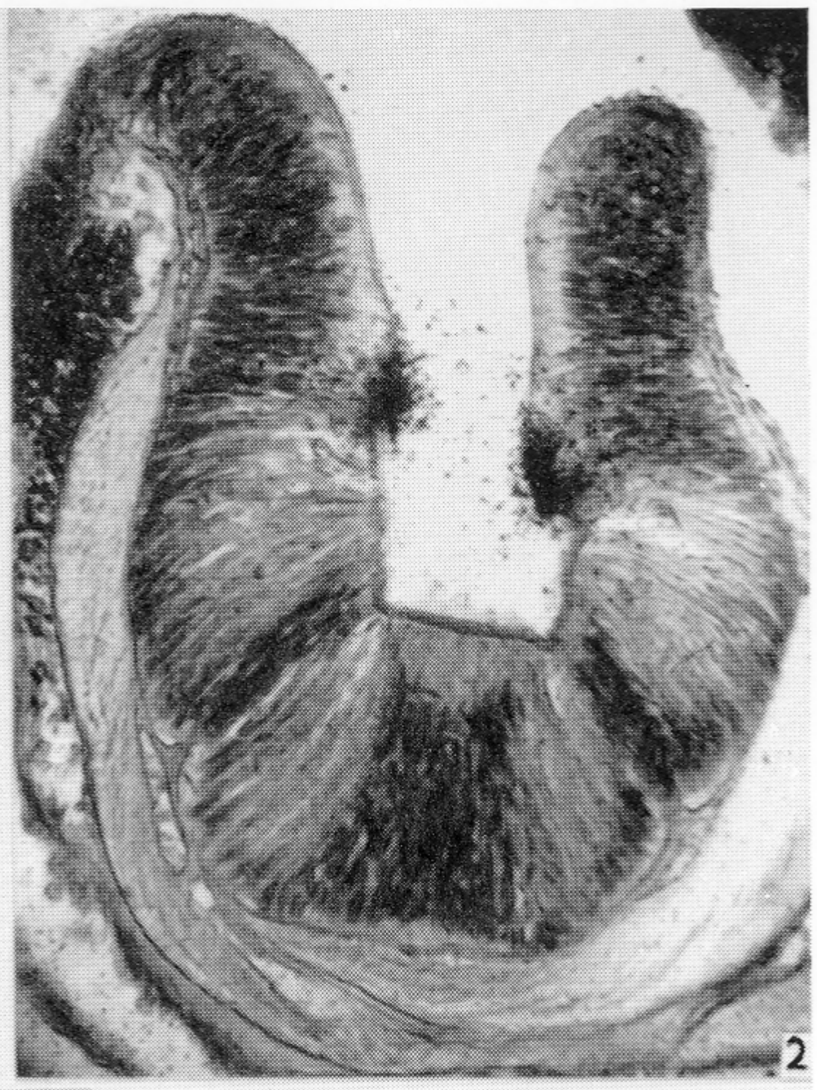
BARRINGTON, E. J. W., 1957. The distribution and significance of organically bound iodine in the ascidian *Ciona intestinalis* Linnaeus. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 1–15.

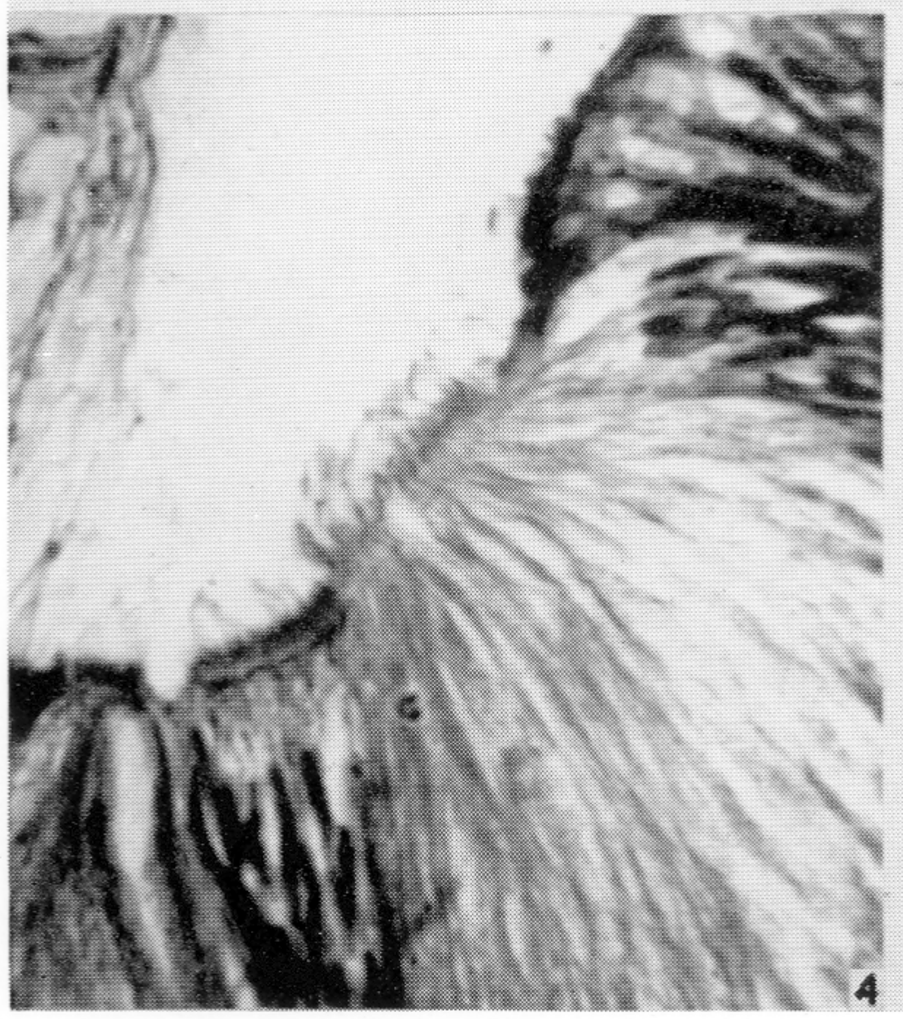
BARRINGTON, E. J. W. & FRANCHI, L. L., 1956*a*. Organic binding of iodine in the endostyle of *Ciona intestinalis*. *Nature, Lond.*, Vol. 177, p. 432.

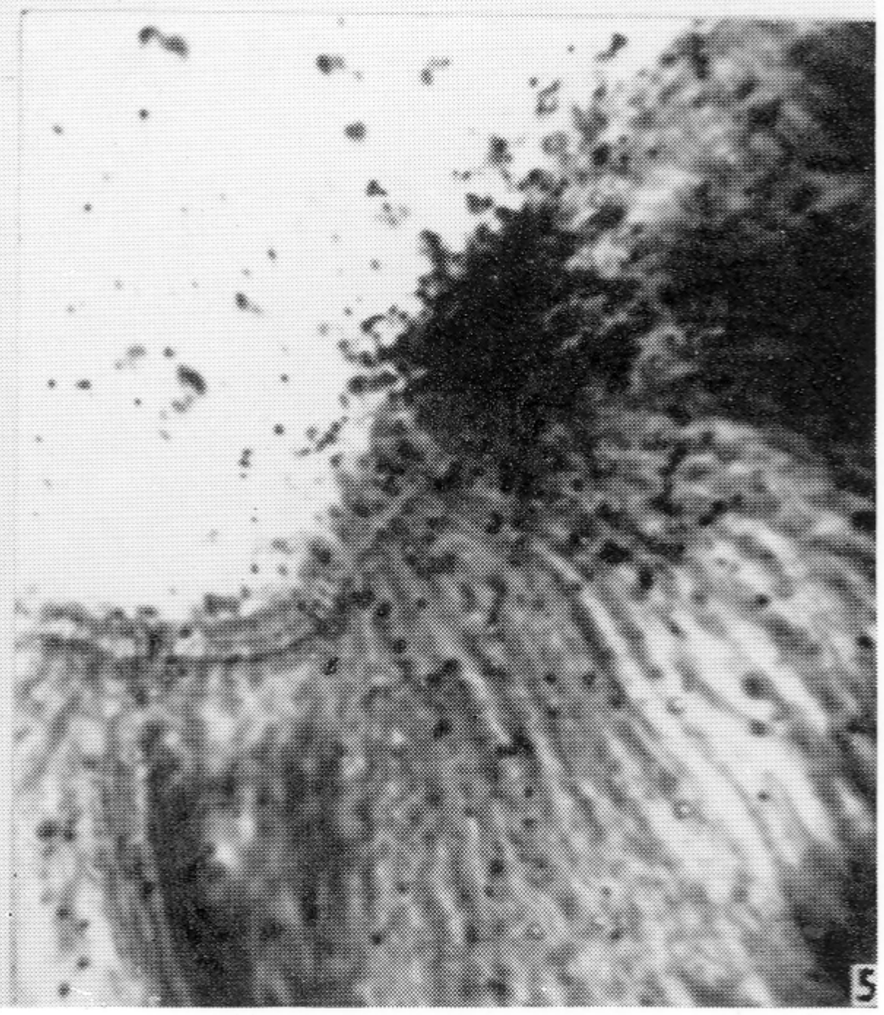
—— —— 1956*b*. Some cytological characteristics of thyroidal function in the endostyle of the ammocoete larva. *Quart. J. micr. Sci.*, Vol. 97, pp. 393–410.

GORBMAN, A., 1955. Some aspects of the comparative biochemistry of iodine utilization and the evolution of thyroidal function. *Physiol. Rev.*, Vol. 35, pp. 336–46.









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GORBMAN, A. & CREASER, C. W., 1942. Accumulation of radio-active iodine by the endostyle of larval lampreys and the problem of homology of the thyroid. *J. exp. Zool.*, Vol. 89, pp. 391–406.

LISON, L., 1954. Alcian blue 8 G with chlorantine fast red 5 B. A technic for selective staining of mucopolysaccharides. *Stain Tech.*, Vol. 29, pp. 131–8.

MOŽEJKO, B., 1913. Mikrotechnische Mitteilungen. X. Karminfütterung des *Amphioxus*. *Z. wiss. Mikr.*, Bd. 30, pp. 59–67.

ORTON, J. H., 1913. The ciliary mechanisms of the gill and the mode of feeding in *Amphioxus*, ascidians and *Solenomya togata*. *J. mar. biol. Ass. U.K.*, Vol. 10, pp. 19–49.

PEARSE, A. G. E., 1954. *Histochemistry, Theoretical and Applied*. London: Churchill.

SEMBRAT, K., 1953. Effect of the endostyle of the lancelet (*Branchiostoma lanceolatus* Pall.) on the metamorphosis of axolotl. *Zool. Polon.*, Vol. 6, pp. 3–19.

STEEDMAN, H. F., 1950. Alcian blue 8 GS: A new stain for mucin. *Quart. J. micr. Sci.*, Vol. 91, pp. 477–9.

THOMAS, I. M., 1956. The accumulation of radioactive iodine by *Amphioxus*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 203–10.

EXPLANATION OF PLATE I

Fig. 1. Transverse section of the endostyle of *Amphioxus*, which should be compared with Text-fig. 1 for the identification of the several zones. Zones 2 and 4 (ventral and dorsal glandular tracts) can be distinguished by the lighter staining and more basal location of their nuclei. The PAS-positive secretion (slightly darker) at the distal ends of the zone 5 cells is clearly visible on the right side of the endostyle at the point where the epithelium swells slightly to the left. PAS, Harris's haematoxylin and tartrazine.

Fig. 2. Autoradiograph of a section adjacent to that of fig. 1. The black iodine image is centred over the tips of the zone 5 cells.

Fig. 3. Autoradiograph of a transverse section of the left side of an endostyle. Zone 3, with dark nuclei, lies at the right-angled bend; zones 2 and 1 lie to its right, while zone 4 (dorsal glandular tract) lies above it. Zone 5 lies immediately above zone 4 and continues into zone 6. An unusually light iodine image has been obtained as a result of the use of a slightly over-age stripping-film, and is clearly seen to extend into the lumen from the tips of the zone 5 cells; the small amount of secretion accumulated at the distal ends of these is represented by a negative image. Note the absence of any iodine image over the mucus-secreting cells of zone 1, which lie at the bottom right-hand corner; the background of scattered spots is a result of fogging.

Fig. 4. Transverse section of the right side of an endostyle, stained with alcian blue (Harris's haematoxylin as counterstain). Zone 4 (dorsal glandular tract) is the light area occupying the middle of the region photographed, and is completely negative. A strong positive response is seen in the distal ends of the zone 5 cells, lying immediately above zone 4; a weaker reaction is just detectable in zones 3 and 2, but a strong one is shown at the right edge of zone 1, which just comes into view at the extreme left margin.

Fig. 5. Autoradiograph of a section adjacent to that of Fig. 4. The centre of the dense black iodine image overlies the tips of the zone 5 cells, the position of which can be gauged by comparison with Fig. 4. Note the complete absence of iodine accumulation elsewhere, even over the edge of zone 1, despite the positive alcian blue (mucin) response which this shows in Fig. 4.

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THE CHEMISTRY OF ETHYLENEDIAMINE TETRA-ACETIC ACID IN SEA WATER

By C. P. SPENCER

Marine Biology Station, University College of North Wales

(Text-figs. 1–10)

In recent years, ethylenediamine tetra-acetic acid (EDTA) has been extensively used in culture media for unicellular algae. This, and other chelating agents are also becoming widely used in toxicity studies with plants and animals, in analysis and for controlling the ionic concentration of certain metal ions in a wide variety of studies.

EDTA forms complexes with a large number of metallic cations, and in the presence of sufficient EDTA only very small amounts of many metals can exist as free ions. The use of EDTA as a metal buffer system has been advocated by numerous workers. These metal buffer systems depend for their operation upon the equilibrium between the metal cations, the EDTA and the chelate. Providing that the concentrations of the chelate and the chelating ion are large compared with that of the free metal ion, such a system will tend to maintain a constant concentration of metallic ions, should these be continuously removed by a biological system.

The available data suggest that EDTA is not readily metabolized by most forms of life, and moreover it does not appear to be markedly toxic except by virtue of its metal-chelating reactions. Its use under the correct conditions for controlling metal ion concentrations in biological systems therefore has much to commend it.

The physical chemistry of metal–EDTA equilibria has been examined by several workers (Chaberek, Bersworth & Martell, 1955; Raaflaub, 1956) and methods for calculating the concentrations of free metal ion at equilibrium outlined. These treatments, however, are limited to simple cases when only one chelating metal ion is present. The biologist is inevitably faced with working with systems containing many reacting metal ions (e.g. in sea water). Data regarding the free metal ion concentrations existing in equilibrium with EDTA in such systems is of obvious importance if the use of EDTA is to be fully exploited. This paper records some data on the equilibria set up when EDTA is added to sea water and on the effect of variations of certain controlling factors.

THE DISSOCIATION OF EDTA

In distilled water EDTA behaves as a typical quadribasic weak acid.

$$H_4Y \rightleftharpoons H_3Y^{-1} + H^+$$
$$H_3Y^{-1} \rightleftharpoons H_2Y^{-2} + H^+$$
$$H_2Y^{-2} \rightleftharpoons HY^{-3} + H^+$$
$$HY^{-3} \rightleftharpoons Y^{-4} + H^+$$

where Y^{-4} represents the fully dissociated EDTA quadrivalent anion. The third and fourth dissociation constants may be determined directly from the titration curve which gives values of $pK_3 = 6{\cdot}1$ and $pK_4 = 10{\cdot}2$. The first and second dissociation constants must be determined indirectly and the reported values are $pK_1 = 1{\cdot}9$ and $pK_2 = 2{\cdot}6$ respectively (Calvin & Martell, 1953).

When chelation of a metallic ion occurs it results in the displacement of the weakly acidic protons, as for example in the reaction:

$$M^{2+} + H_2Y^{2-} \rightleftharpoons MY^{2-} + 2H^+ \qquad \ldots(1)$$

where M^{2+} represents a divalent metal cation.

Thus, when EDTA is added to sea water, chelation of the metallic ions causes a fall in pH. A typical titration curve for EDTA in sea water is shown in Fig. 1 (p. 134).

The curve of Fig. 1 was obtained by titrating sea water of 18·5‰ chlorinity containing 9×10^{-3}M disodium EDTA with 2×10^{-2}N-NaOH. The sea water contained 0·0023 equivalent per litre excess base as determined by the method of Mitchell & Rakestraw (1933). The titration was carried out in a carbon dioxide-free atmosphere. pH measurements were made by means of a glass electrode. Theoretically, two equivalents of alkali should be required to neutralize the EDTA. The discrepancy between the theoretical and the observed end-point is accounted for by the excess base present in the sea water.

The shape of the titration curve obtained is reminiscent of a strong acid–strong base titration and the effect of additions of EDTA to sea water followed by neutralization with alkali is comparable with the results of adding an equivalent quantity of any strong acid. It should be noticed that, due to the operation of the equilibrium (1), the system acts as a pH buffer in the region of pH 4–5. For most biological purposes, however, the pH of EDTA-treated sea water will be restored to pH 7 or above, and in this region the EDTA system has no buffering capacity and the bicarbonate–carbonate buffering system will therefore remain virtually unaffected. The pH of EDTA-treated sea water may therefore be adjusted by considering the EDTA to be acting as a strong acid and adjusting the resultant carbonate alkalinity and partial pressure of carbon dioxide as required.

These considerations apply only when an excess of strongly chelating cations is present. In sea water the concentration is reached at about $6{\cdot}0 \times 10^{-2}$M EDTA. At higher EDTA concentrations the system is more complex and the buffer capacity of the sea water will be affected at physiological pH values.

THEORETICAL TREATMENT OF SYSTEM AND DERIVATION OF FORMULAE

When EDTA is added to sea water a series of equilibria of the following type operate:

$$M_1^{2+} + Y^{4-} \rightleftharpoons M_1Y^{2-}$$

$$M_2^{2+} + Y^{4-} \rightleftharpoons M_2Y^{2-},$$

etc.,

where M^{2+} represents the divalent chelating cations present. In addition, the four dissociations of EDTA must be considered. At pH values above about 3–4, the concentrations of H_4Y and H_3Y^{1-} are not appreciable and the first and second dissociations can therefore be neglected for most biological purposes.

For multi-equilibria systems of this type it is possible to write a number of simultaneous equations.

Thus: $$\frac{[M_1Y^{2-}]}{[M_1^{2+}]\,[Y^{4-}]} = K_{M_1}, \quad \frac{[M_2Y^{2-}]}{[M_2^{2+}]\,[Y^{4-}]} = K_{M_2}, \quad \text{etc.},$$

where the quantities in brackets represent concentrations, and by rewriting the analogous equations for the third and fourth dissociations of EDTA in the form

$$\frac{[HY^{3-}]}{[H^+]\,[Y^{4-}]} = K_A \quad \text{and} \quad \frac{[H_2Y^{2-}]}{[H^+]^2\,[Y^{4-}]} = K_B,$$

where $K_A = 1/K_4$ and $K_B = 1/K_3K_4$, K_3 and K_4 being the dissociation constants for the third and fourth dissociations of EDTA. In addition we have the mass equations:

$$[\text{Total } M_1] = [M_1^{2+}] + [M_1Y^{2-}]$$

$$[\text{Total } M_2] = [M_2^{2+}] + [M_2Y^{2-}]$$

etc.

and $$[\text{Total } Y] = [M_1Y^{2-}] + [M_2Y^{2-}] + \text{ etc. } + [H_2Y^{2-}] + [HY^{3-}] + [Y^{4-}].$$

In general if a system consisting of n competing ions is considered it is possible to set up $(2n+1)$ simultaneous equations. Providing the values of the stability

constants (K_{M_1}, K_{M_2}, etc.) of the metal chelates, the values of the dissociation constants of EDTA (K_3 and K_4) and the total concentrations of EDTA (Total Y) and of the chelating cations (Total M_1, Total M_2, etc.) are known, then the system of equations contains $(2n+1)$ unknowns (i.e. M_1^{2+}, M_1Y^{2-}, M_2^{2+}, etc.) and may be solved for any of these.

When the case of EDTA in sea water is considered the value of n is large and the system of simultaneous equations becomes very cumbersome to solve by direct means. The following alternative treatment was therefore used.

Let $\dfrac{[M_1^{2+}]}{[Y^{4-}]}=a$, then $[M_1^{2+}]=a[Y^{4-}]$.

Similarly $[M_2^{2+}]=b[Y^{4-}]$,

etc.

Also let $\dfrac{[M_1Y^{2-}]}{[Y^{4-}]}=\alpha$, then $[M_1Y^{2-}]=\alpha[Y^{4-}]$.

Similarly $[M_2Y^{2-}]=\beta[Y^{4-}]$.

And let $\dfrac{[H_3Y^{3-}]}{[Y^{4-}]}=\lambda$, then $[HY^{3-}]=\lambda[Y^{4-}]$,

and $[H_2Y^{2-}]=\mu[Y^{4-}]$.

And let $\dfrac{1}{[Y^{4-}]}=f$.

Then from the equations for the stability constants of the metal chelates and for the dissociation constants of EDTA

$$K_{M_1}=f(\alpha/a),\quad K_{M_2}=f(\beta/b),\quad \text{etc.}$$

Also $$K_A[H^+]=\lambda \quad \text{and} \quad K_B[H^+]^2=\mu.$$

Now $$[\text{Total } M_1]=[M_1^{2+}]+[M_1Y^{2-}]$$

$$=a[Y^{4-}]+\alpha[Y^{4-}].$$

$$f[\text{Total } M_1]=a+\alpha$$

$$=\alpha+f\alpha/K_{M_1}$$

$$\alpha=\frac{f[\text{Total } M_1]}{1+f/K_{M_1}}$$

Similarly $$\beta=\frac{f[\text{Total } M_2]}{1+f/K_{M_2}},$$

etc.

Now

$$[\text{Total } Y]=[M_1Y^{2-}]+[M_2Y^{2-}]+\text{ etc. }+[H_2Y^{2-}]+[HY^{3-}]+[Y^{4-}]$$
$$=\alpha[Y^{4-}]+\beta[Y^{4-}]+\text{ etc. }+\mu[Y^{4-}]+\lambda[Y^{4-}]+[Y^{4-}].$$

$$f[\text{Total } Y]\alpha+\beta+\text{ etc. }+\mu+\lambda+1.$$

$$f[\text{Total } Y]=\frac{f[\text{Total } M_1]}{1+f/K_{M_1}}+\frac{f[\text{Total } M_2]}{1+f/K_{M_2}}+\text{ etc. }+K_A[H^+]+K_B[H^+]^2+1.$$

This equation may be solved for f. It will be seen that the function f is by definition the reciprocal of the concentration of the fully dissociated form of EDTA (i.e. Y^{4-}). In a system consisting of several cations each competing for the Y^{4-} anion, it is the concentration of this ion, together with the total amount of a particular metal species present and the appropriate stability constant, which determines the equilibrium concentration of the unchelated metal ions. All the chelating cations present will affect to a greater or less extent the value of f for a certain concentration of EDTA. When the general equation has been solved for f for a particular case the concentration of Y^{4-} can be calculated. Substitutions of this value in the equation

$$[M^{2+}]=\frac{[\text{Total } M]}{K_M[Y^{4-}]+1}$$

together with the appropriate values of [Total M] and K_M allows the calculation of $[M^{2+}]$.

The general equation for f is only directly applicable to a system of any number of competing metallic ions which react with Y^{4-} to give chelates of the type MY^{2-}. If chelates of other structure are found the form of the terms in the general equation corresponding to these chelates alters. For such chelates it is in general a simple matter to recast the equilibrium constant equations so that they can be incorporated in the general formula.

As an example we may consider the case of calcium. This forms chelates with both the Y^{4-} and HY^{3-} anions of EDTA according to the following reactions (Calvin & Martell, 1953):

$$Ca^{2+}+Y^{4-}\rightleftharpoons CaY^{2-}$$
$$Ca^{2+}+HY^{3-}\rightleftharpoons CaHY^{1-}.$$

Hence $$\frac{[CaY^{2-}]}{[Ca^{2+}][Y^{4-}]}=K_1 \quad \text{and} \quad \frac{[CaHY^{1-}]}{[Ca^{2+}][HY^{3-}]}=K_2.$$

The stability constants for both reactions are known and from these and the appropriate proton association constant of EDTA

$$K_A=\frac{[HY^{3-}]}{[H^+][Y^{4-}]}$$

may be derived a fourth constant K′:

$$K' = \frac{[CaHY^{1-}]}{[Ca^{2+}][Y^{4-}][H^+]} \quad \text{where} \quad K' = K_2 \times K_A.$$

The equations for K_1 and K' are now in a suitable form for manipulation by the procedure used in deriving the general formula. It can be shown that this substitution results in two terms

$$\frac{f[\text{Total Ca}]\,K_1}{f+K_1+K'[H^+]} \quad \text{and} \quad \frac{f[\text{Total Ca}]\,K'[H^+]}{f+K_1+K'[H^+]}$$

in place of the usual single term of the form $\frac{f[\text{Total } M]}{1+f/K_M}$ as in the case of ions forming only one chelate.

Insertion of these terms in the general formula and solving for f enables each term to be evaluated and hence the concentration of Ca^{2+}, CaY^{2-} and $CaHY^{1-}$ calculated for a specific case. Calculation shows that in sea-water solutions of EDTA, formation of the $CaHY^{1-}$ chelate is negligible at pH values of 5 and above. At pH 4 the influence of $CaHY^{-1}$ formation on the value of f is only slight and for most purposes can be neglected. This approximation is only valid if the concentration of HY^{3-} is kept low by the presence of a stoichiometric excess of strongly chelated cations. If appreciable formation of $CaHY^{-1}$ does occur it results in a reduction in the concentration of Ca^{2+}.

The general formula includes terms to allow for the effect of the third and fourth dissociation constants of EDTA. If systems at pH values below 4 are to be considered the first and second dissociations will become important. In such cases the pH-dependent terms in the general formula must be extended to include the terms $+K_C[H^+]^3$ and $+K_D[H^+]^4$, where

$$K_C = \frac{1}{K_2K_3K_4} \quad \text{and} \quad K_D = \frac{1}{K_1K_2K_3K_4}.$$

APPLICATION OF THE GENERAL FORMULA TO SEA WATER CONTAINING EDTA

The constants quoted in Table 1 are the apparent stability constants determined in solutions of known ionic strength ($\mu = 0{\cdot}1$). These constants are related to the thermodynamic dissociation constants K by the relationship

$$K = K' \frac{\gamma MY^{2-}}{\gamma M^{2+} \times \gamma Y^{4-}},$$

where K' is the apparent dissociation constant and γMY^{2-}, γM^{2+} and γY^{4-} are the activity coefficients of the metal–EDTA anion, the metal cation and the fully dissociated quadrivalent EDTA anion respectively.

Due to the nature of the ions relatively large variations in the activity coefficients and the apparent value of K with ionic strength can be expected. The reported constants are not therefore strictly applicable to the equilibria set up in EDTA solution in sea water. There is insufficient data for a rigorous treatment to assess the variations in the individual apparent constants with variations of ionic strength. It seems justifiable, however, to attempt an approximate treatment on the basis of the data available.

TABLE 1. STABILITY CONSTANTS OF THE EDTA CHELATES OF SOME BIOLOGICALLY IMPORTANT IONS (CALVIN & MARTELL, 1953)

Na^{+}	1·66	Mn^{2+}	13·4
Sr^{2+}	8·36	Co^{2+}	16·1
Mg^{2+}	8·69	Zn^{2+}	16·1
Ca^{2+}	10·59	Cu^{2+}	18·3
Ca^{2+}	3·51*		

Unless otherwise stated the values given are the log K values for the MY^{2-} chelate.

$$*K=\frac{CaHY^{-1}}{Ca^{2+}\,HY^{-3}}.$$

Carini & Martell (1952) have investigated the changes with ionic strength of the apparent stability constant of the calcium–EDTA chelate. Their results show that in sea water of 19‰ Cl the apparent stability constant will be reduced by about a power of 10 compared with its value at $\mu=0{\cdot}1$. Variations will also be caused in the values of the apparent dissociation constants of EDTA, but these can be neglected for the present approximation.

It seems reasonable to suppose that the variation in the activity coefficients γMY^{2-}, γM^{2+} with changes in ionic strength will be of the same order for all divalent metallic cations and as an approximation the apparent stability constants to be used in sea-water solutions can therefore be reduced by a similar factor. This approximation can only be justified in the case of equilibria involving divalent cations and the quadrivalent EDTA anion. As will be seen from what follows below, this limitation still allows the calculation of some data of value.

The reacting cations present in sea water fall into two groups. On the one hand there are the major constituents, Na^{+}, Mg^{2+}, and Ca^{2+} which are present in concentrations of the order of 10^{-2}M or above, and on the other the remaining cations present in micro- or submicromolar concentrations. Application of the general formula in the specific case of the addition of a certain concentration of EDTA to sea water indicates that for any particular case the value of f is largely controlled by a relatively small number of the chelating ions present. The dominance of an ion in this role is determined by its concentration relative to the concentration of EDTA and by the value of the stability constant of its chelate. It is thus possible to ignore the effects of many of the ions when calculating the value of f for a certain concentration of EDTA. With EDTA concentrations between 10^{-2} and 10^{-6}M the influence of

the chelating reactions involving the cations of magnesium and calcium are predominant. In the case of calcium only the formation of the CaY^{2-} chelate is important, since the term corresponding to the formation of $CaHY^{3-}$ has little effect on the value of f.

Fig. 2 shows the variation in the value of f when various quantities of EDTA are added to sea water at pH 8·0. The values of f plotted in this curve were

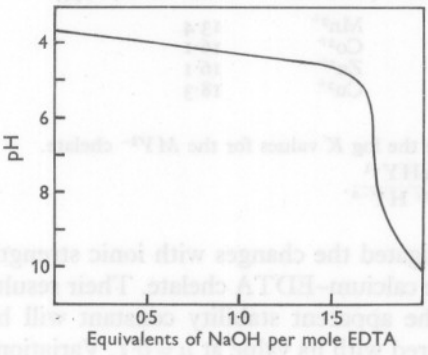

Fig. 1

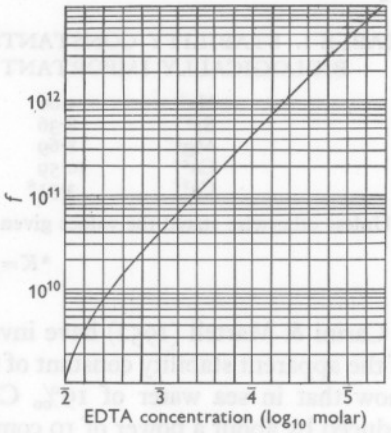

Fig. 2

Fig. 1. Titration curve for a solution of EDTA in sea water.

Fig. 2. Variation of f with the concentration of EDTA in sea water of 19‰ Cl at pH 8·0.

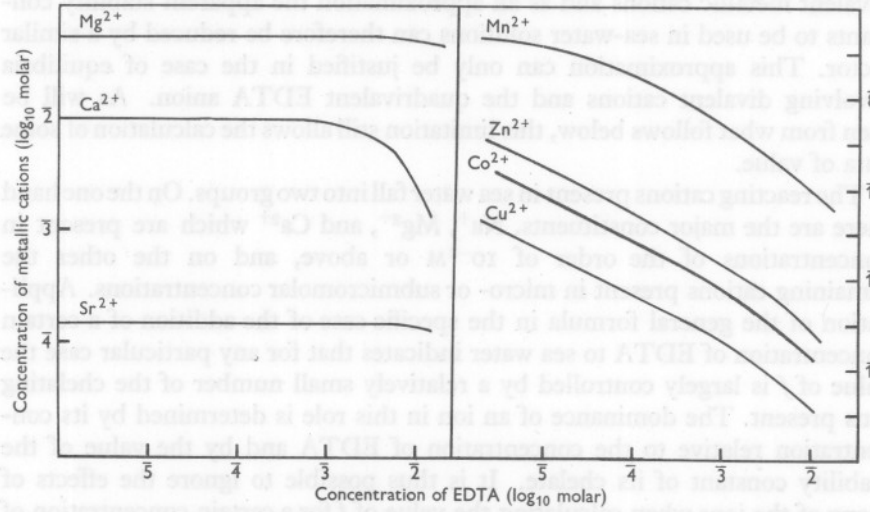

Fig. 3. Variation of the concentration of certain metallic cations in equilibrium with various concentrations of EDTA in sea water of 19‰ Cl at pH 8·0.

obtained by applying the general formula to solutions of EDTA in sea water of 19‰ chlorinity and cationic composition reported as typical by Sverdrup, Johnson & Fleming (1942). As a first approximation the influence on the value of f of all cations other than magnesium and calcium was neglected.

Values of f taken from the curve are of sufficient accuracy for many purposes. If greater accuracy is required the curve may be used for obtaining an approximate root of the equation which may then be further refined to the desired degree.

Using the value of f obtained from the curve in Fig. 1 the concentrations at equilibrium of the unchelated portions of several biologically important cations have been calculated for a range of EDTA concentrations. The results are shown in Fig. 3. The total concentrations of the various cations used in these calculations are taken from the same authority as the data for the construction of Fig. 2.

An examination of the ultra-violet absorption spectrum of appropriate mixtures of calcium and magnesium ions in the presence of EDTA provides some experimental verification of the theoretical results set out in Fig. 3 above. EDTA itself absorbs strongly below about 260 mμ. Chelation with calcium or magnesium causes a shift of the absorption to shorter wavelengths. The absorption maxima cannot be determined at the concentrations which have been used and precise measurements are thus not possible. However, the curves obtained when either the calcium or magnesium chelate is used are separated (Fig. 4). If mixtures of calcium and magnesium ions in the presence of EDTA are examined, at relative concentrations such that the calcium is in stoichiometric excess of the EDTA, it is seen that the absorption spectrum of the mixture approximates to that of the calcium chelate, indicating that little magnesium is chelated under these conditions. This is as predicted by the theoretical treatment. If the concentration of EDTA is increased until it approaches the stoichiometric amount required by the calcium the absorption spectrum of the mixture starts to deviate slightly from that of the pure calcium chelate. Marked deviation, indicating appreciable formation of the magnesium

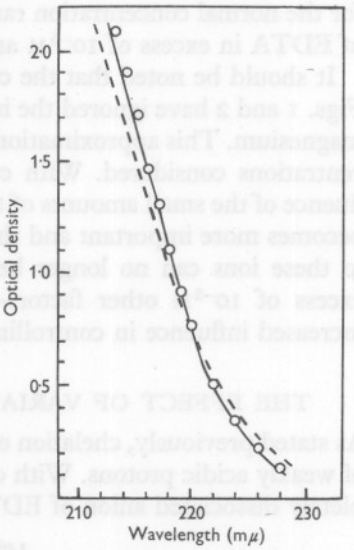

Fig. 4. Absorption spectra of 5×10^{-3} EDTA in M/15 phosphate buffer at pH 7·0 with additions of: (*a*) 5×10^{-3}M-Ca^{2+}, ————; (*b*) 5×10^{-2}M-Mg^{2+}, – – – –; and (*c*) 5×10^{-3}M-Ca^{2+} + 5×10^{-2} M-Mg^{2+}, ○ ○ ○. Absorption measured in 1 cm cuvette against phosphate buffer as blank using a Unicam SP 500 spectrophotometer.

chelate does not, however, occur until the EDTA concentration is equal to or exceeds the concentration equivalent to the calcium.

The case of iron has so far been omitted from the discussion and no data for the concentration of either ferrous or ferric ions which can exist in equilibrium with various concentrations of EDTA in sea water is given in Fig. 3. For several reasons the case of iron is more complicated and it will be discussed elsewhere. For most purposes, however, it is safe to assume that the presence of iron will have little effect upon the other equilibria and this is certainly true for the normal concentration range of iron found in sea water when additions of EDTA in excess of 10^{-5}M are used.

It should be noted that the calculations involved in obtaining the data for Figs. 1 and 2 have ignored the influence of all cations, other than calcium and magnesium. This approximation is reasonable within the range of EDTA concentrations considered. With concentrations of the order of 10^{-6}M the influence of the small amounts of the more strongly chelated ions such as copper becomes more important and the terms in the general formula corresponding to these ions can no longer be ignored. With concentrations of EDTA in excess of 10^{-2}M other factors—especially the pH of the system—have an increased influence in controlling the value of f.

THE EFFECT OF VARIATIONS OF PH ON THE EQUILIBRIA

As stated previously, chelation of cations by EDTA involves the displacement of weakly acidic protons. With certain exceptions chelation involves the completely dissociated anion of EDTA

$$M^{2+} + Y^{4-} \rightleftharpoons MY^{2-}$$

and the tendency for hydrogen ions to associate with Y^{4-}

$$H^{+} + Y^{4-} \rightleftharpoons HY^{3-}$$

may thus be considered to be direct competition with metallic cation. The second proton association

$$H^{+} + HY^{3-} \rightleftharpoons HY^{2-}$$

will compete indirectly. As before, the effect of the third and fourth proton association can be neglected when considering systems at pH values above 4·0.

Fig. 5 shows the variations with pH of the sum of the two pH-dependent terms in the general formula. $K_A[H^+] + K_B[H^+]^2$ increases rapidly with falling pH, reaching values of the order of 10^6 at pH 5. When the general formula is applied to 19‰ sea-water solutions of EDTA, the terms corresponding to the ions of magnesium and calcium, which are of dominant influence in controlling the value of f, are of the order of 10^7 and above. It therefore follows that at pH values of 6 and above the pH will have little effect on the equilibria set up when EDTA is added to sea water. Below a pH

of 6 the two pH-dependent terms will increasingly affect the value of f and hence the equilibrium concentration of the unchelated metallic cations.

The values of the first and second proton association constants are such as to suggest at first sight that the more stable first proton association might be expected to have a marked effect, especially since the value of the association constant for this proton is of the same order as the formation constant of the

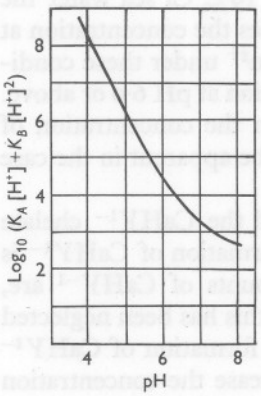

Fig. 5

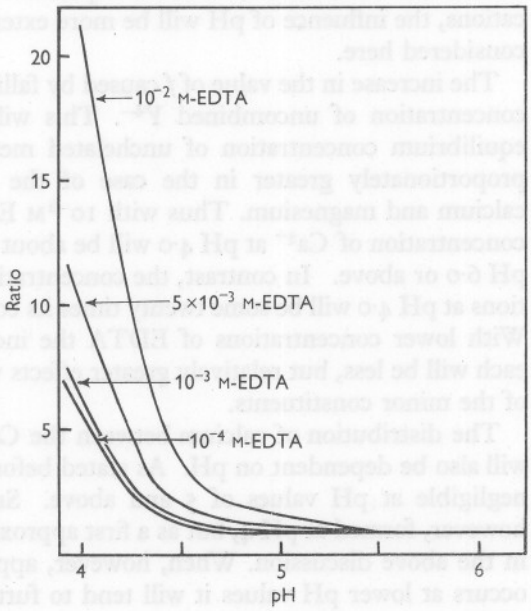

Fig. 6

Fig. 5. Variations with pH of the two pH-dependent terms in the general formula. Vertical axis shows value of $\log_{10}(K_A[H^+]+K_B[H^+]^2)$

Fig. 6. Ratio of f at various pH values to f at pH 8·0 in sea water of 19‰ Cl with various concentrations of EDTA.

calcium chelate and 10^2 greater than that of the magnesium chelate. In fact, over the pH range 8–10 when the effect of the first proton association might be expected to show, the pH has little effect on the value of f and hence on the position of the equilibria. Presumably in this pH range the mass effect of the hydrogen ions is so low compared with the 10^8 times greater amounts of calcium and magnesium that this proton association is of negligible influence in controlling the concentration of Y^{4-} compared with the chelation of calcium and magnesium.

As can be seen from Fig. 6 the relative effect of pH on the value of f varies with the concentration of EDTA added to the sea water. The effect is apparent at higher pH values and is of greater magnitude at the lower pH values, the

more nearly the concentration of EDTA approaches the total concentration of strongly chelated cations present. With 19‰ Cl sea water this represents about 6×10^{-2}M EDTA. Additions of EDTA to 19‰ Cl sea water are in any case limited to about this concentration by solubility considerations. It is, however, possible that EDTA may be used in diluted sea water and in such cases it should be noticed that if the concentration of EDTA is equal to or exceeds the stoichiometric amount required to chelate all the strongly chelated cations, the influence of pH will be more extensive than under the conditions considered here.

The increase in the value of f caused by falling pH reflects a decrease in the concentration of uncombined Y^{4-}. This will result in an increase in the equilibrium concentration of unchelated metal cations, the increase being proportionately greater in the case of the micro-constituents than with calcium and magnesium. Thus with 10^{-2}M EDTA in 19‰ Cl sea water the concentration of Ca^{2+} at pH 4·0 will be about four times the concentration at pH 6·0 or above. In contrast, the concentration of Mn^{2+} under these conditions at pH 4·0 will be some twenty times its concentration at pH 6·0 or above. With lower concentrations of EDTA the increases in the concentration of each will be less, but relatively greater effects will still be apparent in the case of the minor constituents.

The distribution of calcium between the CaY^{2-} and the $CaHY^{1-}$ chelate will also be dependent on pH. As stated before, the formation of $CaHY^{1-}$ is negligible at pH values of 5 and above. Small amounts of $CaHY^{-1}$ are, however, formed at pH 4, but as a first approximation this has been neglected in the above discussion. When, however, appreciable formation of $CaHY^{1-}$ occurs at lower pH values it will tend to further decrease the concentration of Y^{4-}.

THE EFFECTS OF VARIATIONS IN THE SALINITY ON THE EQUILIBRIA

Variations in the salinity of the sea water to which additions of EDTA are made will affect the equilibria due to: (*a*) variations in the ionic strength of the medium, and (*b*) variations in the total concentrations of the chelating cations.

Fig. 7 shows the relative variations of f with three concentrations of EDTA in sea water of various salinities. As the salinity falls the decreasing ionic strength is associated with a rise in the apparent stability constants of the chelates and a concomitant increase in chelation. At the same time the total concentration of the chelating cations decreases, with the result that the EDTA is present in proportionately greater quantities.

As can be seen from the appropriate curves in Fig. 7 the relative importance of these two effects depends upon the EDTA concentration. With EDTA concentrations of 10^{-2}M decreasing salinity causes an immediate fall in the

value of f, the influence of decreasing ionic strength being masked by the mass effect. With lower concentrations of EDTA, however, falling salinity is at first accompanied by an increase in the value of f due to the lowering of the ionic strength. When the total concentration of chelated cations falls below about 6 times the concentration of EDTA, the mass effect becomes dominant and the value of f falls sharply.

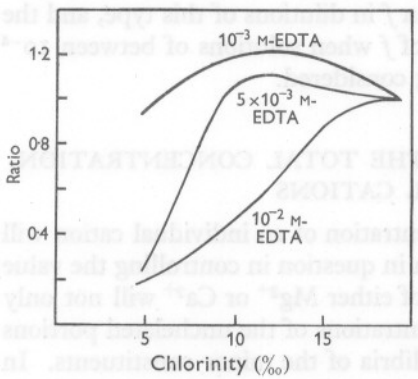

Fig. 7

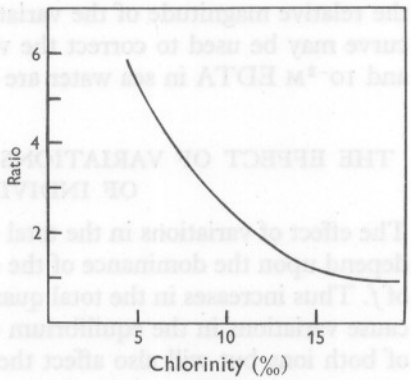

Fig. 8

Fig. 7. Ratio of f in sea waters of various chlorinities to f at 19‰ chlorinity with various concentrations of EDTA.

Fig. 8. Ratio of f at various dilutions of a 10^{-3}M solution of EDTA in sea water of 19‰ Cl at pH 8·0.

Both the increase in the value of f due to decreased ionic strength and the decrease in f caused by the mass effect are accompanied by a decrease in the concentration of unchelated metallic cations. The rise in the value of f between 19 and 10‰ Cl with 5×10^{-3}M EDTA, for example, is a reflexion of the rising stability constants which are paralleled by increasing chelation. Similarly, with the same strength of EDTA below 10‰ Cl, the rapidly falling value of f due to the mass effect is accompanied by a further decrease in metallic cation concentration, since the total concentration of the metals is decreasing.

The fall in metallic cation concentration is only slight, due to the effect of the ionic strength, but more marked due to the mass effect. The combined effect is proportionately greater with the minor constituents than with such ions as calcium and magnesium. For example, with additions of 5×10^{-3}M EDTA to sea water of 19‰ Cl the resulting concentrations of Ca^{2+} and Cu^{2+} are about $5{\cdot}5 \times 10^{-3}$M and $4{\cdot}9 \times 10^{-15}$M respectively. If the same addition is made to 15‰ Cl sea water the concentrations become $3{\cdot}53 \times 10^{-3}$M and $2{\cdot}6 \times 10^{-15}$M. With sea water of 5‰ Cl, however, the concentration of Ca^{2+} will fall to $1{\cdot}3 \times 10^{-4}$M and that of Cu^{2+} to about $5{\cdot}8 \times 10^{-17}$M.

It may sometimes happen that sea water containing EDTA is diluted with

distilled water. In this case not only is the ionic strength of the medium reduced and the total concentration of metallic cation, but also the total concentration of EDTA is proportionately reduced. Fig. 8 shows the variation in the value of f when sea water of 19‰ Cl containing 10^{-3}M EDTA is diluted. The proportionate decrease in the total amount of EDTA with decreases in the concentration of the other constituents causes a progressive increase in the value of f. The concentration of EDTA has little effect upon the relative magnitude of the variations in f in dilutions of this type, and the curve may be used to correct the value of f when solutions of between 10^{-4} and 10^{-2}M EDTA in sea water are being considered.

THE EFFECT OF VARIATIONS IN THE TOTAL CONCENTRATION OF INDIVIDUAL CATIONS

The effect of variations in the total concentration of an individual cation will depend upon the dominance of the cation in question in controlling the value of f. Thus increases in the total quantity of either Mg^{2+} or Ca^{2+} will not only cause variations in the equilibrium concentrations of the unchelated portions of both ions but will also affect the equilibria of the minor constituents. In contrast, a small increase in, for example, the total amount of copper present, will have only a negligible effect on the value of f, and only the copper equilibrium will thus be affected. If, however, the total amount of copper is increased to a point where the appropriate term in the general formula is no longer of negligible influence on the value of f, then further increases in the total copper present will affect not only the Cu^{2+} equilibrium but also all the other equilibria.

The effects of increasing the total concentration of calcium in a 10^{-3}M solution of EDTA in 19‰ Cl sea water at pH 8·0 will be considered first. As the total amount of calcium present is increased the value of f increases. As shown in Fig. 9, f is directly proportional to the total concentration of calcium for a particular concentration of EDTA. The value of the proportionality constant is greater the more nearly the concentration of EDTA approaches the total concentration of strongly chelated cations. In 19‰ Cl sea water, decreases in the concentration of EDTA below 10^{-3} M have little further effect on this proportionality constant.

The increase in f caused by increased total amounts of calcium reflects increases in the equilibrium concentrations of all the strongly chelated cations. Table 2 gives the equilibrium concentration of several cations in a 10^{-3} M solution of EDTA in 19‰ Cl sea water at pH 8·0 appropriate to various total amounts of calcium. The concentrations of free cations increase linearly with increasing total amounts of calcium, the relative increase in each cation being approximately equal, with a given concentration of EDTA, for all cations regardless of the amount present or the value of their respective stability

constants. The greater increases in f with higher concentrations of EDTA reflect greater increases in the equilibrium concentration of each metallic cation. Nevertheless, even with 10^{-2}M solutions of EDTA, the relative increase in free cations caused by addition of calcium remains approximately the same for a considerable range of calcium additions.

TABLE 2. EFFECT OF INCREASING TOTAL AMOUNT OF CALCIUM IN 10^{-3}M SOLUTIONS OF EDTA IN 19‰ CL SEA WATER AT pH 8·0

Total Ca present (molar)	Molar equilibrium concentrations		
	Ca^{2+}	Mn^{2+}	Cu^{2+}
$1{\cdot}0 \times 10^{-2}$	$9{\cdot}8 \times 10^{-3}$	$3{\cdot}0 \times 10^{-9}$	$3{\cdot}9 \times 10^{-14}$
$2{\cdot}0 \times 10^{-2}$	$1{\cdot}9 \times 10^{-2}$	$6{\cdot}0 \times 10^{-9}$	$7{\cdot}8 \times 10^{-14}$
$5{\cdot}0 \times 10^{-2}$	$4{\cdot}9 \times 10^{-2}$	$1{\cdot}5 \times 10^{-8}$	$2{\cdot}0 \times 10^{-13}$

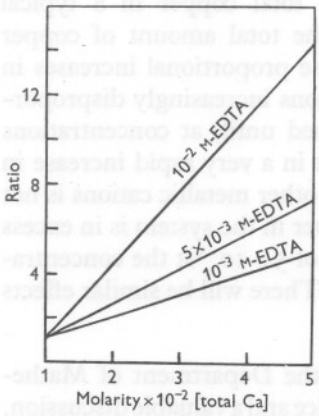

Fig. 9

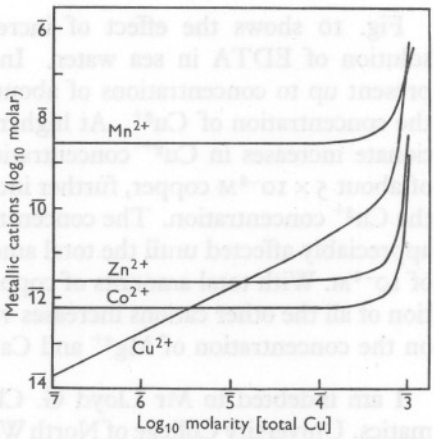

Fig. 10

Fig. 9. Ratio of f at various total calcium concentrations to f at 10^{-2}M total calcium in 19‰ Cl sea-water solutions of EDTA at pH 8·0.

Fig. 10. Variation in the concentration of certain metallic cations in 10^{-3}M solutions of EDTA in 19‰ Cl sea water at pH 8·0 with additions of various amounts of copper.

In contrast, if the total concentration of a minor constituent is increased, small increases have no appreciable effect on the value of f and only the concentration of the free cations of the metal in question will increase. When the total concentration of such a minor constituent is increased above a certain critical value, however, the corresponding term in the general formula will become of significance in controlling the value of f.

Table 3 gives the values of f appropriate to several total concentrations of some biologically important minor constituents. The concentration at which a particular metal will start to affect the other equilibria and the magnitude of the effect produced depend upon the stability constant of the chelate of the

metal in question. Thus the other equilibria will be affected sooner by increases in the total amount of copper than by increases in less firmly bound metals such as manganese.

TABLE 3. VARIATIONS OF f IN 10^{-3}M EDTA SOLUTIONS IN 19‰ CL SEA WATER AT pH 8·0 WITH CHANGES IN THE TOTAL AMOUNTS OF CERTAIN BIOLOGICALLY IMPORTANT MINOR CONSTITUENTS

Total concentration of metal (molar)	f		
	Manganese	Zinc	Copper
10^{-5}	$3{\cdot}9 \times 10^{10}$	$3{\cdot}9 \times 10^{10}$	$3{\cdot}9 \times 10^{10}$
10^{-4}	$3{\cdot}9 \times 10^{10}$	$3{\cdot}9 \times 10^{10}$	$4{\cdot}2 \times 10^{10}$
5×10^{-4}	$7{\cdot}9 \times 10^{10}$	$8{\cdot}1 \times 10^{10}$	$8{\cdot}2 \times 10^{10}$
10^{-3}	$3{\cdot}5 \times 10^{11}$	$7{\cdot}2 \times 10^{10}$	$9{\cdot}2 \times 10^{13}$

Fig. 10 shows the effect of increases in the total copper in a typical solution of EDTA in sea water. Increases in the total amount of copper present up to concentrations of about 10^{-5}M cause proportional increases in the concentration of Cu^{2+}. At higher concentrations increasingly disproportionate increases in Cu^{2+} concentrations are caused until, at concentrations of about 5×10^{-4}M copper, further increases result in a very rapid increase in the Cu^{2+} concentration. The concentration of the other metallic cations is not appreciably affected until the total amount of copper in the system is in excess of 10^{-4}M. With total amounts of copper in excess of 5×10^{-4}M the concentration of all the other cations increases very rapidly. There will be similar effects on the concentration of Mg^{2+} and Ca^{2+}.

I am indebted to Mr Lloyd G. Chambers of the Department of Mathematics, University College of North Wales, for advice and a valuable discussion.

SUMMARY

A general formula has been derived for the quantitative treatment of systems involving a number of interdependent equilibria. The formula has been applied specifically to the equilibria set up between metallic cations and their chelates when EDTA is added to sea water. This system involves competition for the fully dissociated quadrivalent anion of EDTA (the principal ion of EDTA which forms complexes) by the metallic cations and by the hydrogen ions present. The equilibrium concentration of a metallic cation in any particular case will depend upon the equilibrium concentration of the quadrivalent anion of EDTA, in the sense that the higher the concentration of this ion the lower will be the concentration of the metallic cation. The general formula allows the calculation of the equilibrium concentration of the quadrivalent EDTA anion, and from this the concentrations at equilibrium of each metallic cation can be readily found.

When solutions of EDTA in typical sea waters are considered quantitatively, it becomes apparent that relatively few of the metallic cations present contribute significantly to determining the equilibrium concentration of the EDTA quadrivalent anion. The mass action effect of the relatively large concentrations of calcium and magnesium in sea water causes these ions to be dominant in controlling the equilibria. Consequently, changes in the total amounts of either of these molecular species cause marked changes not only in the equilibria in which they are directly involved but also affect the other equilibria. In contrast, changes within certain limits in the total concentration of other metal species have but little effect on the equilibrium concentration of the EDTA quadrivalent anion, and so affect only the equilibrium involving the metal ion in question. The limits within which this applies are controlled by the concentration at which the mass effect of the metal species in question becomes great enough to cause that particular equilibrium to have a significant effect in controlling the concentration of the EDTA quadrivalent anion. The concentration at which this occurs depends in turn upon the magnitude of the stability constant of the metal–EDTA chelate in question. The more stable the chelate, the lower the upper limit of the concentration range over which the total amount of a metal may be altered without appreciably affecting the other equilibria.

Variations in the amount of EDTA present affect all the equilibria. With lower amounts of EDTA (up to about 10^{-4}M) there is an approximately inverse proportionality between the total amount of EDTA present and the concentration of unchelated metallic cations. As the total amount of EDTA approaches a value stoichiometrically equivalent to the total amount of divalent cations present (i.e. about 6×10^{-2}M in a typical sea water), there is a progressive disproportionate decrease in the equilibrium concentration of each metallic cation.

An examination of the effects of pH on the system shows that in sea-water solutions of EDTA, pH will have little effect on the equilibrium concentrations of free metal ions within the range pH 6–10. Below pH 6 increases in the metallic cation concentration will occur. These increases will be proportionately greater in the case of the minor constituents of sea water. The relative pH-independence of the system above pH 6 is limited to systems in which divalent metal cations are in stoichiometric excess of the EDTA.

The equilibria in EDTA–sea water systems are dependent upon the ionic strength of the medium and hence on the salinity. A fall in salinity with its concomitant decrease in ionic strength is associated with increased chelation.

Graphs are presented showing the variations of the reciprocal of the concentration of the quadrivalent EDTA anion (f) with variations in those factors affecting the equilibria which are summarized above. These will allow an approximate evaluation of the ionic concentrations of metallic cations in many cases which may be encountered in biological work. For more precise

calculations the interpolated values of f may be used as a first approximation to the root of the appropriate equation for f, which may then be refined to whatever degree is desired.

The general procedures used are applicable to many biological fluids other than sea water, and for similar systems of competing equilibria other than the formation of complexes.

REFERENCES

CALVIN, M. & MARTELL, A. E., 1953. *Chemistry of Metal Chelate Compounds*. New York: Prentice-Hall, Inc.

CARINI, F. F. & MARTELL, A. E., 1952. The effect of potassium chloride on the equilibrium between ethylene diamine tetra-acetate and calcium ions. *J. Amer. chem. Soc.*, Vol. 74, pp. 5745–8.

CHABEREK, S., BERSWORTH, F. C. & MARTELL, A. E., 1955. Chelating agents as metal buffers in biological systems. I. Principles and use of ethylene diamine tetra-acetic acid. *Arch. Biochem. Biophys.*, Vol. 55, pp. 321–37.

MITCHELL, P. H. & RAKESTRAW, N. W., 1933. The buffer capacity of sea water. *Biol. Bull., Woods Hole*, Vol. 65, pp. 437–51.

RAAFLAUB, J., 1956. Application of metal buffers and metal indicators in biochemistry. *Methods of Biochemical Analysis*, Vol. 3, pp. 301–25. New York: Interscience Publishers, Inc.

SVERDRUP, H. U., JOHNSON, M. W. & FLEMING, R. H., 1942. *The Oceans, their Physics, Chemistry and General Biology*. New York: Prentice-Hall, Inc.

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NEMATODES PARASITIC ON SEA WEEDS OF THE GENERA *ASCOPHYLLUM* AND *FUCUS*

By JOHN W. COLES

Department of Zoology, British Museum (Natural History)

(Text-figs. 1–3)

In 1892 Barton described galls found on the sea weed *Ascophyllum nodosum* from the Isle of Cumbrae, west coast of Scotland, and from Stonehaven, east coast of Scotland. The affected areas on the thallus of the plant appeared as swellings covered with small rounded nodules, and were almost invariably confined to the parts of the thallus just above or below the air vesicles. A transverse section through the swellings showed each nodule as a more or less hollow space containing numerous nematodes, specimens of which were sent to J. G. de Man, who (1892) described the worm as a new species, *Tylenchus fucicola*, the first-known marine tylenchid.

On the basis of de Man's description Cobb (N. A. Cobb in M. V. Cobb, 1933) erected the genus *Halenchus* for this species. Since then, other species of *Halenchus* have been described, but only as free-living forms.

From time to time galls on other sea weeds have been reported, but attributed to other animal agents, bacteria and fungi.

During visits by the author to the Laboratory of the Marine Biological Association at various times during 1955–57 galls were found very commonly on *Ascophyllum nodosum* in the Plymouth area (Wembury Bay). They agree well with Barton's description and contain numerous specimens of a nematode, which appears to be identical with de Man's form. Similar galls were also found at Croyde Bay on the north Devon coast in August 1955 (Fig. 1A).

Galls were also found on the sea weed *Fucus vesiculosus* (Fig. 1B) from Wembury Bay, Devon, and at Hannafore, Looe, Cornwall. They were found only on the older growths of the plant and occurred mainly on the stipes, and less frequently on the base of the fronds. The galls are quite noticeable and are usually a little lighter in colour than the rest of the stipes, but are not so large as some of those seen on *Ascophyllum*. The surface of the swellings is rugose and not so papillate as the galls on the other plant. When the galls were opened nematodes were found, but there were only a few in each in contrast with the galls on *Ascophyllum* which contain large numbers. At first the worms were thought to be identical with *Halenchus fucicola*, except that the anterior end appeared rather larger. On closer examination, however,

several small differences have been noted and are considered to be of sufficient significance to warrant the recognition of a distinct species.

Frequently non-tylenchid nematodes are found in the galls as well as the marine mite *Rhombognathus* (*Rhombognathopsis*) *seahami* (Hodge), kindly identified by Dr G. O. Evans. The nematodes appear to represent a variety of species no one of which appears to be consistently present; thus leading one to conclude that they are not the causal agents.

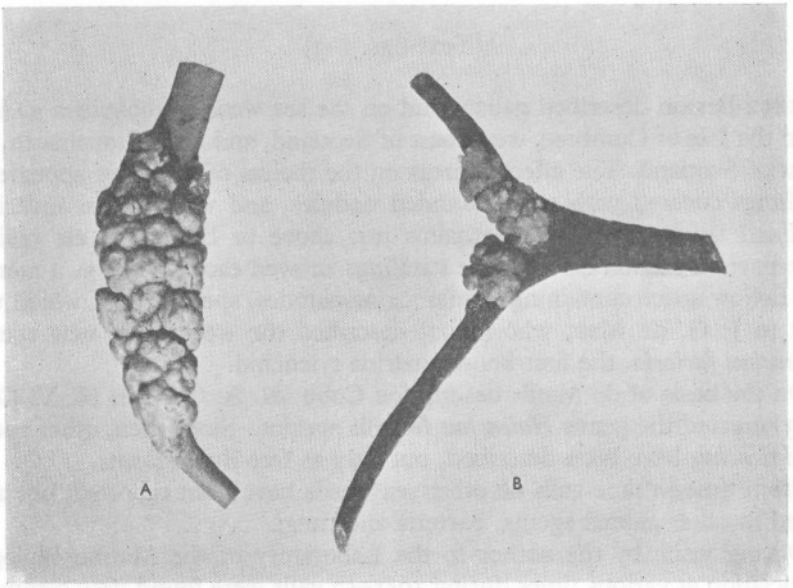

Fig. 1. Galls on (A) *Ascophyllum nodosum*, and (B) *Fucus vesiculosus*. (Approx. natural size.)

During a one-day visit to Lyme Regis, Dorset, in July 1957, galls were seen on the stipes of *Fucus vesiculosus*, and in some plants there were also galls on the fronds. In this locality galls were also seen on the stipes of *F. serratus*. On examination, these were found to be very similar to those on *F. vesiculosus*, and contained the same species of nematode.

The examination of the nematodes in the above material has been based on living specimens, as well as on others killed by heat, fixed in formalin and subsequently cleared in lactophenol and glycerine.

A redescription is given below of *Halenchus fucicola*, together with a description of the new species, *H. dumnonicus*.[1]

[1] From the Dumnonii, the British tribe that inhabited the south-west.

Halenchus fucicola (de Man, 1892) Cobb, 1933

Tylenchus fucicola de Man, 1892. *Festschr. zum Siebenzigsten Geburtstag Rudolf Leuckart*, pp. 121–5.
Anguillulina fucicola, Goodey, 1932. *J. Helminth.* Vol. 10, p. 27.
Halenchus fucicola, Cobb, 1933. *J. Parasit.* Vol. 20, p. 94.

Type host. In galls on the sea weed *Ascophyllum nodosum* Le Jol.

Material studied. From galls on *A. nodosum*, Wembury Bay, Devon, August 1955, June 1956, and May 1957.

Dimensions. Given in Table 1.

Long slender worms, gradually tapering at the extremities, with a characteristic hook-like tail, bent ventrally in both sexes. The cuticle is faintly transversely striated. The striations, which appear to be absent on the head-region, being about 1 μ apart. Lateral fields are present, which are about one-eighth of the body thickness; they appear to start at about half-way between the anterior end of the body and the excretory pore and can be traced to the tail region.

The head is marked off by a slight constriction, this latter being about 6 μ in diameter. There appear to be no distinct lips, the head-framework being divided into six sectors defined by faint ridges. Papillae are present on the two subdorsal and two subventral sectors, and the openings of the amphids are on the lateral ones. The amphidial pouches are situated laterally on either side of the stylet (Fig. 2C). The stylet is fairly well developed (15–16 μ long in the males and 15–18 μ long in the females) and is divided into two portions, a cylindrical posterior portion, with three well-pronounced basal knobs, and a conical anterior portion (Fig. 2C, D).

The excretory pore and its duct are well defined and characteristic, being heavily cuticularized. The pore is situated ventrally about one-eighth of the body length from the anterior end and the duct runs spirally in a posterior direction. The oesophagus is poorly developed. About mid-way along its length there is a slight swelling of the oesophageal wall associated with which is a somewhat ellipsoid widening of the lumen, probably representing a middle bulb. The lumen of the oesophagus can be seen in living specimens to have a wavy outline. The junction of the oesophagus and intestine is not clear, and was only seen in living specimens, where it occurred at about 20–25 μ anterior to the excretory pore. The granulated material of the intestine was seen to commence at this region and sometimes could be seen to run anteriorly into the oesophagus. Close behind the nerve ring, which is situated at the posterior end of the oesophagus, a dorsal oesophageal gland emerges from the wall of the oesophagus as a glandular diverticulum running alongside the intestine. This gland has a fairly well-defined nucleus and opens into the lumen of the oesophagus about 2–3 μ behind the base of the stylet. The distance of the extremity of the gland from the anterior

TABLE 1. DIMENSIONS OF *HALENCHUS FUCICOLA* (DE MAN, 1892) FROM WEMBURY BAY

Sample		Body length (μ)	Body length / Body thickness	Body length / Excretory pore	Body length / Length of tail (anus to tip)	Body length / Length of stylet	Body length / Length of spicules	V (%)
August 1955, ♂♂	Range	800–970	34·58–48·50	7·17–8·18	10·90–12·93	51·90–64·67	36·36–43·00	—
	Mean	891	38·10	7·78	11·73	58·67	40·70	—
	Standard deviation	±55·26	±7·08	±0·28	±0·63	±4·16	±2·18	—
	No. of specimens	10	10	10	10	10	10	—
August 1955, ♀♀	Range	970–1120	32·50–46·67	7·30–9·55	10·18–13·75	60·62–70·00	—	60·00–68·04
	Mean	1052·70	40·52	8·51	12·95	65·45	—	62·17
	Standard deviation	±54·50	±5·45	±0·56	±1·49	±3·31	—	±2·44
	No. of specimens	11	11	11	8	11	—	9
June 1956, ♂♂	Range	820–970	35·38–51·11	7·39–8·94	10·90–13·14	45·55–64·67	37·27–45·00	—
	Mean	883	42·06	8·11	11·63	56·50	41·68	—
	Standard deviation	±48·31	±4·69	±0·44	±0·75	±5·45	±2·12	—
	No. of specimens	10	10	10	9	10	10	—
June 1956, ♀♀	Range	980–1100	33·44–49·52	8·00–9·48	12·22–15·00	61·25–73·33	—	59·09–64·00
	Mean	1056	41·69	8·85	13·48	66·46	—	61·37
	Standard deviation	±41·35	±4·96	±0·48	±1·06	±3·55	—	±1·55
	No. of specimens	10	10	10	6	10	—	10
May 1957, ♂♂	Range	920–1060	35·92–45·91	7·36–7·92	11·05–12·80	57·50–67·33	40·00–55·71	—
	Mean	963	42·20	7·67	11·87	62·18	44·08	—
	Standard deviation	±43·72	±3·57	±0·17	±0·57	±3·19	±5·07	—
	No. of specimens	10	10	9	8	10	9	—
May 1957, ♀♀	Range	1040–1430	33·94–49·26	7·47–8·22	12·18–14·05	61·17–79·44	—	59·61–65·76
	Mean	1159	42·51	7·84	12·99	68·43	—	63·76
	Standard deviation	±46·80	±4·98	±0·28	±0·71	±6·17	—	±1·99
	No. of specimens	10	9	8	8	10	—	8

The ratio body length/excretory pore has been used instead of the usual '*b*' (body length/oesophagus) because of the difficulty of determining the posterior limit of the oesophagus. *V* is the distance of the vulva from the anterior end of the body expressed as a percentage of the body length.

TABLE 2. DIMENSIONS OF *HALENCHUS DUMNONICUS* SP.NOV. FROM WEMBURY BAY, 1955, 1956, 1957, AND HANNAFORE, LOOE, 1956

Sample		Body length (μ)	Body length / Body thickness	Body length / Excretory pore	Body length / Length of tail (anus to tip)	Body length / Length of stylet	Body length / Length of spicules	V (%)
♂♂	Range	850–1200	33·85–46·15	6·44–7·84	9·56–13·33	39·54–57·14	35·42–50·00	—
	Mean	989·10	40·09	7·20	11·12	47·57	40·45	—
	Standard deviation	±38·15	±3·27	±0·52	±1·23	±6·12	±4·26	—
	No. of specimens	11	11	10	10	11	11	—
♀♀	Range	900–1480	30·86–47·62	6·43–9·31	9·69–11·95	42·86–61·36	—	64·76–88·89
	Mean	1091·20	39·41	7·52	10·96	50·76	—	69·94
	Standard deviation	±166·55	±4·79	±0·76	±0·75	±5·08	—	±6·20
	No. of specimens	16	16	12	10	16	—	12

end of the body varies from 155 to 253 μ. The intestine terminates in a short rectum.

Deirids were not seen, while phasmids are present on the tail in the posterior region of the lateral caudal alae in the males, and occur in the region about half-way between the anus and the tip of the tail in the females.

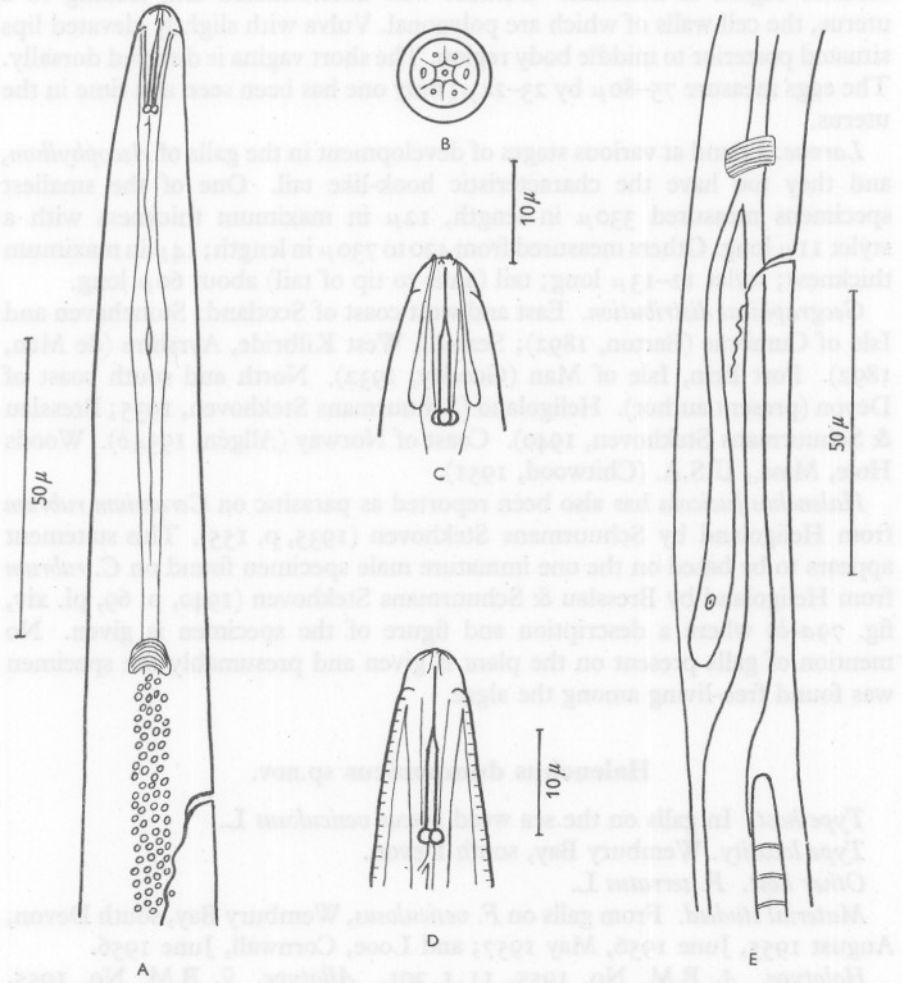

Fig. 2. A, *Halenchus fucicola*, anterior end; B, *H. fucicola*, head (*en face*); C, *H. fucicola*, head (ventral view showing papillae and amphids) (B and C to same scale); D, *H. fucicola*, head (lateral view); E, *H. dumnonicus*, posterior oesophageal region (lateral view).

Male. Testis single and straight, commencing at the anterior region of the intestine, opening without any apparent constriction into a vas deferens, which ends in a short ejaculatory duct. Spicules paired and equal, arched,

with slight variations, 20–24 μ in length (Fig. 3C, F and G). Gubernaculum trough-like, about 6–7 μ in length in a lateral view. Lateral alae present, commencing just anterior to proximal end of spicules and extending to about mid-way between cloaca and tip of tail.

Female. Ovary single and straight, anterior to vulva, and commencing at anterior region of intestine. Oviduct well differentiated and leading to a uterus, the cell walls of which are polygonal. Vulva with slightly elevated lips situated posterior to middle body region. The short vagina is directed dorsally. The eggs measure 75–80 μ by 23–24 μ; only one has been seen at a time in the uterus.

Larvae: Found at various stages of development in the galls of *Ascophyllum*, and they too have the characteristic hook-like tail. One of the smallest specimens measured 330 μ in length, 12 μ in maximum thickness with a stylet 11 μ long. Others measured from 420 to 730 μ in length; 14 μ in maximum thickness; stylet 12–13 μ long; tail (anus to tip of tail) about 60 μ long.

Geographical distribution. East and west coast of Scotland: Stonehaven and Isle of Cumbrae (Barton, 1892); Seamill, West Kilbride, Ayrshire (de Man, 1892). Port Erin, Isle of Man (Goodey, 1932). North and south coast of Devon (present author). Heligoland (Schuurmans Stekhoven, 1935; Bresslau & Schuurmans Stekhoven, 1940). Coast of Norway (Allgén, 1934*b*). Woods Hole, Mass., U.S.A. (Chitwood, 1951).

Halenchus fucicola has also been reported as parasitic on *Ceramium rubrum* from Heligoland by Schuurmans Stekhoven (1935, p. 155). This statement appears to be based on the one immature male specimen found on *C. rubrum* from Heligoland by Bresslau & Schuurmans Stekhoven (1940, p. 69, pl. xiv, fig. 79*a–b*) where a description and figure of the specimen is given. No mention of galls present on the plant is given and presumably the specimen was found free-living among the algae.

Halenchus dumnonicus sp.nov.

Type host. In galls on the sea weed *Fucus vesiculosus* L.

Type locality. Wembury Bay, south Devon.

Other host. F. serratus L.

Material studied. From galls on *F. vesiculosus*, Wembury Bay, south Devon, August 1955, June 1956, May 1957; and Looe, Cornwall, June 1956.

Holotype. ♂, B.M. No. 1955, 11.1.301. *Allotype.* ♀, B.M. No. 1955, 11.1.302. *Paratypes:* B.M. No. 1955, 11.1.303–311 (5 ♂♂, 4 ♀♀); 1957, 8.1.1–12 (6 ♂♂, 6 ♀♀). Host material registered under B.M. No. 1955, 11.1.312–325. 1957, 8.1.13–16.

Dimensions. Given in Table 2.

The shape of the body is very similar to that of *Halenchus fucicola* except that the anterior end is much more strongly developed in proportion to the

total length of the worm, and the stylet, although of similar shape to the other form, is much longer (20–22 μ long in the males and 20–25 μ long in the females). The tip of the tail is again hook-like, bent ventrally in both sexes.

The transverse striations of the cuticle, lateral fields, structure of the head and papillae are the same as in *H. fucicola*.

The excretory pore and its duct bear a considerable resemblance to those seen in *H. fucicola*, although the duct does not appear to be quite so heavily cuticularized and is situated ventrally about one-eighth of the body length from the anterior end. The structure of the oesophagus is also similar to that of the other species and the nerve ring is situated at the posterior end of it. The connexion of the oesophagus with the intestine is not clear in this species also and occurs just posterior to the nerve ring, about 20–25 μ anterior to the excretory pore. The dorsal oesophageal gland usually appears more slender than it does in the previous species, but this structure is somewhat variable in size. In some of the preserved and cleared specimens the connexion of the gland and the wall of the oesophagus is more easily seen (Fig. 2E). The opening of the gland into the lumen of the oesophagus occurs at about 2–3 μ from the base of the stylet. The distance of the extremity of the gland from the anterior end of the body is about 200 μ. The intestine is as in *H. fucicola*.

Deirids have not been seen. The phasmids have not been seen in this form.

Male. Testis and vas deferens as in *H. fucicola*. Spicules are longer, stouter and differ in shape from those in the other form (21–27 μ in length). In lateral view a well-pronounced protuberance can be seen raised in the form of a hump on the ventral side in mid-region; towards the proximal end there is also a greater curvature (Fig. 3D, H). Gubernaculum is trough-like, about 7 μ in length in a lateral view. Lateral alae are present as in *H. fucicola*.

Female. Ovary, oviduct and uterus similar to that in *H. fucicola*. Two specimens were found each bearing an egg; in one case the egg measured 66 by 22 μ and in the other 108 by 27 μ. Vulva with slightly elevated lips, situated posterior to the middle body region. Seen in a lateral position the vagina appears to have a greater cuticular development than in *H. fucicola* (Fig. 3E). A short post-vulvar uterine sac is present.

Larvae. Not seen.

Geographical distribution. South Devon and south Cornwall; Lyme Regis, Dorset.

The new species is a slightly larger form and differs from *H. fucicola* principally in the greater development of the stylet and in the shape of the spicules. In *H. fucicola* the males average about 900 μ in length and the females, including those bearing an egg, average just over 1000 μ. Only one specimen was found as long as 1430 μ and specimens over 1100 μ are not common. The maximum body thickness varies from 18 to 28 μ in the males and 20–33 μ in the females. In *H. dumnonicus* the males average about 1000 μ in length and the females about 1100 μ. Only two gravid specimens were

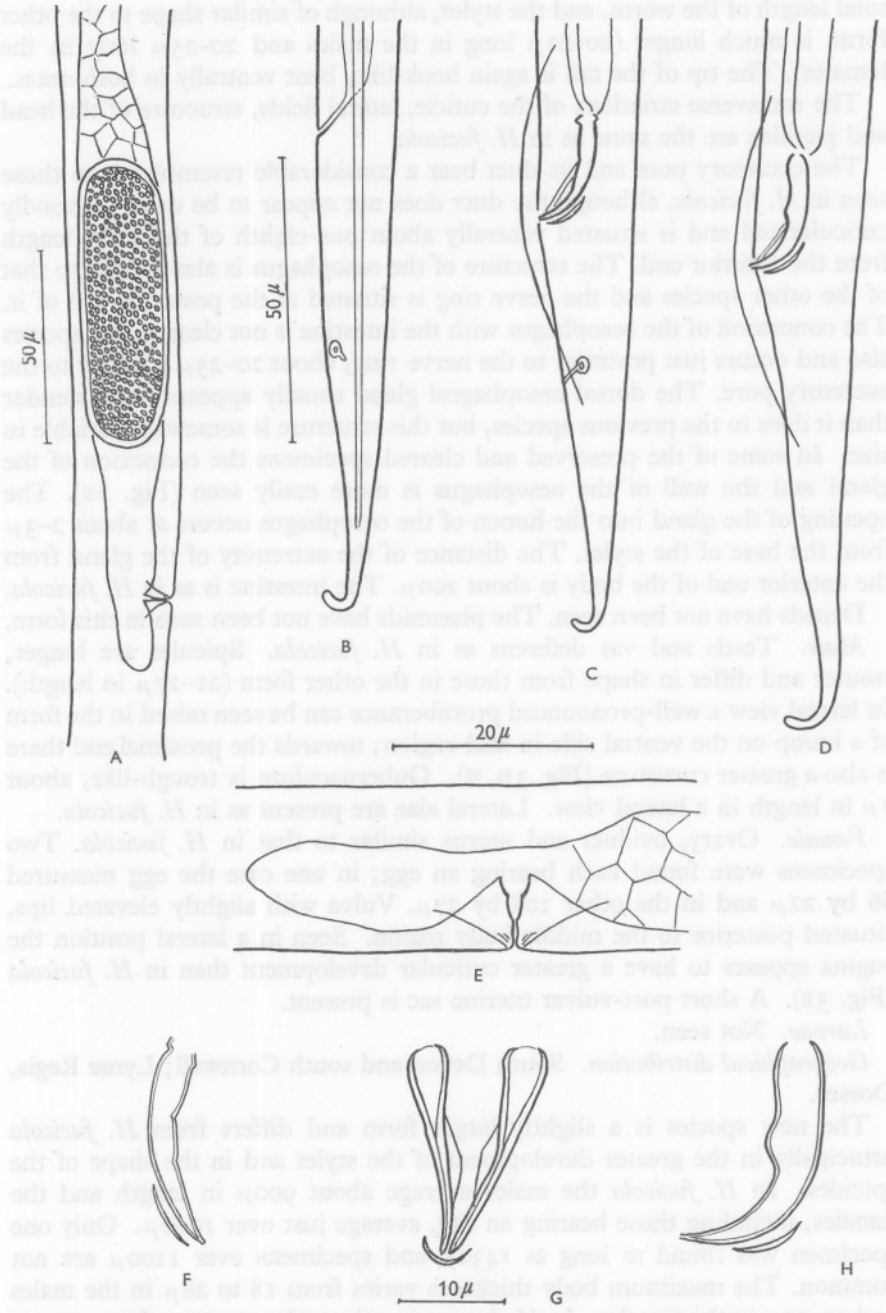

Fig. 3. A, *Halenchus fucicola*, posterior uterine region with egg; B, *H. fucicola*, female tail (showing lateral field and phasmid); C, *H. fucicola*, lateral view of male tail; D, *Halenchus dumnonicus*, lateral view of male tail (B, C and D to same scale); E, *H. dumnonicus*, posterior uterine region; F, *H. fucicola*, lateral view of spicule and gubernaculum; G, *H. fucicola*, ventral view of spicules and gubernaculum; H, *H. dumnonicus*, lateral view of spicule and gubernaculum (F, G and H to same scale).

found, each bearing one egg and measuring 1240 and 1270 μ in length respectively. The maximum body thickness varies from 20 to 31 μ in the males and 21–38 μ in the females.

The samples considered here are not random because the intention during sampling was to get some idea of the full range of size, so that the number of large and small specimens measured is probably biased in favour of such individuals. As a result of this it is valueless to compare the means of the absolute measurements. The difficulty, however, can be overcome by comparing ratios: while appreciating that ratios may also vary due to differential growth. For example, in *H. fucicola* the ratio body length/stylet length is 30 at a larval body length of 330 μ, 35 at 420 μ and 56 at 730 μ. In a sample of ten adults, mean body length 970 μ, the ratio is 59·60; and in another sample of 10 adults, mean body length 1159 μ, it is 68·43. These values were obtained by dividing a sample of twenty individuals into two halves on the basis of body length. From these figures it can be seen that the body grows at a greater rate than the stylet.

Ratios have been calculated for body length/body thickness, body length/distance of excretory pore from anterior end, body length/tail length, body length/stylet length, body length/length of spicules, and the distance of the vulva from the anterior end expressed as a percentage of the body length, i.e. vulva × 100/body length. The mean body lengths, given in the tables, are in all cases not significantly different, so that the ratios calculated against them should be strictly comparable. The ratios were calculated for all specimens and means and standard deviations calculated for them. Of these only the ratios of body length/length of stylet are significantly different in the two forms.

Due to the low number of specimens, the measurements of *H. dumnonicus* have been lumped, and it is this total which has been compared to each sample of *H. fucicola*.

As mentioned earlier, the specimens in the galls of *Fucus vesiculosus* are much scarcer, and when living specimens are removed from the galls they are usually more active than those of *Halenchus fucicola* from *Ascophyllum*. Specimens are also scarce in the galls on *Fucus serratus*.

Of the remaining species of *Halenchus*,[1] *H. dumnonicus* differs from *H. mediterraneus* (Micoletzky, 1922) in the length of the stylet, the position of the caudal alae, which do not extend so far posteriorly, and in the size and shape of the gubernaculum, which is smaller and has a wavy outline.

Finally, the new species differs from *H. mexicanus* Chitwood, 1951, in the shape of the tail, which is not hook-like.

[1] *Tylenchus* (*Chitinotylenchus*) *zostericola* Allgén (1934*a*) found associated with *Zostera* in Holland was transferred to *Halenchus* by Chitwood (1951), but is now considered to belong to the genus *Radopholus* (see Allen, 1955).

These tylenchid nematodes occur on the fucoid algae which predominate in the middle zone of the shore, and at Wembury Bay much time has been spent by the writer looking for galls on *Fucus spiralis* and *F. serratus* without success, although on a later visit to Lyme Regis galls were seen on the latter species.

Ascophyllum nodosum and *Fucus vesiculosus* occur at approximately the same height on the shore, and at Wembury Bay galls were sometimes seen on both plant species where they were growing near to each other. It would therefore seem possible for both species of nematodes to infest either plant, since at some stage in the life cycle some of the worms presumably emerge from the galls to infest new host plants. An obstacle to the elucidation of the life history of the worms by experimental means, however, arises from the difficulty of maintaining the large brown sea weeds under laboratory conditions.

Sometimes *Halenchus fucicola* and *Halenchus* sp. are quoted as parasites of red sea weeds, *Furcellaria fastigiata*, *Chondrus crispus* and *Rhodymenia palmata*. It appears, however, that these statements are based on the reports in two other papers by Barton (1891, 1901). Barton's material of *Furcellaria* and *Chondrus*, which was found washed up at Lyme Regis in 1900, is in the algal collections of the British Museum (Natural History) and the writer has had the opportunity of examining it. Although the nematodes in the galls of these plants are in very poor condition they certainly do not appear to be tylenchids. According to Barton (1891), the nematodes from *Rhodymenia palmata* were found in galls attributed to a copepod, and in a recent paper by Harding (1954), in which a description is given of the life history of the copepod from galls on *Rhodymenia palmata*, nematodes were again reported in some of the galls.

Thanks are due to Dr H. W. Parker who suggested this line of research, to Mr W. G. Inglis and Mr S. Prudhoe for much help and advice during the course of the work, and to the staff of the Laboratory at Plymouth, especially to Dr M. W. Parke.

SUMMARY

The nematode *Halenchus fucicola* (de Man), causing galls on the thallus of the sea weed *Ascophyllum nodosum*, has been found in the Plymouth area (Wembury Bay) and at Croyde Bay on the north Devon coast, and a redescription of the worm is given. A new species of *Halenchus* (*H. dumnonicus*) causing similar growths on the sea weeds *Fucus vesiculosus* and *F. serratus* is described. This latter form is also found at Wembury Bay; at Looe, Cornwall, and at Lyme Regis, Dorset.

REFERENCES

ALLEN, M. W., 1955. A review of the genus *Tylenchorhynchus*. *Univ. Calif. Publ. Zool.*, Vol. 61, pp. 129–66.

ALLGÉN, C. A., 1934*a*. Freilebende marine Nematoden aus Hallands Väderö und der nahegelegenen Küste Schonens (Südschweden). Eine Beitrag zur Kenntnis der Nematoden fauna des südlichen Kattegatts. *Folia zool. hydrobiol., Riga*, Vol. 6, pp. 49–86.

—— 1934*b*. Die marinen Tylenchen. *Folia zool. hydrobiol., Riga*, Vol. 6, pp. 86–7.

BARTON, E. S., 1891. On the occurrence of galls on *Rhodymenia palmata* Grev. *J. Bot., Lond.*, Vol. 29, pp. 65–8.

—— 1892. On malformations of *Ascophyllum* and *Desmarestia*. *Phycol. Mem., Lond.*, Part 1, pp. 21–4.

—— 1901. On certain galls in *Furcellaria* and *Chondrus*. *J. Bot., Lond.*, Vol. 39, pp. 49–51.

BRESSLAU, E. & SCHUURMANS STEKHOVEN, J. H., Jr., 1940. *Marine Freilebende Nematoden aus der Nordsee*, 74 pp. Bruxelles: Musée royal d'Histoire naturelle de Belgique.

CHITWOOD, B. G., 1951. North American marine nematodes. *Texas J. Sci.*, Vol 3, pp. 617–72.

COBB, M. V., Ed., 1933. N. A. Cobb: New nemic genera and species, with taxonomic notes. *J. Parasit.*, Vol. 20, pp. 81–94.

GOODEY, T., 1932. The genus *Anguillulina* Gerv. and v. Ben., 1859, vel *Tylenchus* Bastian, 1865. *J. Helminth.*, Vol. 10, pp. 75–180.

HARDING, J. P., 1954. The copepod *Thelastris rhodymeniae* (Brady) and its nauplius, parasitic in the sea weed *Rhodymenia palmata* (L.) Grev. *Proc. zool. Soc. Lond.*, Vol. 124, pp. 153–61.

MAN, J. G. DE, 1892. Über eine neue in Gallen einer Meeresalge lebende Art der Gattung *Tylenchus* Bast. *Festschr. zum Siebenzigsten Geburtstag Rudolf Leuckart*, pp. 121–5. Leipzig: Wilhelm Engelmann.

MICOLETZKY, H., 1922. Neue freilebende Nematoden aus Suez. *S.B. Akad. Wiss. Wien, Math-Naturwiss. Kl.*, Abt. I, Bd. 131, pp. 77–103.

SCHUURMANS STEKHOVEN, J. H., Jr., 1935. Nematoda errantia. *Tierw. N.- u. Ostsee*, Teil 5, b, 173 pp. (Lief. 28).

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THE DISTRIBUTION OF INTERTIDAL ORGANISMS ALONG THE COASTS OF THE ENGLISH CHANNEL

By D. J. CRISP
Marine Biology Station, Menai Bridge, Anglesey

and A. J. SOUTHWARD
The Plymouth Laboratory

(Text-figures 1–7)

INTRODUCTION

Our investigations on the distribution of barnacles (Crisp & Chipperfield, 1948; Crisp, 1950; Southward, 1950, 1951; Norris & Crisp, 1953; Southward & Crisp, 1952, 1954*a*; Bishop & Crisp, 1957, 1958) were prompted by the lack of quantitative information on the distribution of most species. During these investigations we noted that the distribution of barnacles and of other species of shore animals had certain features in common. We have therefore extended our studies to include many of the commoner intertidal organisms in the belief that we may be able to discover which are the most important of the many possible factors controlling the distribution of littoral species generally, and of barnacles in particular.

We have already described the main features of the distribution of the commoner intertidal animals along the Irish coast (Southward & Crisp, 1954*b*). The present paper, the second of a series, deals with the English and French sides of the Channel. These two coasts are most conveniently treated together for several reasons. In the first place the Channel presents a simpler picture, both hydrographically and faunistically, than that afforded by the seas bounding the other coasts of the British Isles. It therefore offers a useful introduction to further contributions which we are preparing on the British Isles.

The fauna and flora of the Atlantic coast of France will be described elsewhere by one of us (D.J.C.) in collaboration with Prof. E. Fischer-Piette.

In describing distributions along the Channel coasts we have included the whole region lying between Cape Cornwall and North Foreland on the English side and the region between Calais and Le Conquet on the French side, together with Ile d'Ouessant, the Channel Islands and the Isles of Scilly. In this area we studied some of the commoner animals and plants found on predominantly rocky shores (including large boulders, piers and breakwaters). As far as possible the dominant organisms were treated quantitatively. In

previous papers (Southward & Crisp, 1954*a*, *b*, 1956) we have stressed the importance of a quantitative approach to biogeography. Some measurement of the abundance of the organisms is essential if the results are intended to be used later to check changes in distribution, and is equally important for the purpose of revealing the main controlling factors.

In two previous studies of the area, that of Moore & Kitching (1939) on barnacles and the more general work of Fischer-Piette (1936), some quantitative information is given for certain of the species we have investigated. However, Moore & Kitching investigated only the distribution of *Chthamalus stellatus*, and to a lesser extent that of *B. balanoides*, on the English side. Fischer-Piette, although he included species (especially of plants) that we ourselves did not investigate, omitted a detailed account of the common periwinkles and did not consider the top-shells. He concerned himself mainly with details of the distribution in the western half of the Channel, and considered particularly the effects of comparatively local factors such as tidal conditions and wave action. We have investigated the fauna throughout the Channel, and have tried to relate the patterns of distribution both to general trends in the physical environment and to such topographical details as seem to be significant.

The species investigated are listed in Table 1, together with brief references to their distribution outside the Channel and their periods of breeding. The species in brackets are those that were not investigated as fully as the other species, or those for which we had to rely upon previous observations. The terms of abundance employed in the table have exact quantitative meanings for each species, as explained in Table 2.

THE PHYSICAL ENVIRONMENT

The features of the environment most likely to influence the geographical distribution of the plants and animals of the shore can be divided into two groups. The primary factors include the temperature of the sea and the air, the salinity of the sea and its content of nutrient salts, and the currents and water movements of the sea. The variation of these factors in the Channel has been discussed by Russell (1953); they are readily defined and measured, though the information regarding them may not be complete or satisfactory as far as the intertidal zone is concerned. The other factors, including the degree of exposure to wave action and 'biological factors' attributed to water masses of different past history, are at present difficult even to define, let alone to measure.

Temperature

The monthly means of sea and air temperatures for February and August are shown in Fig. 1. These months represent the overall extremes of average

temperature for the year. At some stations slightly greater extremes of air temperature may be found in January and July.

We have already commented (Southward & Crisp, 1954*b*) on the difficulty presented by the differences between the temperature of the sea and the air.

TABLE 1. THE SPECIES INVESTIGATED, THEIR GENERAL DISTRIBUTION AND MAIN BREEDING SEASONS*

	Abundance in Channel†		North European limits	South European (or other) limits	Months or season of main breeding period in Channel
	West	East			
Southern forms:					
Anemonia sulcata (Pennant)	A	R	N.W. Scotland	Med., Canaries	
Chthamalus stellatus (Poli)	A	N	N.E. Scotland	(W. Africa)	May–Sept.
Balanus perforatus (Bruguière)	A	N	S. Wales	(W. Africa)	July–Sept.
Patella depressa (Pennant)	A	N	N. Wales	(N. Africa)	July–Aug.
P. aspera (Lamarck)	A	N	Scotland, N.E. England	Spain	Sept.–Nov.
(*Haliotis tuberculata* (L.))	+	N	N. France	Mediterranean	July–Sept.
(*Gibbula magus* (L.))	C	O–R	N. Scotland	Mediterranean	?
G. pennanti Philippi	A	N	N. France	?	?
G. umbilicalis (da Costa)	A	C–R	N. Scotland	Mediterranean	Autumn
Monodonta lineata (da Costa)	A	N	N.W. Ireland	(Madeira)	Autumn
Littorina neritoides (L.)	A	R	Scotland, N.E. England	(N. Africa)	Winter
(*Paracentrotus lividus* (Lamarck))	+	N	N.W. Ireland, S.W. Scotland	(Azores)	Probably summer
(*Laminaria ochroleuca* (la Pylaie))	+	N	S. England	(N. Africa)	Aug.–Nov.
(*Saccorhiza polysichides* (Lightf.))	+	N	W. Norway, N. Scotland	(N. Africa)	Probably all year
(*Bifurcaria rotunda* (Huds.))	+	N	Ireland, S. England	(N. Africa)	Spring
Intermediate forms:					
Actinia equina L.	A	A	Murman Sea	(Africa)	Viviparous; probably all year round
Verruca stroemia (O. F. Müller)	A	C–R	S. Norway, Iceland	Mediterranean	Nov.–May
Balanus improvisus Darwin	A	A	Baltic	(Tropics)	May–Sept.
Elminius modestus Darwin	A	A	S.W. Scotland	France	May–Oct.–Jan.
Hemioniscus balani Spence-Bate	A	R	60–62° N. in *B. balanus*	?	?
Patella vulgata L.	A	A	N. Norway	Mediterranean	Oct.–Mar.
Gibbula cineraria (L.)	A	A	N. Norway	Mediterranean	Probably winter
(*Calliostoma zizyphinum* (L.))	+	+	S. Norway	Mediterranean	Probably winter and spring
Littorina obtusata (L.)	A	A	Murman Sea	Portugal	Winter and spring
L. littorea (L.)	A	A	Murman Sea	Portugal	Probably all year
Fucus spiralis L.	+	+	N. Norway, Iceland	(N. Africa)	Summer
(*Himanthalia elongata* (L.))	+	N	N. Norway (not Iceland)	Spain	May–Oct.
Northern forms:					
Balanus balanoides (L.)	C–R	A	Arctic	N. Spain	Feb.–Apr.
B. balanus (L.)	N	?	Arctic	Ireland, S. Wales, S.E. England	Feb.–Apr.
B. crenatus Bruguière	A	A	Arctic	W. France	Feb.–May
Littorina saxatilis (Olivi)	A	A	Arctic	Portugal	All year
(*Laminaria digitata* (Huds.))	+	+	Arctic	N.W. Spain	All year
(*L. hyperborea* (Gunn.))	+	+	N. Norway	N.W. Spain, Portugal	Nov.–Apr.
(*L. saccharina* (L.))	+	+	Arctic	W. France	Winter, but some all year
Alaria esculenta (L.)	+	N	Arctic	W. France	Jan.–Apr.
Ascophyllum nodosum (L.)	+	+	Arctic	Spain	Spring
Fucus serratus L.	+	+	Murman Sea, White Sea	N. Spain	Autumn
F. vesiculosus L.	+	+	Murman Sea, White Sea	(N. Africa)	All year
Pelvetia canaliculata (L.)	+	+	White Sea	Portugal	June–Sept.

* Details of distribution outside the Channel and information on breeding periods have been obtained from the following works, supplemented by personal observations where available: Forbes & Hanley, 1853; Darwin, 1854; Sars, 1878; Woodward, 1880; Børgeson & Jónsson, 1908; Stephenson, 1924, 1935; Mortensen, 1927; Crofts, 1929; Nobre, 1932; Hamel, 1931–39; Fischer-Piette, 1935, 1936, 1955; Fischer-Piette & Prenant, 1956; Stephensen, 1938; Bassindale, 1941; Lami, 1941, 1943, 1954; Thorson, 1941, 1946; Station Biologique de Roscoff, 1951*a*, *b*, 1954; Lilly *et al.* 1953; Orton *et al.* 1956; Marine Biological Association, 1957.

† For meanings of symbols refer to Table 2. Parentheses round certain species indicate that our observations were incomplete.

TABLE 2. MEANINGS OF THE NOTATION USED TO DESCRIBE THE ABUNDANCE OF CERTAIN INTERTIDAL ANIMALS

For all organisms the letters involved represent the same word: A, abundant; C, common; F, frequent; O, occasional; R, rare; and N, not found. The exact quantitative basis of these categories varies with each species, as follows:

Anemones

A Many in almost every pool and damp place
C Groups in pools and damp places
F Isolated specimens in few pools
R A small number, usually under 10, found after 30 min searching

Barnacles

For *Chthamalus stellatus*, *Balanus balanoides* and *Elminius modestus*, we have usually recorded numbers per square centimetre of rock. For these and other species except *Balanus perforatus* the categories have the following meanings:

A More than 1 per cm^2; rocks well covered
C 0·1 to 1·0 per cm^2; up to one-third of rock space covered
F 0·01 to 0·1 per cm^2; individuals never more than 10 cm apart
O 0·0001 to 0·01 per cm^2; few within 10 cm of each other
R Less than 1 per m^2; only a few found in 30 min searching

For *B. perforatus*, which is a much larger species, the values are:

A Over 0·1 per cm^2; close groups on most vertical faces, often up to MTL
C 0·01 to 0·1 per cm^2; adjacent groups, not always above LWN
F Less than 0·01 per cm^2; adjacent in crevices
O Less than 0·01 per cm^2; rarely adjacent even in crevices
R Only a few found in 30 min searching

Limpets

A Over 50 per m^2 or more than 50 % of limpets at certain levels
C 10 to 50 per m^2, 10 to 50 % at certain levels
F 1 to 10 per m^2, 1 to 10 % at certain levels
O Less than 1 per m^2 on average, less than 1 % of population
R Only a few found in 30 min searching

Top-shells

A Exceeding 10 per m^2 generally
C 1–10 per m^2, sometimes very locally over 10 per m^2
F Less than 1 per m^2, locally sometimes more
O Always less than 1 per m^2
R Only one or two found in 30 min searching

Periwinkles

For the smaller species *Littorina neritoides*, and the small *L. saxatilis* found among barnacles, the ratings are:

A Over 1·0 per cm^2 at HW, extending down the midlittoral zone
C 0·1 to 1·0 per cm^2, mainly in the supralittoral fringe
F Less than 0·1 per cm^2, in crevices
R Only a few found in 30 min searching

For the larger littorinids the ratings are:

A More than 50 per m^2
C 10 to 50 per m^2
F 1 to 10 per m^2
R Only a few found in 30 min searching

The other animals, and the plants, are recorded only as present (+) or absent (N), though there may be a differentiation between forms in different habitats.

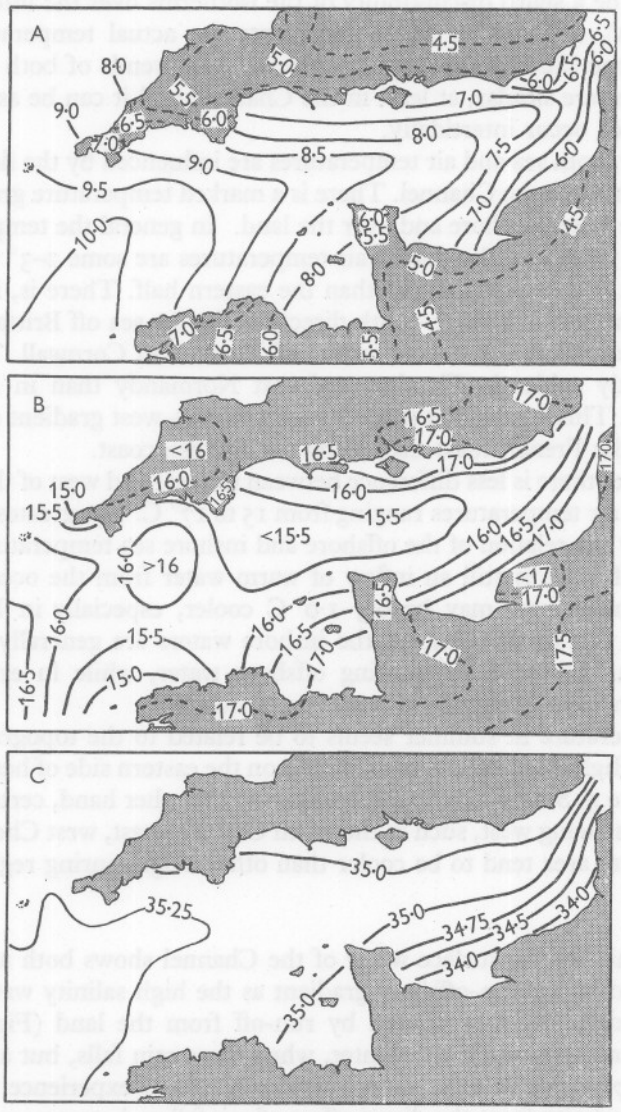

Fig. 1. Some environmental factors in the Channel. A, mean sea and air temperatures for February; B, mean sea and air temperatures for August; C, mean salinity, year. Sea temperatures and salinities are taken from Lumby (1935) and represent conditions pertaining over 30 years ago. Air temperatures have been abstracted from Air Ministry (1936) and Office Nationale Météorologique (1940, 1942*a*, *b*) and isotherms drawn to show actual temperatures not corrected to sea-level; again conditions pertaining some time ago (at least 20 years) are shown.

There may be a sharp discontinuity of the isotherms over the intertidal zone (cf. Fig. 1A), and neither figure represents the actual temperature of the intertidal zone (Southward, 1958). However, the trends of both sea and air temperatures are similar, at least in the Channel, and it can be assumed that similar trends occur intertidally.

In winter, both sea and air temperatures are influenced by the flow of water eastwards through the Channel. There is a marked temperature gradient from the offshore water, inshore and over the land. In general the temperatures of the inshore water and the coastal air temperatures are some 2–3° C higher in the western half of the Channel than the eastern half. There is, in addition, a further gradient in a north-south direction, for the sea off Brittany is about 0·5° C warmer than the coastal waters of Devon and Cornwall. The coastal air is slightly colder in Flanders and east Normandy than in Sussex and south Kent. This means that there is a steeper east–west gradient of temperature along the French coast than along the English coast.

In summer there is less difference between the east and west of the Channel, the sea and air temperatures ranging from 15 to 17° C. The greatest difference is shown by the relation of the offshore and inshore sea temperatures. In the western half there is still an inflow of warm water from the ocean, and the inshore temperatures may be 0·5–1·0° C cooler, especially in Brittany: in the eastern part of the Channel the inshore waters are generally 1·0–1·5° C warmer than the eastward flowing offshore water, while in estuaries and shallow bays even greater local warming can occur.

Air temperature in summer seems to be related to the topography of the coastline. Higher temperatures are found on the eastern side of headlands and peninsulas, e.g. Start Pt. and Cherbourg. On the other hand, certain exposed coastal areas facing west, such as the north Cornish coast, west Cherbourg and the Le Havre area tend to be cooler than other neighbouring regions.

Salinity

The salinity of the surface water of the Channel shows both an east–west gradient and an inshore–offshore gradient as the high salinity water entering from the ocean becomes diluted by run-off from the land (Fig. 1C). The differences are greatest in the winter, when more rain falls, but are probably of little importance to most shore organisms, which experience larger local changes in salinity from the direct effect of rainfall and streams on the shore.

Nutrient salts

As far as can be ascertained there are no great differences in the phosphate and nitrate resources of the main body of water in different parts of the Channel proper. Locally rich patches of lower salinity and high silicate content, however, occur in the vicinity of estuaries, while richer high salinity water may be found off the Channel mouth (Harvey, 1930; Kalle, 1937).

Tides, currents and water movements

If we divide the Channel by a line running from Swanage to Cherbourg we find that in the two basins thus formed the tides are out of phase. In the western basin the maximum spring tides occur at midday and midnight, while in the eastern basin they occur in the early morning and evening. Fast tidal currents are found where the two basins join, especially off headlands such as St Catherine's Pt., The Needles, St Alban's Head, Portland Bill, Cap de la Hague and Alderney. The greatest and smallest tides of the Channel are found in this middle section also. The least tides occur on the English side, where the shallow water effects are large and produce the 'double' or 'abnormal' tides of Portland, Swanage and the Isle of Wight. On the French side very big tides occur in the Gulf of St Malo.

The main residual current is up-Channel, the direction of the prevailing winds. Water enters at the western end and passes eastwards through the Straits of Dover. Carruthers (1930) has described the routes taken by drift bottles liberated in the western Channel, while more recently measurements of the total flow through the Straits of Dover have been made by Bowden (1956). It need scarcely be pointed out that the rate of exchange of water with the Atlantic is much greater in the western section of the Channel than it is in the eastern part. Little is known of the minor eddies and counter currents which may exist near the coast and which may be of greater importance in dissemination of larval stages of many intertidal species. A counter eddy has been established in Pevensey Bay from current measurements made at the Varne lightship (Carruthers, 1935; Carruthers, Lawford & Veley, 1950). Counter currents are also indicated on either side of the main drift through the Straits of Dover by Van Veen (1936). It is possible that a counter current sometimes flows eastwards along the south coast of Cornwall and flows clockwise round the south-west peninsula towards the Bristol Channel, but current measurements at the Seven Stones Light Vessel, between Scilly and Land's End, show a residual drift in the direction E.S.E. (Carruthers, Lawford, Veley & Gruning, 1951).

On theoretical grounds the water movements up-Channel would be expected to be deflected southwards very slightly by Corioli's force, while those down-Channel would be deflected northwards. Thus the main residual flow from west to east would have a tendency to hug the French coast, while counter currents might be expected to develop more readily on the English side. However, Corioli's force influences mainly the movement of large water masses which are moving freely with their own inertia. The surface layers of water, and especially those close to the shore where friction with the sea bed is important, however, should tend to follow the direction most closely approximating to that of the wind consistent with the lie of the shoreline.

Wave-action and substratum

The effects of wave-action on the plants and animals of the shore are modified by the type and configuration of the shoreline and the depth of water offshore, and these factors are interrelated. This fact does not seem to have been realized by previous workers, who assessed wave-action on the basis of topography or orientation to prevailing winds (Moore, 1935; Lysaght, 1941). Over small areas of coastline it is sometimes possible to assess wave-action by relating a series of measurements of wave wash above normal tidal height with winds of a certain direction and strength (Southward, 1953), but this method fails on coasts open to ocean swell (Southward & Orton, 1954).

On gently shelving rocky ledges wave wash may surge great distances up the shore, while on very broken rocky reefs splash may be the main effect. The mechanical action of waves also depends upon the nature of the shore; on steep slopes there will be great dynamic shock, while on shelving shore there will be mainly a tearing or shearing effect. Moreover, the presence of submerged reefs or bars offshore may reduce wave-action on the shore or may modify the type of wave reaching the shore (Bigelow & Edmonson, 1947).

We are thus as far as ever from the attainment of some measure of wave-action, since so much depends on local factors. Local factors are even more important on depositing shores, or where large amounts of mud, sand or gravel are found offshore. These particles are moved or brought into suspension, according to their size and the power of the waves, and can scour the rocky parts of the coast. In general, the greater the wave-action at a place, the larger the particles remaining to be moved or affected by wave-action, for the smaller particles have been removed (cf. de Martonne, 1935). Thus headlands jutting out into deep water, though they receive greater pounding by waves, generally show little scour, while bays with accumulations of sand or gravel, though often receiving less wave-action, may be exposed to greater damage by scour. The scouring action of gravel seems to be especially severe, and predominantly gravel shores are virtually intertidal deserts.

The western half of the Channel is bounded by a steep rocky shoreline indented at intervals by drowned valleys and sandy bayhead beaches, and deep water is never far offshore. Largely because its shores consist of softer material the eastern half of the Channel has a more even coastline, and the great accumulations of sand and shingle form gently shelving beaches. The eastern basin is shallow and wave-induced movements must often disturb the sea bed. Thus in the eastern Channel the water carries much suspended matter, and near the shores the waves cause continual abrasion through the sand and shingle that they churn up. This abrasion is most marked near high-water mark where the largest stones are found and where the waves break with greatest force. In the western basin, however, the water is generally clearer, and carries little particulate material; the shores are wave-beaten rather than

scoured. This generalization is true only in so far as these shores are of harder rock; where the rock is more friable, or where much material is brought down by rivers, coastal conditions approximate to those of the eastern end of the Channel. Thus the coasts surrounding the Helford River, the Fal and Fowey, Plymouth Sound, Salcombe and the Dart are locally silty and muddy with turbid water at spring tides and after rain. Mounts Bay is extensively sand-scoured, while much of Lyme Bay has accumulations of shingle, culminating in the famous Chesil Bank. The scour experienced by many of the coasts lying between the Exe and Chesil Bank must surely equal or exceed that suffered anywhere to the east of Portland. Nevertheless, the Channel water as a whole remains very clear throughout most of its length as far as the Isle of Purbeck. Only where the Channel narrows and shallows to the east of the Isle of Wight does the water near the shore become almost continuously turbid.

Similar conditions apply to the French coast. The long estuaries of Finistère and the Côtes du Nord produce locally turbid conditions, and the scour is probably greater than in corresponding drowned valleys on the English side, on account of the greater tidal range. The Gulf of St Malo, particularly towards its south-eastern corner, is in some respects analogous to West Bay. Its coasts have been eroded to produce much sand, gravel and shingle, which is prevented from being driven eastwards by the Cherbourg peninsula. Hence this loose material has accumulated both in the bay, reducing its depth, and on the west-facing shores south of Carteret where the scour appears to be severe.

Two small but important differences between the French and English coasts should be referred to here. First, at the extreme western end of the Channel the coast of north Finistère is less steep than that of south Devon and Cornwall, and is protected by numerous offshore reefs and islands. Moreover, the predominant wind at the mouth of the Channel, which is south of west, blows almost directly on to the coast of south-west England, while northern Finistère lies slightly in the lee of the Armoricaine peninsula. Hence a considerable part of the latter coast, excluding the offshore islands, is less exposed to wave-action; in general, it retains more loose material in the shape of sandy beaches and boulder spits (e.g. Sillon de Talbert) than corresponding regions of the English coast. Secondly, at the extreme eastern end of the Channel, the English side is composed entirely of soft chalks, sands and alluvium, yielding intertidally only chalk reefs and sand or shingle beaches. On the Boulonnais coast, however, exposures of oolite face the prevailing winds and give rise to more typical rocky shores below H.W.N., even though banks of shingle may form at high-water mark.

It is worth pointing out that geological factors profoundly influence the formation and character of tide pools, which are the main habitat of some animals and plants. In the eastern part of the Channel the rocks are usually too friable or too flat-bedded to allow the formation of other than shallow pools, which are often filled with sand or other deposits.

The main differences between the two halves of the Channel are summarized in Table 3.

TABLE 3. MAIN ENVIRONMENTAL DIFFERENCES BETWEEN THE WESTERN AND EASTERN PARTS OF THE CHANNEL

Factor	West	East
Summer temperature	Little lower	Little higher
Winter temperature	Higher	Lower
Average temperature	Higher	Lower
Tendency to frosts	Slight	Considerable
Average sea salinity	Higher	Lower
Nutrient salts	Little difference	Little difference
Biological factors	Decreasing from west to east?	Not known
Tidal currents	Less	More (very great in middle)
Scour	Little	Much
Silt in suspension	Little	Much
Rocky substratum	Much	Less
Pollution	Some	More
Wave-action	More	Less, but bottom disturbance greater
Depth of water	More	Less
Thermocline in summer	Probably stable	Probably disturbed in shallower water by currents

THE DISTRIBUTIONS

The main trends in distribution of the organisms investigated have already been summarized in Table 1. The detailed distribution of the commoner animals is set out in Table 7 (appendix). We have not tabulated in detail our results for the algae, as these were not as completely investigated as the animals on the English side of the Channel. The algal distributions, and those of some of the less common animals also investigated in less detail, are commented upon below, after the commoner animals.

COMMONER ANIMALS

Anemonia sulcata

The records for *Anemonia sulcata* in Table 7 refer mainly to positive occurrences, as the species may be found only at extreme low water and its complete absence from a locality cannot always be stated with certainty. The records refer to both ecological forms of the species, the one in pools in the upper midlittoral and the other among algae in the infralittoral fringe. Both forms are common in the western part of the Channel, but only the low-water form occurs towards the east. The species penetrates sporadically into the eastern basin, the most easterly records being at Brighton and Grandcamp.

Actinia equina

The commoner of the two species of sea-anemone investigated, *Actinia equina*, occurs throughout the Channel. Fischer-Piette (1936) regarded the distribution of the species as 'capricious', and his charts show areas of greater

or lesser abundance. Our results differ: we found the species common at some localities where he did not find it, for example Tremazan and Cancale, and no general trend in distribution could be made out, save that the greater area of suitable rocky substratum at the western end of the Channel resulted in the species being found at a greater proportion of the stations than in the eastern basin.

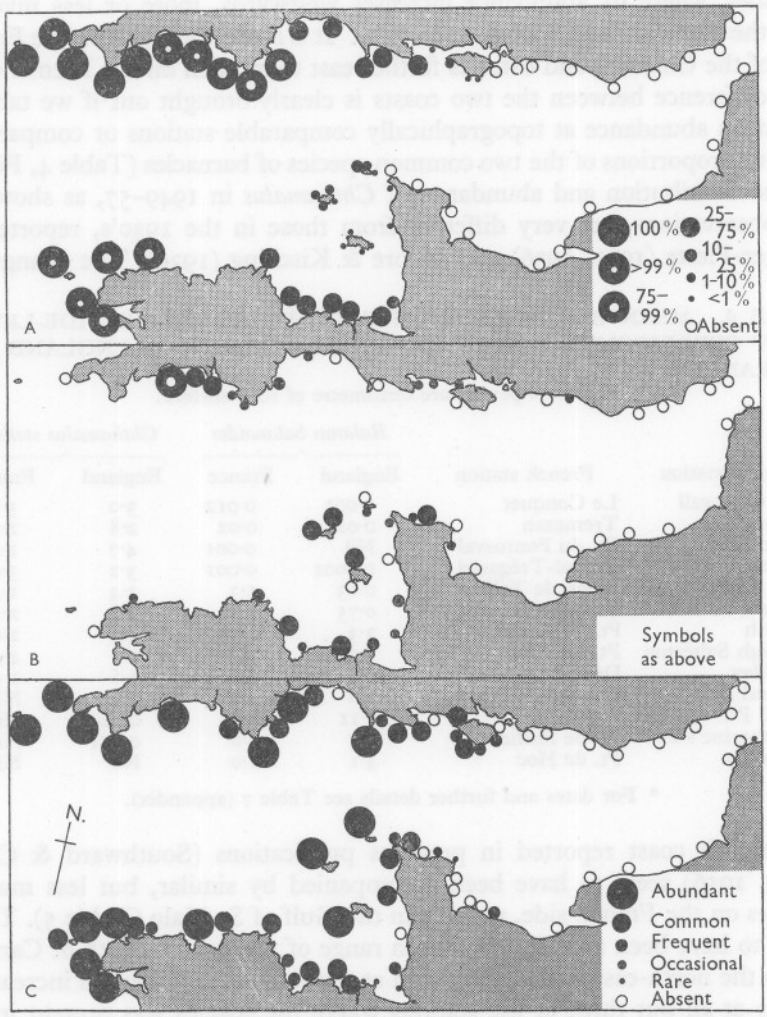

Fig. 2. Distribution of some representative species in the Channel. A, confined exclusively to the western part; *Chthamalus stellatus*, shown as percentage of population of *Chthamalus* and *Balanus balanoides*; B, common in the west and rare in the east; *Hemioniscus balani*, shown as percentage infection of *Balanus balanoides*; C, present in the eastern basin on the French side only; *Littorina neritoides*.

Possibly the abundance of *Actinia* varies greatly from year to year, or over quite small distances on the shore. No doubt the viviparous habit of breeding would contribute to such variation.

Chthamalus stellatus

The barnacle *Chthamalus stellatus* is confined to the western half of the Channel, where its abundance increases westwards, more or less inversely with the abundance of *Balanus balanoides*. It is more abundant on the English side of the Channel, and extends farther east there than on the French coast. The difference between the two coasts is clearly brought out if we tabulate the mean abundance at topographically comparable stations or compare the relative proportions of the two common species of barnacles (Table 4, Fig. 2).

The distribution and abundance of *Chthamalus* in 1949–57, as shown by our observations, are very different from those in the 1930's, reported by Fischer-Piette (1932, 1936) and Moore & Kitching (1939). The changes on

TABLE 4. MEAN BARNACLE COUNTS TAKEN AT THREE TIDE-LEVELS AT CORRESPONDING STATIONS ON THE COASTS OF ENGLAND AND FRANCE*

Numbers per square centimetre of rock surface.

		Balanus balanoides		*Chthamalus stellatus*	
English station	French station	England	France	England	France
Cape Cornwall	Le Conquet	0·002	0·012	3·0	3·8
Sennen Cove	Tremazan	0·02	0·02	2·8	2·3
Lizard Head	Pt. du Pontusval	Nil	0·001	4·7	2·0
Polkerris	Primel-Trégastel	0·0001	0·001	3·2	3·0
Hope Cove	Sillon de Talbert	0·03	1·3	3·4	1·4
Brixham	St Quay-Portrieux	0·75	2·5	2·8	2·1
Dawlish	Pt. du Roselier	2·3	1·3	3·5	2·6
Budleigh Salterton	Pt. de Pléneuf	3·0	1·4	2·5	4·0
West Bay	Dinard	0·9	2·1	2·6	1·9
Portland Bill	Cap de la Hague	1·0	> 1·0	2·9	Nil
Peveril Pt.	Cap Lévy	0·11	> 1·0	0·12	Nil
St Catherine's Pt.	Pt. de Barfleur	0·5	> 1·0	0·001	Nil
Bembridge	Pt. de Hoc	4·1	> 1·0	Nil	Nil

* For dates and further details see Table 7 (appendix).

the English coast reported in previous publications (Southward & Crisp, 1954*a*, 1956) seem to have been accompanied by similar, but less marked changes on the French side, notably in the Gulf of St Malo (Table 5). There seems to have been a slight increase in range of the species north of Carteret and to the north-east of Alderney, and at the same time a marked increase in density at all but three of the stations where the species was present in the 1930's. The increase appears to have been most marked in the outermost Channel Islands, Alderney and Guernsey, where *Chthamalus* is now present in greater numbers than in Jersey, a reversal of the previous situation.

TABLE 5. CHANGES IN THE ABUNDANCE OF *CHTHAMALUS STELLATUS* AT STATIONS IN THE GULF OF ST MALO

As maximum density per square centimetre, or ratio to *Balanus balanoides*. Brackets indicate nearby stations, to be compared with each other.

	1930–32 (from Fischer-Piette, 1932, 1936)	1954–6
Cap de la Hague	Absent	Absent
Nez de Jobourg	Absent	—
Vauville	—	0·0005
Flamanville	Absent	—
Carteret	0·01	0·01
Granville	1:20 at H.W.N.	3:4 at H.W.N.
Clonque	0·0005	0·5
Chateau a l'Etoc	Absent	—
Pt. Quesnard	—	0·004
La Moye Pt.	0·006	0·36
Fort Doyle	0·0001	0·01
Montorgueil Castle	Very rare, just above upper limit *B. balanoides*	0·01
Plemont Pt.	Very rare, just above upper limit *B. balanoides*	0·04
La Corbière	Very rare, just above upper limit *B. balanoides*	0·015
Cancale	1:20 at M.T.L.	2:1 at M.T.L.
Dinard	—	5:4 at M.T.L.
Pt. du Decollé	1:1 at M.T.L.	—
Pt. de Pléneuf	1:1 at M.T.L.	2:1 at M.T.L.
Pt. de Pordic	3:1 at M.T.L.	4:5 at M.T.L.
Sillon de Talbert	2:3 at M.T.L.	1:3 at M.T.L.

Balanus balanoides

The common acorn barnacle *Balanus balanoides* is present throughout the Channel. Our records agree generally with those of Fischer-Piette (1932, 1936; personal communications) except for south-west England where the species is less abundant than formerly. During 1949–57 *B. balanoides* was rare west of Plymouth and was absent from the Isles of Scilly and the extreme south-west of Cornwall (Figs. 2, 6). The species was more common on the French coast opposite, and was found without difficulty at most stations examined between Roscoff and the Rade de Brest, though frequently confined to low water and never very common. Comparison of representative stations on both sides of the Channel (Table 4) shows that the species is generally more abundant on the French side. Only at two pairs of stations is the incidence of *B. balanoides* less on the French side. The French stations in these cases, Pt. du Roselier and Pt. de Pléneuf, are relatively exposed headlands, while the corresponding English stations do not stand out from the coastline.

On both coasts of the western basin the abundance of *B. balanoides* increases towards the east, but not uniformly. There is a region of reduced density on the English coast, from West Bay to St Catherine's Pt., that is not paralleled by a corresponding reduction on the opposite coast. As *Chthamalus stellatus* is also reduced in abundance at many of the stations in the same area, the

reduction cannot well be attributed to effects of temperature or wave-action. It seems possible that the small tidal range in the region may reduce the stocks of adults to a point where there is difficulty in producing sufficient larvae for more abundant settlement. The strong tidal currents may also be detrimental to settlement of larvae and may hinder recruitment from more distant stocks of adults.

Our observations support and extend previous conclusions as to the influence of habitat on the abundance of barnacles in the Channel (Fischer-Piette, 1936; Southward & Crisp, 1954*a*, 1956). In the western basin *B. balanoides* thrives better and grows to a larger size in the estuaries and harbours than on the open coast. In the eastern part of the Channel it is dominant on the open coast, and in sheltered bays and harbours is replaced by *Eliminius modestus*. On shores composed largely of chalk rocks, as for example at Lulworth Cove, Rottingdean, North Foreland, Cap Blanc Nez and Fécamp, *B. balanoides*, in common with other species of barnacles present, was reduced in number and occurred only sporadically or in sparsely scattered groups. Similar low barnacle counts were found on other soft rocks, such as the Wealden clays exposed at Brook and Fairlight. The relative paucity of barnacles on soft substrata has been commented on previously (Southward & Crisp, 1954*b*), and has been attributed to porosity, with consequent desiccation through the basis (Moore & Kitching, 1939) or to the enhanced erosive activities of limpets on such rocks (Hawkshaw, 1878). The poor adhesion of the base on soft rocks must be a contributory factor, together with the scouring effects of the loose flints that are almost always present on chalk shores, and all these factors probably operate together on the open coast. In sheltered waters, however, where there is little wave-action, where limpets are less abundant and where loose substrata do not move, barnacles may be found in good number on soft materials.

Balanus perforatus

Like *Chthamalus*, *Balanus perforatus* is confined to the western half of the Channel, and is not found east of the Isle of Wight or Cotentin. *B. perforatus*, in recent years, seems also to have undergone an increase in abundance, which may have begun in the 1930's or earlier, for Fischer-Piette (1936) remarked on an increase in the Channel Islands between 1930 and 1935. No doubt, as a southern species, *B. perforatus* might be expected to benefit from any general increase in temperature, such as occurred in the Channel in recent years (Southward & Crisp, 1954*a*; Cooper, 1958). It breeds and settles in a limited period of the late summer and autumn (Norris & Crisp, 1953), and would receive most benefit from the warm autumns that have been a marked feature of the climatic amelioration. Nevertheless, the evidence for increased abundance of this species must be viewed with caution. It is a barnacle that may

be surprisingly locally distributed, and one that is profoundly influenced by wave-action and the configuration of the shore. Observations of its abundance may be very subjective, and we ourselves have noted considerable differences in our individual assessments of its abundance at a station. For these reasons it is difficult to compare our results with those of Fischer-Piette (1936), though we have tried to do so (Table 6). In considering the earlier observations, it is hard to believe that in 1932 *B. perforatus* was absent from Wembury (it was recorded by Colman, 1933) or the Isles of Scilly, though the abundance may have been less than it is now. This throws some doubt on the apparent changes that have occurred at the other English stations.

TABLE 6. RELATIVE ABUNDANCE OF *BALANUS PERFORATUS* AT STATIONS ON THE ENGLISH COAST

	1930–2 (from Fischer-Piette, 1936)	1949–57
St Catherine's Pt.	N	O
Portland Bill	O–R	A
Straight Pt.	A	—
Budleigh Salterton	—	A
Brixham	C	C
Prawle	O–R	A
Wembury ('Blackstone Pt.')	N	A
Rame Hd.	C	A
Lizard Hd.	O–R	C
Lamorna Cove ('Carn du Pt.')	C	A
Cape Cornwall	O–R	C
Isles of Scilly	N	F–A

These terms of abundance are explained in Table 2. We have converted Fischer-Piette's terms as follows: Grande abondance, A; Abondance moyenne, C; Faible, F; Très faible, O–R.

We can, however, be certain that Fischer-Piette's evidence that *B. perforatus* was commoner on the French side of the Channel no longer holds good. In fact, at the western extremity, the species is commoner on the English side. Notable changes seem to have taken place in the Channel Islands, as with *Chthamalus stellatus*. Thus, in Alderney and Guernsey, where *Balanus perforatus* was reported 'très faible' or absent in 1931, we found it to be quite common at most exposed places and extremely abundant on the outer edges of the reefs. In Jersey, on the other hand, the species appeared to be rather uncommon, and only in the north-west of the island did we find it in numbers approaching those present on the outer two islands. We did not see the species at two stations where it was reported previously to be 'faible' or 'très faible', probably because our visit did not allow each station to be worked on a very low tide.

The revised distributions of *B. perforatus* and *Chthamalus* in the Gulf of St Malo agree well with one another (Fig. 5, p. 184). Both are relatively common on the coasts of Ille-et-Vilaine and Côtes-du-Nord, and become scarce along the western coast of Cotentin. They are both rather uncommon in

Jersey, and more abundant in Alderney and Guernsey, although *Chthamalus* is not there as abundant as on the coasts of Ille-et-Vilaine and Côtes-du-Nord. If we compare the extreme limits of *Balanus perforatus* in 1930–32 and 1949–57, there would seem to have been an extension of range eastwards along the English coast, corresponding to the extension of *Chthamalus* during the same period. Fischer-Piette (1936) did not find the species east of St Alban's Head: it is now common in Poole Harbour (Norris & Crisp, 1953), and can be found without difficulty in parts of south Hampshire and the Isle of Wight. Dr H. G. Stubbings informs us that occasionally he has found spat of *Balanus perforatus* on settlement panels exposed at the entrance to Chichester harbour, but so far we have been unable to find any adult specimens in Sussex.

B. perforatus is sometimes locally much commoner in bays and fairly saline estuaries than on the open coast as, for example, in the Halford and Fal estuaries as compared with the Lizard peninsula (Fig. 6, p. 191). However, it can be very abundant in some exposed situations, and there is no general rule that the species is commonest in embayed situations, as might be inferred from Fischer-Piette's evidence. The species is extremely abundant on the Eddystone rocks, off Plymouth, and on the outermost blocks of the Plymouth breakwater, both isolated sites where wave-action is severe and prolonged. The species is also common on sand-scoured reefs in Mounts Bay and Whitsand Bay. The action of the waves, whether accompanied by scouring effects or not, does not therefore seem to be directly inimical to the species.

Balanus crenatus

We have not been able to investigate *Balanus crenatus* in sufficient detail because, on the open coast, it is often present only at extreme low-water mark. Our records, as far as they go, indicate that the species is generally distributed along the Channel, though possibly more in evidence at the eastern end. In the western half of the Channel *B. crenatus* resembles *B. balanoides* and *Elminius* in growing better in estuaries and harbours than on the open coast. Specimens from the Dart, Plym, Penzé and l'Aber Beniot estuaries commonly reach 15–20 mm in diameter, and may occur up to M.T.L., compared with sizes of 6–9 mm at extreme low water on the open coast.

This species, of course, is very common in the infralittoral zone and may be very abundant on the shells of molluscs and crustaceans dredged from shallow water.

Balanus improvisus

We have included the estuarine species *Balanus improvisus* in our records for comparison with *Elminius modestus*, which is replacing it in many of its habitats. *Balanus improvisus* was present throughout the Channel in the estuaries, from the Helford and l'Aber Beniot in the west, to the Rother and Boulogne in the east.

Elminius modestus

Detailed accounts of the spread of the immigrant Australasian barnacle *Elminius modestus* along the Channel coasts will be found elsewhere (den Hartog, 1956; Crisp, 1958; Bishop & Crisp, 1957, 1958). During the later part of the survey the species was abundant in the eastern part of the Channel, but common only in the estuaries and harbours in the west. On both sides of the Channel there was, in 1954–56, an intermediate region in which *Elminius* had not established itself. On the English side it was absent from the greater part of Lyme Bay: on the French side the uncolonized area was larger, and included the whole of the Gulf of St Malo and the Channel Islands.

Verruca stroemia

We have attempted to study the distribution of the low-water species *Verruca stroemia* in more detail than the other species found only at the lower levels of the shore, since its general European distribution is intermediate between the distributions of the typically northern or southern species. *Verruca* is most commonly found in shallow water below the tide marks, but may be quite abundant in the infralittoral fringe in places. Its most favoured habitat is on the undersides of rocks and stones where there is little movement of the substrate, but where there is enough water movement to prevent the deposition of large amounts of silt. Incomplete observations on *Verruca* suggest that it may be restricted by its feeding habits to places with some water currents, for it does not always set up a current by rapid beating of the cirri as other barnacles do (cf. Southward, 1955*c*).

Where conditions are satisfactory the species can be very abundant in the intertidal zone, as for example at Brixham, as noted by Fischer-Piette (1936), on the stones and boulders of the breakwater. At Plymouth it can be found in widely distinct habitats such as the outer reefs at Wembury, and the fairly silty conditions at Hen Pt. in the Tamar. *Verruca* is common on most shores at the western end of the Channel, but seems to become sparser eastwards. The most easterly record, where two or three specimens only were found, is Fairlight, Sussex. The relatively scanty records may perhaps be filled in by use of planktonic occurrences of the larvae, which are quite distinctive (Bassindale, 1936), but this method inevitably takes account of infralittoral populations also. It seems probable that the species is absent from the southern North Sea, for larvae have not been seen in inshore plankton samples from Essex. The absence of *Verruca* nauplii from hauls at Brighton confirms that the adult cannot be very common in the eastern half of the Channel. At Brixham, Plymouth, and farther west in the Channel (see p. 193) *Verruca* is often the most abundant cirripede larva in the plankton in spring.

Hemioniscus balani

We became interested in the parasitic isopod *Hemioniscus balani* since there appeared to be some relation between its distribution and abundance and the abundance of its common host, *Balanus balanoides*. The latter is not, of course, the only host in the Channel since *Hemioniscus* has been found in *Elminius* and *Balanus improvisus* as well as in *B. balanus* (Perez, 1923; Crisp & Molesworth, 1951). Our records relate to the larger gravid female stage only, as the smaller male stages are often overlooked in the field.

It has already been shown (Southward & Crisp, 1954*a*) that the incidence of the parasite in the western part of the English side of the Channel in 1949–52 was much higher than farther east; the later observations confirm and extend these results. The species was found almost everywhere in the western basin, often at very high percentages of infection, but was very sparse or absent altogether in the eastern basin where the host was more abundant.

Since *Hemioniscus* occurs in the North Sea, some explanation other than the influence of climate alone must be found for its relative sparsity in the eastern part of the Channel. It is perhaps significant that there was an apparent increase in the rate of infection by *Hemioniscus* in Devon and Cornwall between 1949 and 1952 (Southward & Crisp, 1954*a*), while the host *Balanus balanoides* decreased in abundance. More recent observations show a smaller percentage of infection in the following years, coincident with a general resurgence of the host (Southward & Crisp, 1956).

Just as a change in average temperature may alter the balance between competing species such as *Chthamalus stellatus* and *Balanus balanoides*, it may influence a host-parasite relationship. On this theory we have only to postulate that *Hemioniscus* passes through its life cycle more quickly at higher temperatures, *Balanus balanoides* being limited in its reproduction to a single annual cycle. Further work is clearly necessary before a definite conclusion can be reached on the dynamics of the relationship between *Hemioniscus* and its hosts.

Patella vulgata

The common limpet, *Patella vulgata*, was found on all solid substrata throughout the channel. On the English side it was the only limpet present east of the Isle of Wight, as reported by Evans (1953).

Patella depressa

Patella depressa (=*P. intermedia*) is confined to the western half of the Channel. The eastern limits are at Ventnor and Culver Cliff (Fischer-Piette, 1936) on the English side, and Barfleur on the French side. At these stations the range of the species ends abruptly, without undergoing any gradual

diminution in abundance such as occurs with other animals. At most wave-beaten localities in the western basin it constitutes from 10 to 50%, or frequently more, of the limpet population around mid-tide level. It is scarce or absent on part of the west coast of Cotentin, where the rocks seem to be made unsuitable for limpets by sand scour. On both sides of the Channel it is found in less abundance at the extreme western end. It is absent from the Isles of Scilly, rare in Ushant (Fischer-Piette, 1936), and the abundance on the open coast does not exceed 50% at any place west of Plymouth. In Finistère our records differ from those of Fischer-Piette (1936) in showing that the species is not entirely absent from Tremazan. A few specimens were found in a limited region towards low water, but the shore appeared generally unsuitable to limpets and barnacles, possibly because of the dense algal growths. At other open coast stations farther east *P. depressa* constituted more than half the limpet population at some levels.

On extremely wave-beaten rocks the limpet population tends to be dominated by *P. aspera*, which seems more tolerant of excessive wave-action or wetting. For example, on the Eddystone rocks off Plymouth the larger limpets are almost all *P. aspera*; *P. depressa* has not been found and *P. vulgata* is represented only by smaller specimens towards the upper midlittoral. At Shipman Head, Bryher, Scilly, over 90% of the limpets below H.W.N. were *P. aspera*. These effects of wave-action may explain the reduced numbers of *P. depressa* at some of the more western stations, but larval dispersal may also be involved (see p. 194).

Patella aspera

The distribution of *Patella aspera* corresponds closely with that of *P. depressa*, and there is a similar sharp cut-off at the identical stations which mark the eastern limits of the species. *P. aspera* is abundant at most exposed sites in the western basin. We could not detect any general trends in distribution except that the species was scarce on the coast of Cotentin where sand scour is severe.

It must be noted that the agreement in distribution of *P. depressa* and *P. aspera* does not hold good outside the Channel. *P. aspera* extends up the west coast of Britain, and penetrates southward into the North Sea on the east coast of England (evidence to be published later), while *P. depressa* has its northern limit in Anglesey (Crisp & Knight-Jones, 1955) and is absent from Ireland (Southward & Crisp, 1954*b*).

Monodonta lineata

Monodonta lineata is the most conspicuous of the top-shells, since it is common at the upper limit of the midlittoral zone and can be found in the open on bare rock. Like the other top-shells, however, the greatest numbers are found on partly sheltered shores of boulders or broken rock, and it is less

common on steep slopes (see Southward & Crisp, 1954*b*). *Monodonta* is abundant throughout most of the western half of the Channel, but extends considerably farther east on the French coast. On the English side the limit

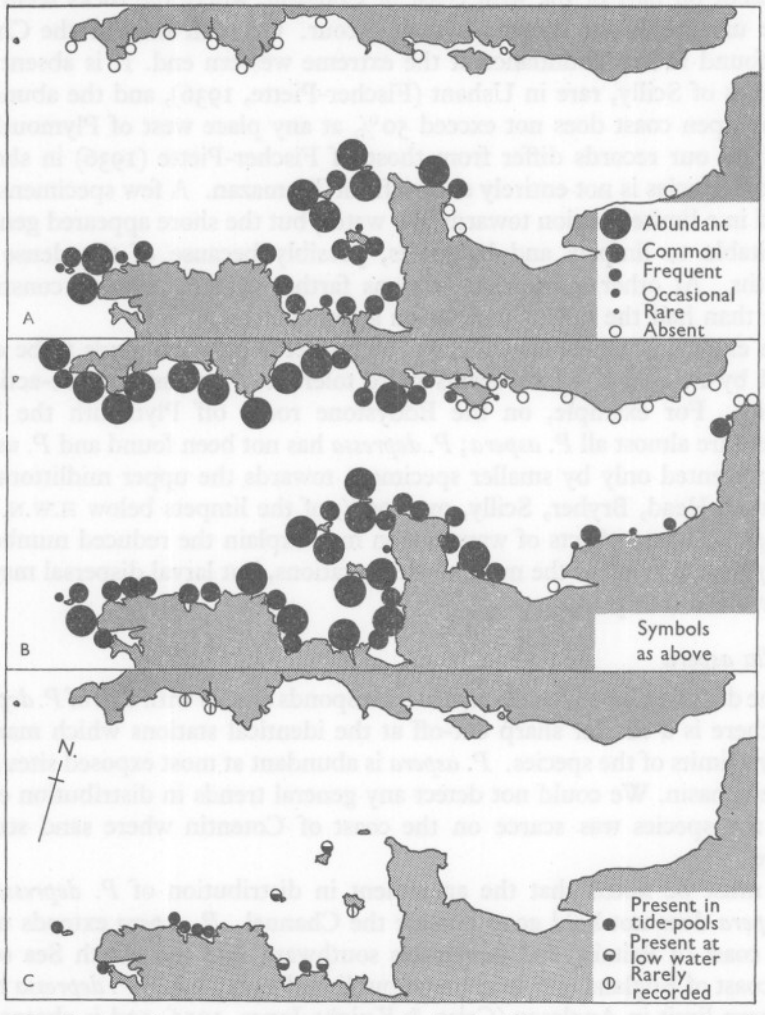

Fig. 3. Distribution of some representative species in the Channel. A, confined exclusively to the French side of the western basin; *Gibbula pennanti*; B, quite common along the whole French coast, but confined to the western basin on the English side; *G. umbilicalis*; C, confined to the western basin, but not at all common in the Channel, and rarely found on the English side; *Paracentrotus lividus*.

is at Lyme Regis; on the French side it is present in small numbers east of the Cotentin peninsula, but is absent from most of the Normandy coast. The boundaries of this species imply that it is limited by factors other than the

configuration of the shore, for many apparently suitable shores are found to the east of its range. For example the Dorset coast between Weymouth and Swanage, and the Normandy coast east of Arromanches, both offer very suitable habitats for top-shells if the abundance there of *Gibbula umbilicalis* is taken as a guide. We have unsuccessfully searched the Dorset area several times, since there are old records (Mansell-Pleydell, 1898) of the occurrence of *Monodonta* there. It seems probable that the records are based on casual findings of dead shells; the finding of a 'recently vacated' dead shell at Whitstable, Kent (el Maghraby & Perkins, 1956) shows how misleading such records may be.

Gibbula umbilicalis

The distribution of *Gibbula umbilicalis* follows the pattern of that of *Monodonta*, and although it penetrates somewhat farther east than the latter on both sides of the Channel, there is again a considerable difference between the English and French sides (Fig. 3, p. 176). The most easterly occurrence of *Gibbula umbilicalis* on the English coast is at Bembridge, while on the French side it occurs as far as Calais. The areas where the species was not found on the French coast, around Le Havre and Le Tréport, are rather unfavourable to top-shells, the few rocks being separated by wide stretches of sandy beach. On the English coast the absence east of the Isle of Wight cannot be attributed entirely to the absence of suitable shores, for there are apparently suitable habitats at Seaford, Brighton and near Beachy Head.

Gibbula pennanti

The validity of the species *Gibbula pennanti*, noted as an unnamed variety of *G. umbilicalis* by Forbes & Hanley (1853), has been established by Gaillard (1954). We ourselves did not learn the species until the later part of the survey. For this reason our maps are not as complete as could be desired (Fig. 3A). The adults can be separated easily from *G. umbilicalis* by the closure of the umbilicus and, once learnt, the two are usually distinguishable on shell pattern alone. The uninitiated would be easily misled, for although *G. pennanti* may be commoner at low water in some places, in localities where it is abundant it tends to occur over most of the midlittoral zone and may replace *G. umbilicalis* as the most abundant top-shell. Unlike *G. umbilicalis*, however, *G. pennanti* is confined to the French side of the western half of the Channel. It was found at most stations on the Cotentin peninsula, the Gulf of St Malo, and the Brittany coast, and was common in the Channel Islands, especially in Alderney and Guernsey, but was absent east of Barfleur. Careful searches in the south-west of England, in places where the habitat appeared to be essentially similar to that in which the species is found in France, have failed to disclose a single specimen.

Gibbula cineraria

The top-shell commonly found at low water, *Gibbula cineraria*, was found throughout the Channel. Although it favours broken rocky shores like other top-shells, the species is much less sensitive to the presence of sand, and may sometimes be common in predominantly sandy places. Unlike the other species of *Gibbula* discussed above, it is quite common infralittorally, especially among algae.

Littorina species

The common small periwinkle, *Littorina saxatilis*, is abundant throughout the Channel. It seems very tolerant of scouring and of low salinity. *L. obtusata* is also common everywhere where fucoids are plentiful.

The edible winkle, *L. littorea*, is present throughout the Channel on the more sheltered shores. It is intolerant of wave-action, and is scarce at the western extremities of both sides of the Channel, and very rare in the outer Channel Islands. We did not find it in the Isles of Scilly, but it was recorded there by Clark (1906), and Mr G. M. Spooner tells us that he found two specimens in 1936.

L. neritoides is abundant on exposed shores throughout the western half of the Channel. On the English side it is present in the Isle of Wight, where the eastern limit is Sandown. On the French coast it penetrates somewhat farther into the eastern basin; it was present in small numbers on the Normandy coast east of Cotentin, and two specimens were collected at Calais.

Paracentrotus lividus

The sea-urchin, *Paracentrotus lividus*, which is such a noticeable feature of intertidal pools on the Atlantic coasts of Ireland and France, is much rarer in the Channel, where it is often confined to extreme low-water mark or the infralittoral zone. For this reason we have few positive records, and have relied on earlier observations to compile Fig. 3c. *Paracentrotus* can be regarded as virtually absent on the English side, specimens having been found only occasionally at Looe and Wembury (Marine Biological Association, 1957; personal recollections of the Plymouth Laboratory staff; preserved material in the faunal collection at Plymouth). On the French side our observations confirm that the species is common only in the neighbourhood of Roscoff (Station Biologique de Roscoff, 1951*a*) where it is present in pools towards low water. It is less common in the Bay of St Brieuc, at the Roches Douvres and in Guernsey (Koehler, 1884; Fischer, 1928). No specimens were found during our survey in the extreme north-west of Brittany, but observations were not made on the more exposed islands offshore where *Paracentrotus* may be more common, as it is in Ushant.

OTHER ANIMALS

Balanus balanus

Balanus balanus (=*B. porcatus*) is not common intertidally, but its distribution has always interested us, as it is a species of markedly northern character. It is present on the shore, at extreme low water, in Anglesey and the Isle of Man, and there are recent records of its occurrence at low water, or just within the infralittoral zone in south-west Ireland (Lilly, Sloane, Bassindale, Ebling & Kitching, 1953). It has been found on test panels from Milford Haven, and has been recorded by Bassindale (1941) in other parts of the Bristol Channel. The species seems to be common in the North Sea, but we have been unable to confirm any of the records of its occurrence in the Channel. It has not been seen on the shore, and has never been found on dredged material brought into Plymouth or Brixham. There exist specimens in the Jeffreys collection, now in the U.S. National Museum, labelled as from Exmouth (Pilsbry, 1916). The authenticity of some of the Jeffreys material is doubtful, however, since the collection includes specimens of *B. perforatus* labelled as from Irish and Scottish localities where the species does not occur (Norris & Crisp, 1953; Southward & Crisp, 1954*b*). The most reasonable view of these discrepancies in the Jeffreys collection is that some of the labels have been exchanged.

At the moment we conclude from the limited evidence that *B. balanus* is absent from the western half of the Channel and scarce or absent in the eastern half.

Haliotis tuberculata

The ormer, *Haliotis tuberculata*, found mainly at extreme low water or infralittorally, is restricted entirely to the western half of the French side of the Channel, where the limits are Alderney and Cherbourg (Wegmann, 1884; Sinel, 1906; Stephenson, 1924; Crofts, 1929; Tomlin, 1937; Station Biologique de Roscoff, 1951*b*). We have few personal records, but local collectors confirmed that the species is still quite abundant in Guernsey and Alderney, where it may be commoner than in Jersey, and in north Brittany.

Gibbula magus

The top-shell *Gibbula magus* is predominantly infralittoral, but is sometimes common at extreme low-water mark in the Channel. Its distribution intertidally appears to follow the trend of the other top-shells of southern character, for the species is much more frequent on the French side. It is common on sandy shores. We found it in some number on the Cotentin peninsula, as far east as Grandcamp, and it appears to be abundant around Roscoff (Station Biologique de Roscoff, 1951*b*). *G. magus* sometimes occurs intertidally near Plymouth (Marine Biological Association, 1957) but numbers approaching those found on the French coast have been encountered only in the Isles of Scilly.

Calliostoma zizyphinum

A further top-shell, *Calliostoma zizyphinum*, has been included in the detailed table (Table 7, Appendix), but the distribution is not clear. Since the species can occur infralittorally only positive records have been noted. *Calliostoma* is present throughout the Channel, and has been recorded from the vicinity of Ramsgate (Woodward, 1880). That more records from the western basin than the eastern basin are included in the table may be due to the greater amount of rocky shores available in the west, and it is not possible to be sure of any trends in distribution.

PLANTS

Only the commoner species of large *Phaeophyceae* could be investigated (Table 1, p. 159). No doubt the other classes of algae have species with corresponding types of distribution (cf. Fischer-Piette, 1936).

Laminaria ochroleuca

Probably the most southern in distribution of the laminarians, *L. ochroleuca* has been recognized in the Channel only in more recent years. There seems good evidence for believing that it has increased its range and abundance in the Channel (Parke, 1948). It is now common in south-west Cornwall and around Plymouth, and extends as far east as the Salcombe estuary (Spooner, 1950), but is apparently absent from Torbay and farther east (personal communications, Mr G. M. Spooner). On the French side the distribution corresponds with that of the southern types of animals. It is abundant at the western end, and has its eastern limit about Alderney and Barfleur, but is apparently absent in the Gulf of St Malo, Jersey, Guernsey and most of Cotentin (Hamel, 1931–39; Lami, 1943, 1954; Station Biologique de Roscoff, 1954).

Laminaria spp.

The three other species of *Laminaria* present in the Channel, *L. digitata*, *L. hyperborea* and *L. saccharina*, occur throughout. *L. digitata* and *L. hyperborea*, which grow best on firm substrata where there is some exposure to wave-action, may be less common in the eastern basin.

Saccorhiza polysichides

The laminarian *Saccorhiza polysichides* is of relatively southern character; like *Laminaria ochroleuca* it seems to be absent from the eastern half of the Channel. On the French side the eastern limit is just north of Barfleur (Hamel, 1931–39).

Alaria esculenta

The only laminarian to favour very wave-beaten sites, *Alaria esculenta*, is found exclusively in the western basin of the Channel. On the French side it occurs as far east as Alderney and Cherbourg (Fischer-Piette, 1936; Hamel, 1931–39). We were unable to visit many of the really exposed sites that the species favours, but have confirmed that it is abundant in Alderney. On the English side *Alaria* does not seem to penetrate as far east. We have found the species as far east as Portland Bill, but it is not common to the east of Start Pt. Its southern limit is just south of the entrance to the Channel (Dizerbo, 1947).

Ascophyllum nodosum

Ascophyllum nodosum is present throughout the Channel, but appears to be commoner in the western half (Hamel, 1931–39), especially in estuaries and sheltered bays.

Fucus and *Pelvetia*

The fucoids *Fucus spiralis*, *F. vesiculosus*, *F. serratus* and *Pelvetia canaliculata* are present throughout the Channel.

Himanthalia elongata

We have confirmed that *Himanthalia elongata* is confined to the western half of the Channel (Fischer-Piette, 1936; Hamel, 1931–39). The eastern limits are Peveril Pt. and Barfleur, but the species is absent from the inner parts of Lyme Bay and the Gulf of St Malo. This distribution has been discussed in some detail by Fischer-Piette (1936).

Bifurcaria rotunda

There is little to add to previous accounts of the distribution of *Bifurcaria rotunda*. It is present in pools at most exposed sites on the English coast as far east as Start Pt.; it is not now absent at Wembury ('Blackstone Pt.') as stated by Fischer-Piette (1936). On the French side it is quite abundant in the two outer Channel Islands, Alderney and Guernsey, and on the north coast of Cotentin, but does not occur east of Barfleur. It is often found as a distinct zone at the upper limit of *Himanthalia* in France and the Channel Islands, but we have not observed this form in England, although it was noted at a few stations by Fischer-Piette.

GENERAL TRENDS OF THE DISTRIBUTIONS

East-west trends

There is a clearly marked east-to-west trend in the distributions of many of the animals and plants which have been described. In nearly all such species not only is the general trend from east to west the same, but the range

and even the detailed features show that a remarkable similarity exists between the British and French coasts. For example, *Balanus balanoides* becomes gradually less common west of the Cherbourg peninsula and of the Isle of Wight as *Chthamalus stellatus* increases, and markedly less common west of Start Point and Sillon de Talbert. The eastern limits on the two sides of the Channel of *Patella intermedia*, *P. aspera*, *Anemonia sulcata* and *Balanus perforatus* are almost identical. Perhaps the most striking feature of the general similarity between the two coasts is the sharp faunal and floral discontinuity existing on both sides at the junction of the eastern and western basins. On the British side the limits of *Chthamalus stellatus*, *Balanus perforatus*, *Littorina neritoides*, *Gibbula umbilicalis*, *Patella intermedia*, and *P. aspera* occur at or very close to the Isle of Wight, the limits of *Monodonta lineata* and *Himanthalia* somewhat farther to the west, while *Anemonia sulcata*, though very common in parts of the Isle of Wight, has been found at only one station farther east. The critical nature of these limits is indicated in Fig. 4. A similar critical change occurs at Alderney and on the Cotentin peninsula, where *Chthamalus stellatus*, *Balanus perforatus*, *Patella depressa*, *P. aspera*, *Paracentrotus lividus*, *Haliotis tuberculata*, *Laminaria ochroleuca*, *Saccorhiza polysichides*, *Alaria esculenta*, *Himanthalia elongata* and *Bifurcaria rotunda* all have their eastern limits, although some species, e.g. *Monodonta*, *Littorina neritoides* and *Anemonia* extend a little to the east (Fig. 5).

These sharp biological limits midway along the Channel coincide with the topographical limits separating the two basins. Thus the western basin has a rich fauna including many Atlantic and Lusitanian–Mediterranean forms together with Boreal and Celtic species, while the eastern basin has a depleted fauna from which most of the southern elements are missing.

North-south differences

Differences between distributions of animals on the north and south coasts of the Channel are less conspicuous than those between the east and west basins. The differences that exist are due to the presence of more southern forms on the French coast. *Gibbula umbilicalis* and *Littorina neritoides* have a greater range eastwards on the French side: other forms which are generally more abundant are *Bifurcaria tuberculata*, *Laminaria ochroleuca* and *Gibbula magus*. Several species are found exclusively or almost exclusively on the French side, such as *G. pennanti*, *Haliotis*, *Paracentrotus*, and also species not included in the present survey such as *Pollicipes cornucopiae*[1] and possibly *Pachygrapsus marmoratus*, which was at one time present in the Roscoff area. The only species which appears to be more abundant on the British coast is *Chthamalus stellatus*. It is interesting to note that this species can occur very high up in the intertidal zone, while those forms restricted to the French side are mainly found towards low-water mark.

[1] There is a single record of *Pollicipes* from Land's End (specimens in British Museum).

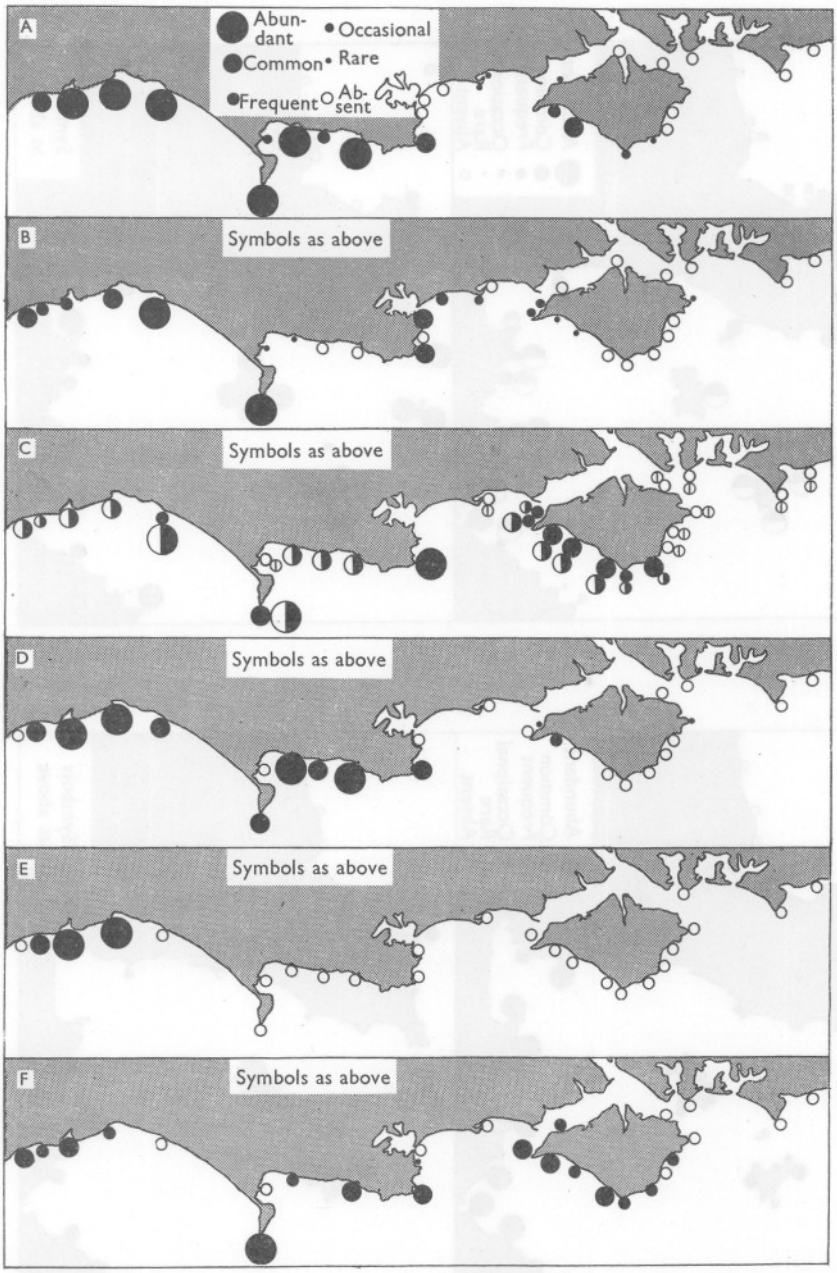

Fig. 4. The critical region on the English coast where many of the southern forms have their eastern limits. A, *Chthamalus stellatus*; B, *Balanus perforatus*; C, *Patella aspera* and *P. depressa*, the latter shown by half-black or barred symbols; D, *Gibbula umbilicalis*; E, *Monodonta lineata*; F, *Littorina neritoides*.

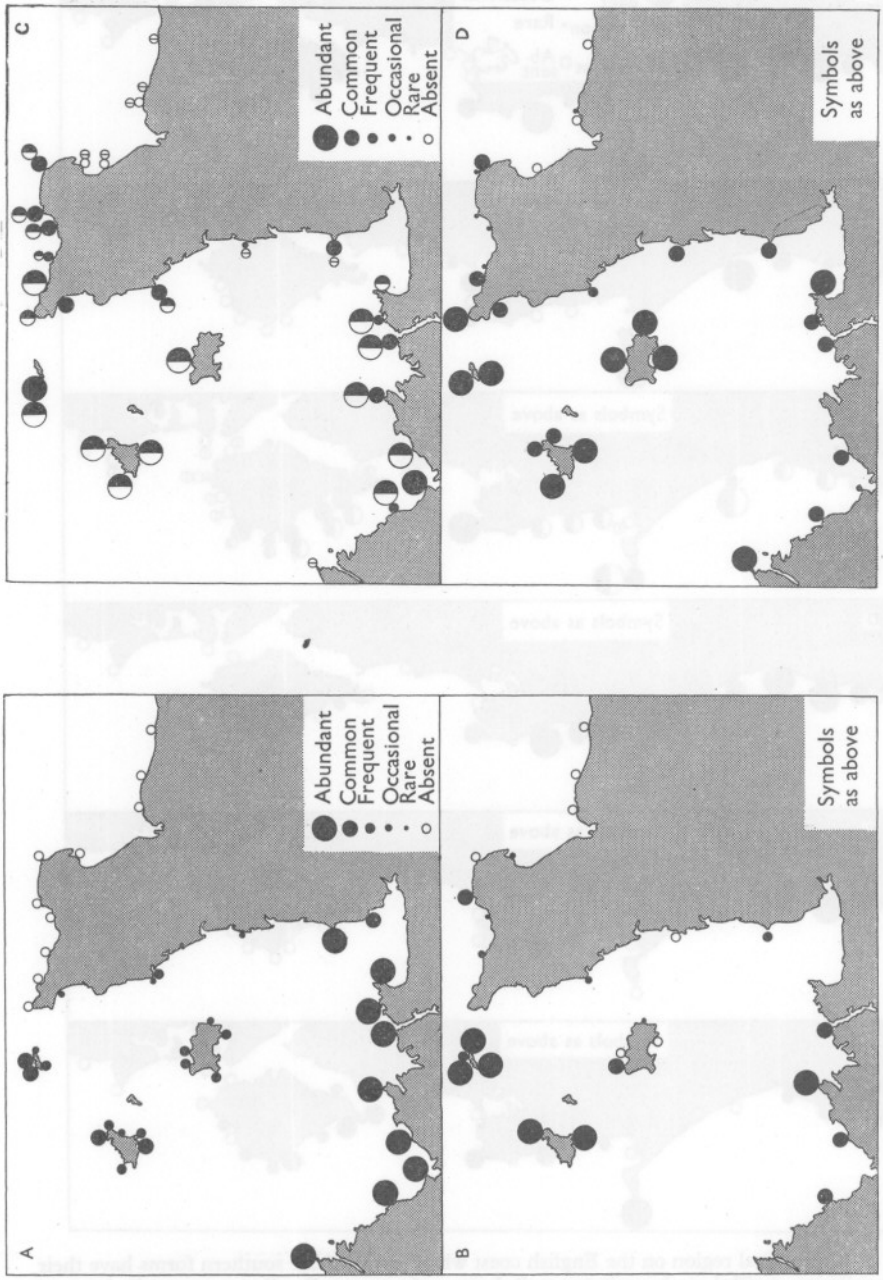

Fig. 5. The critical region on the French coast, showing the limits of: A, *Chthamalus stellatus*; B, *Balanus perforatus*; C, *Patella aspera* and *P. depressa*; and D, *Monodonta lineata*. The half-black or barred symbols refer to *Patella depressa*.

RELATION BETWEEN DISTRIBUTIONS AND ENVIRONMENTAL FACTORS

We have already described the major environmental variables found in the Channel. In this section they are discussed as controlling influences on the distribution of the organisms.

The animals and plants which share the main trend in distribution from east to west are varied in many respects. First, their requirements differ in regard to exposure. Some, such as *Littorina neritoides*, *Chthamalus stellatus*, *Paracentrotus lividus*, *Patella aspera*, and *Alaria esculenta*, grow best in rocky wave-beaten situations, while others, such as top-shells *Monodonta lineata*, *Gibbula umbilicalis*, *G. pennanti* and *G. magus* occur predominantly in some degree of shelter. The greater expanse of rocky shore, and the heavier swell at the western end of the Channel cannot therefore be the only, nor indeed the main factors. At least one species not included in our survey, which requires the proximity of a sandy or gravelly environment, *Sabellaria alveolata*, is abundant in many areas at the western part of the Channel and is replaced by *S. spinulosa* to the east.

Secondly, they differ in their relation to tide level. Some of those confined to the western half of the Channel, such as *Anemonia sulcata*, *Gibbula magus*, *Paracentrotus lividus* and *Bifurcaria tuberculata*, are found mainly at the lower levels or in pools, while others such as *Chthamalus stellatus* and *Littorina neritoides*, are found high up the shore. It is not possible therefore to attribute the general trend to differences in the tidal regime such as the occurrence of equinoctial spring tides at midday in the western basin and at morning and evening in the eastern basin.

Thirdly they differ in their nutrition. The plants are, of course, autotrophic. Many of the animals, such as the limpets *Patella aspera* and *P. depressa*, the periwinkle *L. neritoides*, the top-shells *Monodonta lineata*, *Gibbula umbilicalis* and *G. pennanti*, and the urchin *Paracentrotus lividus*, are surface scrapers, though their nourishment must differ considerably with their tidal zonation and particular habitat. Others, such as the barnacles, can feed on suspended matter including a wide range of particle size (Southward, 1955*a*). *Anemonia sulcata* is a macrophagous carnivore, and *Hemioniscus balani* a parasite. The distributions cannot therefore be attributed to a greater abundance of food organisms in any particular habitat.

Lastly the organisms showing the east-west trend differ in their life histories. The great majority have planktonic stages, but there are without doubt great differences between them in the periods of their development in the sea, although the precise duration of many is not known. All the barnacles, *Paracentrotus*, and probably most of the littorinids have relatively long planktonic phases, while the limpets take only a few days to metamorphose (Lebour, 1937; Thorson, 1946; Dodd, 1957). The anemones are viviparous,

while some of the algae have sporophyte generations which alternate with a small gametophyte. It follows that biological factors present in the sea which influence larval development cannot exercise an important influence on the distributions as a whole.

Effect of temperature

Of the few environmental factors which can be considered as having possible influences on such widely varying organisms, temperature, which may affect all stages of the life history, is probably the most important. Since the western end of the Channel is warmer on average and certainly less severely cold in winter, the species which are plentiful there should be those which are either unable to compete successfully with cold hardy forms or which suffer directly from exposure to the cold; that is forms with a southern or Lusitanian distribution. Conversely, since the eastern end of the Channel is much colder in winter, the forms predominating there must be tolerant of cold conditions. Table 1 (p. 159), which summarizes the known distributions and other relevant details of the species which have been studied, shows that the majority of the forms confined to the western basin of the Channel are in fact those of southern distribution. Of the species whose distribution extends well to the north of the British Isles, the majority are found in similar abundance in both basins, though Arctic forms such as *Balanus balanoides* are less common in the extreme west. The Arctic form *Alaria esculenta* is exceptional in occurring only in the western part of the Channel. It is possibly so dependent upon wave-beaten sites that it is excluded from the eastern basin, even though it has its southern limits just south of the Channel.

The differences between the north and south coasts of the Channel may also be attributed to temperature differences. The sea temperature is higher in winter on the French side of the western basin, and in summer is distinctly higher in the Gulf of St Malo and the Baie de Seine. Thus a number of animals and plants of southern origin penetrate for varying distances along this coast, although they are not represented, or are very sparse, on the coasts of South Devon and Cornwall. Others are able to extend farther eastwards on the French side than on the English side (see above).

There are certain objections, however, which might be raised against the view that the temperature gradient is the predominant factor influencing distributions in the English Channel.

The first difficulty arises from the generally accepted view, put forward by Apellöf (1912), Orton (1920), and Runnström (1929), that marine organisms are restricted primarily by the temperature prevailing during the breeding season, for most animals can live at considerably greater extremes of temperature than those which are tolerated during the reproductive period. Thus, for example, many animals found near their northern limits in south-west Britain breed only for a short period in the summer months, so that their fecundity is

progressively reduced with fall in summer temperature. There is, moreover, little doubt that land-locked areas, having increased summer temperatures, offer especially favourable environments (Southward & Crisp, 1954*b*). Yet, as will be seen by comparing the summer and winter isotherms with the general trend of the distribution maps, the correlation between distribution and temperature applies not to the maximum reached in summer, which would be thought to be critical for reproduction, but rather to the winter minima. Winter conditions can have little relevance to the reproductive state or to the larval stages, for as shown in Table 1 all but three southern forms breed in summer. The difficulty cannot satisfactorily be resolved by assuming that winter temperatures in the eastern half of the Channel are lethal to the adults of these species, for there is no evidence of such mortality. Experimentally it has been demonstrated that some of the southern forms are killed at temperatures that are not uncommon on the land at the eastern end of the Channel. But, as the accompanying paper on this subject shows (Southward, 1958), the more extreme values of low air temperature recorded will be rarely experienced by the animals on the shore. Possibly the less extreme temperatures that are found on the shore might be shown to have debilitating effects in long-term experiments, but against this is the fact that one species at least (*Chthamalus stellatus*) can survive transplantation to the north and east of Britain, overwinter, and produce nauplii the following summer (Crisp, 1950). If mortality occurred as a result of cold, species which extend throughout the greater part of the intertidal range in south Devon, such as *Chthamalus stellatus* and *Littorina neritoides*, would be expected to be killed predominantly at their upper limits where the duration of frost exposure is greater. In fact, however, these two species are absent from the lower levels in cooler localities and at their extreme eastern limits are found only high up the shore.

These difficulties arise through seeking a temperature-vulnerable phase in the life history, probably an unwarranted simplification. All stages in the life history are influenced by temperature. Not only must the adults attain a breeding condition and the larvae develop successfully, but the post-larvae and young adults of most southern forms must also survive and grow sufficiently during the coldest part of the year. Therefore an equably warm environment such as occurs in the western half of the Channel may be better for southern forms than one of extremes, despite possibly higher temperatures at the breeding season. There may, in fact, be no direct influence of temperature at all. If the survival of the species depends upon its rate of growth or feeding in competition with other forms, temperature may operate decisively on this competition well above the level at which it would be directly lethal (cf. Southward, 1955*b*, 1957). We have described elsewhere how the generally warmer conditions in south-west Britain may favour a southern form *Chthamalus stellatus*, while towards the north and east it is displaced by *Balanus*

balanoides, a northern form occupying a similar niche (Southward & Crisp, 1956). Barnes (1956) has evidently reached the same general conclusion. The evidence therefore suggests that the presence of some, perhaps the majority, of the southern forms in the western half of the Channel but not farther east is not simply because they can survive there during the milder winter, but rather because the temperature is favourable to them for a sufficiently long period during the year. That the recent climatic amelioration seems to have been accompanied in the Channel by an increase in range and abundance of at least three southern species tends to support this view. Further, the hypothesis that temperature influences animals and plants throughout the year, and during all stages in their life history, is necessary to explain certain minor trends in distribution as, for example, the variation in the abundance of *Chthamalus stellatus* and *Balanus perforatus* in the Gulf of St Malo (p. 171). In this area there is considerable variation from place to place in the range of temperature throughout the year, but it must on average be sufficiently high for southern forms. The abundance of these two southern species in the two outer islands, Alderney and Guernsey, can be attributed to the more equable temperatures there during the cooler part of the year; this probably assists them in competition with the northern species, *B. balanoides*, which is somewhat less abundant than it is in Jersey and other parts of the Gulf. However, the same two southern species are also abundant on the southern shores of the Gulf; their abundance there must be attributed to the beneficial effects of heating-up of the shore and shallow water during the summer, and its influence on breeding and larval output (cf. Southward & Crisp, 1954*b*). The beneficial effect must be so great that it enables the species to overcome the difficulties imposed by the very much colder conditions during the winter. Jersey lies between the two areas in temperature regime; its shores are cooler in summer than those of the mainland, and it is colder in winter than the outer islands. Presumably the summer temperature is not high enough to allow sufficient increase in breeding activity to compensate for conditions during the rest of the year.

Critical limits

There are other aspects of distribution that cannot be explained by the temperature hypothesis alone, notably the existence of sharp discontinuities affecting several species together. These discontinuities are found at the Isle of Wight and the north coast of Cotentin, at the region of separation of the eastern and western basins. Examples of similar critical limits are found in Anglesey (Crisp & Knight-Jones, 1955).

The critical limits in the Channel do not coincide with closely approximating isotherms (Figs. 1, 4, 5). If temperature alone were controlling distribution of the southern species the limits of different species would be expected to lie at a series of geographically separated regions, the position of

each limit being related to the temperature tolerance of the species in its natural habitat. It is very unlikely that the tolerance limits would then be nearly identical for so many of the species studied. Therefore these sharp faunistic limits point to some factor additional to temperature acting as a barrier.

Effect of substratum

The shores of the eastern basin of the Channel differ from those farther west (p. 164), the infrequent outcrops of harder rock and the prevalence of beaches of scouring material making conditions less suitable for rock-living organisms. These conditions influence adversely all such organisms, but any which are near extinction through other factors, such as temperature, may be unable to extend beyond the point where suitable rock becomes scarce. That this is so is strongly suggested by the fact that the fauna of the eastern section of the Channel is an impoverished one. All the species represented there, so far as we know, are found in the western basin, though some in less abundance. Many species found in the west are, however, absent from the eastern part of the Channel. These species are mainly, but not all, of southern origin. Similarly, many species of algae, for example *Bifurcaria*, *Himanthalia*, *Saccorhiza* and *Alaria*, the latter a northern form, are absent. This poverty of algae must surely be related in part to lack of sufficient rock substratum, to opacity of the water, and to the damage due to scour. The relative lack of rocky substrata and the extent of the scouring that may occur in the eastern basin probably contributes to the absence of the more southern barnacles, and to the scarcity or absence of forms such as *Patella aspera* and *Littorina neritoides*, which can live under the cold conditions of the Yorkshire coast. Thus, it may be that to forms approaching their limit of tolerance of other conditions, including temperature, a change in the nature of the shore may present a simultaneous physical barrier.

However, although the critical faunistic limits correspond roughly with the beginning of extensive areas of sand and shingle, certain of the species terminate to the west of the point where the shore character changes. For example, *Chthamalus* is not found on the north coast of Cotentin, where ample rock is available, while apparently suitable beaches of large boulders occur in Dorset and Normandy eastwards of the limits of *Monodonta*. In the Isle of Wight several of the species studied do not penetrate beyond the southern tip, though apparently suitable reefs exist at the eastern end of the island. Populations growing near the eastern boundary of a rocky shore can clearly be recruited only from the west, but this should not in theory reduce the population density below about half that of regions where recruitment can occur from both directions, assuming even dispersal of larvae.

There are two other factors which may account for the position of these critical faunistic limits more precisely: aspect and dispersal.

Aspect

The need to shelter half-hardy garden plants from north and east winds is well known. The same is likely to hold for the sessile or nearly sessile plants and animals of the intertidal zone. Indeed the effect is probably more pronounced on the shore than it is inland because the cooling effect of strong cold winds from east or north will be enhanced by evaporation as the sea ebbs and the animals are exposed to the air. On a south- or west-facing shore the strongest winds will be associated with mild weather, but on a north-, and especially on an east-facing, shore the strongest onshore winds will be from a cold quarter, so that the greatest cooling by evaporation will occur simultaneously with low air temperature. North-facing shores will also receive less warmth on account of the lower angle of incidence of the sun; they may be almost without sunlight in winter if the slope is steep. The distribution maps show that many of the limits of southern forms coincide with a change in aspect of the coast. *Chthamalus stellatus*, *Balanus perforatus*, *Gibbula umbilicalis*, *Littorina neritoides* and *Patella intermedia* all disappear near St Catherine's Pt., Isle of Wight, where the aspect of the coast changes from south-west to south-east; on the Cherbourg peninsula *Chthamalus* disappears near Cap la Hague, where the aspect changes from west to north, while *Patella depressa*, *P. aspera*, *Himanthalia*, *Bifurcaria* and *Saccorhiza*, and to a large extent *Balanus perforatus* disappear around Barfleur, where the aspect changes from north to east. A notable example of the apparent influence of aspect on a species of northern character is found in west Cornwall (Fig. 6). Here *B. balanoides* is virtually absent from the south coast, rare on the west-facing portion, and abundant on the north-facing region of the coast. Although not shown on the map, the species becomes less common farther east on the north Cornish coast, where it gains a more westerly aspect.

Effects of currents

Another factor which may cause several species to undergo a sudden change in abundance is the influence of currents on dispersal of the planktonic larvae. Intertidal animals with free-swimming larvae must suffer loss of recruitment whenever the larvae are carried too far out to sea and fail to return to the coast. The amount of loss will depend on how far the tidal or residual currents carry water masses offshore, and will increase with the strength of these currents. When the loss of recruitment becomes critical the species must disappear, for there are usually minimal densities of adults below which the population cannot maintain itself without outside assistance (Crisp, 1950; Crisp & Southward, 1953; Crisp, 1958: see also Darwin, 1872; Andrewartha & Birch, 1954).

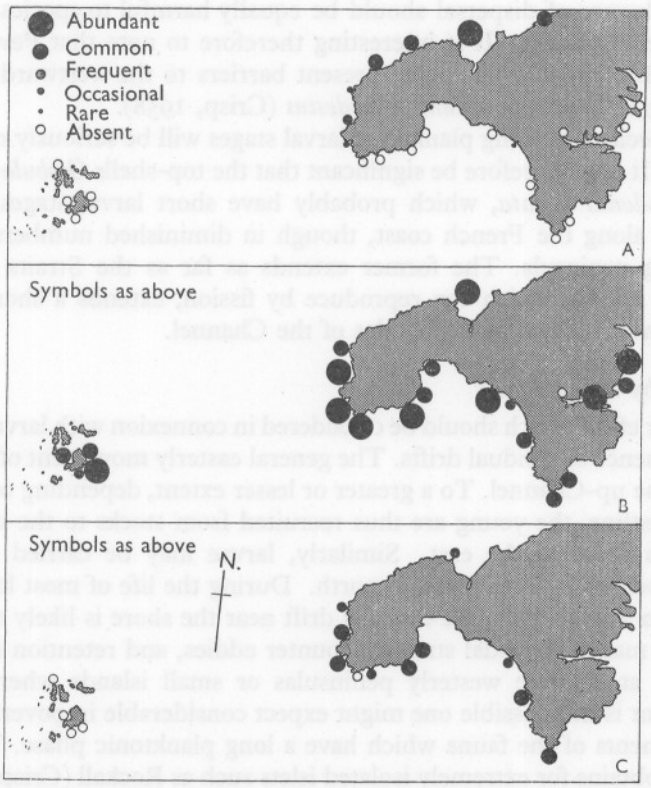

Fig. 6. Part of west Cornwall and the Isles of Scilly, showing the distribution of: A, *Balanus balanoides*; B, *B. perforatus*; and C, *Patella depressa*.

Dispersal by tidal streams

In the Channel, the main tidal streams are greatest between the two basins (see p. 163), while offshore races with turbulence occur at headlands. Therefore the greatest loss of larvae by dispersal would be anticipated at headlands such as St Catherine's Pt., Portland Bill, and off the points of the Cherbourg peninsula and Alderney. Not only must dispersal be increased in this region, but also the diminished tidal range, especially on the English side, may restrict the available stocks of adults, since these are necessarily confined to a narrower zone on the shore.

Change in aspect and increased dispersal are probably both important in determining faunistic boundaries. The fact that both on the south-facing British shore and on the north-facing French shore many of the critical faunistic limits coincide with headlands lying midway between the two main basins suggests that larval dispersal may be a more important factor than aspect.

The influence of dispersal should be equally harmful to species spreading from either direction. It is interesting therefore to note that Peveril Point, Portland Bill, and Cap la Hague present barriers to the westward spread of the immigrant barnacle *Elminius modestus* (Crisp, 1958).

Only species with long planktonic larval stages will be seriously reduced by dispersal. It may therefore be significant that the top-shells *Gibbula umbilicalis* and *Monodonta lineata*, which probably have short larval stages, continue eastwards along the French coast, though in diminished numbers, past the Cherbourg peninsula. The former extends as far as the Straits of Dover. *Anemonia sulcata*, which can reproduce by fission, extends a short distance into the eastern basin on both sides of the Channel.

Dispersal by residual drift

Another effect which should be considered in connexion with larval dispersal is the influence of residual drifts. The general easterly movement of water will carry larvae up-Channel. To a greater or lesser extent, depending on the form of the coastline, the young are thus recruited from stocks to the west rather than from those to the east. Similarly, larvae may be carried round the Brittany peninsula from south to north. During the life of most larvae (anything from 2 to 30 days) the residual drift near the shore is likely to be small and to be masked by tidal streams, counter eddies, and retention in pockets. However, at extreme westerly peninsulas or small islands where westerly recruitment is not possible one might expect considerable impoverishment of those elements of the fauna which have a long planktonic phase. This effect certainly obtains for extremely isolated islets such as Rockall (Crisp, 1956). It is surprising that so little impoverishment occurs at such places as north-west Brittany, Cape Cornwall, Lizard Head, Eddystone Rocks, etc. The presence, for example, of good barnacle settlements in these areas suggests that there are coastal drifts running counter to the main eastward drift, and able to carry larvae round these headlands. Certain species, however, become sparse at the western extremity both of Brittany and Cornwall, and more particularly in Ushant and the Scilly Isles, notably *Balanus balanoides* (absent from Scilly), *B. perforatus* (not at all abundant on Ushant, Scilly, or the Lizard), *Patella depressa* (absent from Scilly, rare on Ushant, not abundant in Finistère or west Cornwall), *Littorina littorea* (rare in Scilly and Ushant), and *Mytilus* (not studied in this survey, but known to be uncommon in Scilly and Ushant). *Balanus balanoides* and *Littorina littorea* are relatively northern in distribution and may be affected by the warmer maritime conditions in these areas, but this is not likely for the other species.

One of us (A.J.S.) has recently obtained further evidence relating to larval dispersal off west Cornwall and the Isles of Scilly, where a series of plankton samples were taken over a limited period in March 1956 (Fig. 7). The samples were taken for the specific purpose of determining whether the larvae of

Balanus balanoides were present off Scilly during the short breeding period of the species. However, the distributions of the larvae of other species of barnacles found in the samples proved to be pertinent to the present work and are also illustrated. The boundaries of both *Verruca stroemia* and *Balanus*

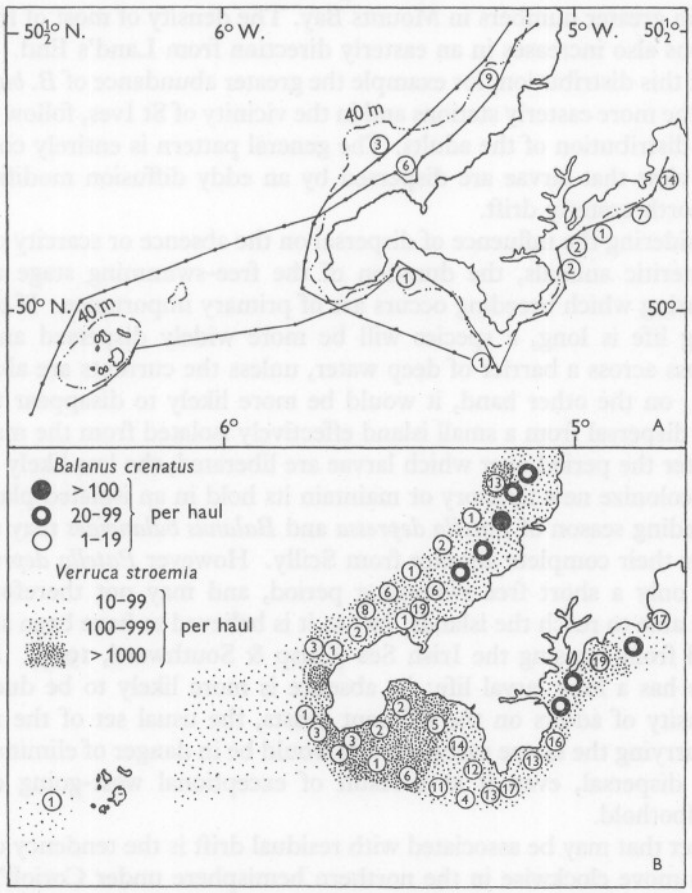

Fig. 7. Distribution of cirripede nauplii off west Cornwall and the Isles of Scilly as shown by samples taken with a standard Hardy plankton indicator, 27–29 March, 1956, towed at approx. 20 m deep at 8·5 knots. A, numbers of *Balanus balanoides* per haul of 4 nautical miles; B, numbers of *B. crenatus* and contours for *Verruca stroemia*. Positions of hauls, and depth contours are shown on A.

crenatus were very sharp, few larvae being found at distances from the coast exceeding a few miles, even though easterly winds of force 3–6 prevailed during the survey. The western limits of the cirripede larvae coincided with the main boundary between relatively oceanic plankton organisms (*Sagitta elegans*, *Candacia*) and neritic forms (*Podon*, *Evadne*, *Sagitta setosa*). The adults

of both *Balanus crenatus* and *Verruca* are found in the infralittoral zone, but the larval distribution indicates that while *Verruca* may extend some way offshore in this region, *Balanus crenatus* is almost entirely confined to the coastal belt. The maps show that larval populations of all three species form only a narrow belt at extreme western points, but accumulate over a broader area and in greater numbers in Mounts Bay. The density of most of the larval populations also increases in an easterly direction from Land's End. Certain aspects of this distribution, for example the greater abundance of *B. balanoides* larvae at the more easterly stations and in the vicinity of St Ives, follow directly from the distribution of the adults. The general pattern is entirely consistent with the view that larvae are dispersed by an eddy diffusion modified by a general north-easterly drift.

In considering the influence of dispersal on the absence or scarcity of intertidal or neritic animals, the duration of the free-swimming stage and the period during which breeding occurs are of primary importance. If the free-swimming life is long, a species will be more widely dispersed and more readily pass across a barrier of deep water, unless the currents are always set against it; on the other hand, it would be more likely to disappear through excessive dispersal from a small island effectively isolated from the mainland. The shorter the period over which larvae are liberated, the less likely will the animal recolonize new territory or maintain its hold in an isolated place. The short breeding season of *Patella depressa* and *Balanus balanoides* may account in part for their complete absence from Scilly. However *Patella depressa* has probably only a short free-swimming period, and may not therefore have ever been able to reach the islands, just as it is believed to have been similarly prevented from crossing the Irish Sea (Crisp & Southward, 1953). *Balanus balanoides* has a long larval life; its absence is more likely to be due to the small density of adults on the adjacent coasts, the usual set of the residual current carrying the larvae eastwards. It would be in danger of elimination by excessive dispersal, even if as a result of exceptional west-going drifts it gained a foothold.

An effect that may be associated with residual drift is the tendency of water masses to move clockwise in the northern hemisphere under Corioli's force. This might result in a greater eastward movement, and better retention of larvae, near the French coast. Southern forms should therefore be carried farther westward on this side of the Channel, though the magnitude of the effect is difficult to determine.

Faunal continuity

Intertidal forms can spread much more readily along a continuous shoreline than across sea passages (Crisp & Southward, 1953; Southward & Crisp, 1954*b*). Hence the continuity of the French coast of the Channel with the Atlantic coasts of France and Spain may allow the survival in the Channel

of some species which, in isolation, would disappear. The case of *Pachygrapsus marmoratus*, which was able to establish itself locally only during favourable periods (Prenant, 1929), illustrates the importance of continuity. It is possible that species such as *Gibbula pennanti* and *Paracentrotus lividus*, which are absent from the English side, can be replenished more easily on the French side by larvae from outside the Channel after periods of unfavourable temperature. Purely infralittoral forms, such as *Octopus vulgaris* (Rees & Lumby, 1954), may also be influenced strongly by continuity.

Effect of suspended particles in the water

We have noted briefly that some of the algae may be excluded from the eastern part of the Channel partly by the opacity of the water. To these organisms such a factor may be more important than aspect or dispersal, and needs examining in more detail. The reduced amount of light received in the lower intertidal zone, where the water is silty, is probably most detrimental to the infralittoral and lower midlittoral species. The fucoids can withstand greater exposure to air, and hence can occur at higher levels on the shore, where they remain sufficiently illuminated for photosynthesis (cf. Burrows & Lodge, 1951). Forms such as *Himanthalia* and *Bifurcaria* cannot occur at higher levels out of the water, and will thus be unable to compensate for the reduced illumination of silty water. They will grow best where the water is clear and the tidal range smallest, for under these conditions they can remain permanently immersed with minimum loss of illumination (cf. Fischer-Piette, 1936). Any effects of reduced illumination caused by particulate matter in the eastern basin will be slightly accentuated by differences in the tidal regime. In the western basin low water of spring tides, and high water of neap tides occur at midday, while the opposite is true for the eastern half of the Channel. This difference means that, on average, there is a slightly thicker layer of water over the intertidal zone during daylight hours in the eastern basin in places where the tidal range is identical.

It is interesting to note that the absence or scarcity of *Himanthalia* and *Bifurcaria* in the eastern part of the Channel is paralleled in the inner parts of Lyme Bay and the Gulf of St Malo (Fischer-Piette, 1936; Lami, 1941). The shores of these two regions are erodible or scoured, there is more silt in suspension, and the tidal range is greater than farther east (in Dorset, north Cotentin and the Channel Islands) where these algae reappear in some abundance.

Effects of other factors

Other factors have so far not been adequately demonstrated, and at present there appears no need to invoke them to account for differences in distribution (see p. 158), though their possible existence has been frequently postulated. Smith (1953) has suggested that adsorption of trace constituents on particulate

matter may contribute to the poverty of the fauna of the eastern part of the Channel and southern North Sea. Because of the limited adsorption capacity of such particles it is difficult to see how this process could continue unless the adsorption was a prelude to destruction or transformation of the substances adsorbed. This is by no means impossible, for particulate matter is the normal site of bacterial action.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

It has been shown that the general distribution of many of the organisms investigated may be explained by differences in temperature along the Channel. The existence of critical limits for several species rather than a series of graded boundaries for each species can be accounted for by the modifying effects of additional environmental factors, especially the character of the substratum, the effect of water movement on the dispersal of larvae and the aspect of the shoreline.

It seems unlikely that further faunistic work can establish with certainty our views as to the causes of the distribution. There are too many independent and unmeasurable variables. However, careful and regular observations in certain areas, and further complete surveys from time to time may make it possible to relate the abundance of certain animals to climatic trends, and hence to temperature, or with some other variable.

Further work on the distribution of animals in other habitats would be useful, especially habitats such as sands, muds, crevices, which offer similar conditions and escape certain of the possible modifying influences such as wave-exposure, scour, and aspect. In particular, investigations could well be made of the bottom fauna of representative stations along the length of the Channel. It is clear from the work of Rees (1950) and Rees & Lumby (1954) that the distribution of *Octopus vulgaris*, a southern form, shows east-west and north-south trends similar to those of many intertidal organisms. This species, moreover, shows fluctuations in abundance of adults and larvae correlated with fluctuations of sea temperature, especially of the winter months (Rees & Lumby, 1954).

East-west distribution trends are also found among planktonic animals in the Channel. There is, for example, a sharp boundary in the western basin between relatively oceanic species to the west and neritic species to the east (Russell, 1935). This line, however, lies well to the west of the boundary of the intertidal organisms, with which it appears to have little relation. A somewhat nearer approach to the intertidal boundaries is shown by the line dividing the copepod *Centropages typicus* in the western basin from the related species *C. hamatus* which is largely confined to the eastern basin and the Gulf of St Malo (Cushing, 1957). The different distribution of these two species has been attributed by Cushing to differences in water currents and bottom

deposits in the two basins, factors we have already suggested may be responsible in part for some of the distributions of intertidal organisms. More correlation between temperature and east-west trends may be found with the pilchard, *Sardina pilchardus*, which is largely confined to the western basin (and Celtic sea) in winter, migration into, and spawning in the eastern basin occurring only during the warmer summer months (see Cushing, 1957).

Only further work on the fluctuations of the boundaries of the planktonic organisms can show whether there is, in fact, any relation between the distributions of these species and of intertidal and bottom-living organisms.

It seems unlikely that experiments on the tolerance of animals to temperature extremes will be immediately relevant to understanding the effect of temperature in distribution. This is more likely to be furthered by studies of the behaviour, nutrition and reproduction of the various animals and plants with special regard to their efficiency and success in natural competition. The latter calls for investigations of suitable animals kept for prolonged periods at a series of temperatures and with various conditions of feeding.

The effects of dispersal on the free-swimming stages is a problem that has been given too little attention in the past and one which offers results equally valuable to the biologist and the hydrographer. Each larva is a clock, set in motion at the moment of liberation from the adult and carried passively from its place of origin by tides and currents. Taken in conjunction with the distribution of adults, quantitative measurements of planktonic larvae can give information on the dynamics of distribution and dispersal of the animals as well as on the direction of local water movements.

During the course of the work we have had the assistance, from time to time, of many friends and colleagues to whom thanks are due: in particular we should like to mention Dr Eve C. Southward, Mr L. W. G. Jones and Mr E. Norris. We are indebted to the Browne Fund of the Royal Society for grants which assisted us in carrying out parts of the field work. Additional financial aid was received by one of us (A. J. S.) from the Department of Scientific and Industrial Research (Senior Research Award), the University of Liverpool and the Marine Biological Association.

SUMMARY

The English Channel may conveniently be divided into a western basin and an eastern basin separated by a line drawn approximately between the Cherbourg peninsula and the Isle of Wight. In these basins, the environmental factors are very different. Thus the water of the western basin is on average warmer and slightly more saline than that of the eastern basin and its temperature variation is less extreme. The French side of the Channel is warmer than the English coast only in the western basin. The tidal oscillations in the two basins

are out of phase with each other and the strongest tidal currents occur where the two basins join. The main residual flow is up-Channel with minor coastal eddies.

The shores at the western end of the Channel are generally rocky, they are close to deep water, and so are exposed to frequent heavy swells. The shores of the eastern basin are generally erodible, and accumulations of sand and shingle tend to reduce the force of the waves. The water of the eastern basin generally contains more suspended matter and the shores are usually scoured by sand and shingle.

The distribution of the following animals is given in some detail and compared with earlier records: *Anemonia sulcata*, *Actinia equina*, *Chthamalus stellatus*, *Balanus balanoides*, *B. perforatus*, *B. crenatus*, *B. improvisus*, *Elminius modestus*, *Verruca stroemia*, *Hemioniscus balani*, *Patella vulgata*, *P. depressa*, *P. aspera*, *Monodonta lineata*, *Gibbula umbilicalis*, *G. pennanti*, *G. cineraria*, *Littorina saxatilis*, *L. littoralis*, *L. littorea*, *L. neritoides*, *Paracentrotus lividus*. The distribution of certain other species is also discussed: *Balanus balanus*, *Haliotis tuberculata*, *Gibbula magus* and *Calliostoma zizyphinum*. The distributions of the following plants are briefly discussed: *Laminaria ochroleuca*, *L. digitata*, *L. hyperborea*, *L. saccharina*, *Saccorhiza polysichides*, *Alaria esculenta*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus spiralis*, *F. vesiculosus*, *F. serratus*, *Himanthalia elongata* and *Bifurcaria rotunda*.

The main trend in distribution is from east to west. Northern forms are mostly found throughout the Channel though commoner at the eastern end. Many southern forms common in the western basin fail to penetrate the eastern basin, and the fauna at the eastern end of the Channel is therefore impoverished as compared with the richer fauna of the western basin. Certain southern forms are found in the western basin only on the French side of the Channel; others extend farther eastwards on the French side than on the English side.

The trends in distribution are considered to be due to temperature differences. The eastern limits of southern forms are considered to result, not from a lethal effect of a minimum temperature, but from the generally lower efficiency of these animals in comparison with northern forms as the temperature falls. The substratum of most of the eastern basin of the Channel, especially on the English side, is inimical to many rock-living organisms, and it seems probable that this reinforces any adverse effects due to the lowering of average temperature.

The limits of many predominantly southern species occur somewhat abruptly on both north and south coasts of the Channel in the region lying roughly midway between the two basins. This concentration of faunistic limits is believed to be due partly to the changes in the aspect of the coast, which expose it to the north and east winds, and partly to excessive dispersal of larvae by tidal currents which are maximal in these regions.

The influence of residual drift may be important to the distribution of certain organisms at the extreme west of the Channel, viz. *Balanus perforatus*, *B. balanoides*, *B. crenatus*, *Patella depressa*, *Littorina littorea* and *Mytilus edulis*. *Balanus balanoides* and *Patella depressa* are absent from the Isles of Scilly.

The clarity of the water, the tidal range, and the tidal regime (i.e. time of high and low spring tides) appear important to certain algae such as *Himanthalia* and *Bifurcaria*. Heavy swell appears essential to *Alaria esculenta*, and this rather than temperature confines it to the western part of the Channel.

Further work on the behaviour, nutrition and breeding of certain of the animals from rocky shores at a series of temperatures seems desirable, together with a study of distributions of animals from other habitats. The mechanism of dispersal and the distribution of larvae of shore animals also deserve more attention.

REFERENCES

AIR MINISTRY, 1936. *Averages of Temperature for the British Isles for Periods Ending 1935*. London: H.M.S.O.

ANDREWARTHA, H. G. & BIRCH, L. C., 1954. *The Distribution and Abundance of Animals*. Chicago: University Press.

APELLÖF, A., 1912. Invertebrate bottom fauna of the Norwegian Sea and the north Atlantic. In MURRAY, J. & HJORT, J., *The Depths of the Ocean*, pp. 457–560. London: Macmillan.

BARNES, H., 1956. *Balanus balanoides* L. in the Firth of Clyde: the development and annual variation of the larval population and the causative factors. *J. Anim. Ecol.*, Vol. 25, pp. 72–84.

BASSINDALE, R., 1936. The developmental stages of three English barnacles, *Balanus balanoides* (Linn.), *Chthamalus stellatus* (Poli) and *Verruca stroemia* (O. F. Müller). *Proc. zool. Soc. Lond.*, 1936, pp. 57–74.

—— 1941. The invertebrate fauna of the southern shores of the Bristol Channel and Severn estuary. *Proc. Bristol Nat. Soc.*, Ser. 4, Vol. 9, pp. 143–201.

BIGELOW, H. B. & EDMONSON, W. T., 1947. *Wind Waves at Sea, Breakers and Surf*. Washington: U.S. Navy Department, Hydrographic Office.

BISHOP, M. W. H. & CRISP, D. J., 1957. The Australasian barnacle, *Elminius modestus*, in France. *Nature, Lond.*, Vol. 179, pp. 482–3.

—— —— 1958. The distribution of *Elminius modestus* in France. (In the Press.)

BOWDEN, K. F., 1956. The flow of water through the Straits of Dover related to wind and differences in sea level. *Phil. Trans.* A, Vol. 248, pp. 517–51.

BURROWS, E. M. & LODGE, S. M., 1951. Autecology and the species problem in *Fucus*. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 161–76.

BØRGESEN, F. & JÓNSSON, H., 1908. The distribution of the marine algae of the Arctic Sea, and of the northernmost part of the Atlantic. *Botany of the Faeröes*, Pt. 3, Appendix, 28 pp.

CARRUTHERS, J. N., 1930. Further investigations upon the water movements in the English Channel. Drift-bottle experiments in the summers of 1927, 1928 and 1929, with critical notes. *J. mar. biol. Ass. U.K.*, Vol. 17, pp. 241–75.

—— 1935. The flow of water through the Straits of Dover as gauged by continuous current meter observations at the Varne lightvessel (50° 56′ N., 1° 17′ E.). Pt. II. Second report on results obtained. *Fish. Invest., Lond.*, Ser. 2, Vol. 14, No. 4.

CARRUTHERS, J. N., LAWFORD, A. L. & VELEY, V. F. C., 1950. Studies of water movements at various light vessels in 1938, 1939 and 1940. I. At the 'Varne' lightship and her successors. *Ann. biol., Copenhague*, T. 6, pp. 115–21.

CARRUTHERS, J. N., LAWFORD, A. L., VELEY, V. F. C. & GRUNING, J. F., 1951. Studies of water movements and winds at various lightvessels. II. At the Seven Stones light vessel near the Scilly Isles. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 587–608.

CLARK, J., 1906. Marine Zoology. In *The Victoria History of the County of Cornwall*, pp. 113–59. London.

COLMAN, J., 1933. The nature of the intertidal zonation of plants and animals. *J. mar. biol. Ass. U.K.*, Vol. 18, pp. 435–76.

COOPER, L. H. N., 1958. Sea temperatures in Plymouth Sound. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 1–3.

CRISP, D. J., 1950. Breeding and distribution of *Chthamalus stellatus*. *Nature, Lond.*, Vol. 166, p. 311.

—— 1956. The intertidal zoology of Rockall. In FISHER, J., *Rockall*. London: Bles.

—— 1958. The spread of *Elminius modestus* in north-west Europe. *J. mar. biol. Ass. U.K.*, Vol. 37 (in the Press.)

CRISP, D. J. & CHIPPERFIELD, P. N. J., 1948. Occurrence of *Elminius modestus* (Darwin) in British waters. *Nature, Lond.*, Vol. 161, p. 64.

CRISP, D. J. & KNIGHT-JONES, E. W., 1955. Discontinuities in the distribution of shore animals in North Wales. *Rep. Bardsey Observatory*, Year 1954, pp. 29–34.

CRISP, D. J. & MOLESWORTH, A. N. H., 1951. Habitat of *Balanus amphitrite* var. *denticulata* in Britain. *Nature, Lond.*, Vol. 167, p. 489.

CRISP, D. J. & SOUTHWARD, A. J., 1953. Isolation of intertidal animals by sea barriers. *Nature, Lond.*, Vol. 172, p. 208.

CROFTS, D. R., 1929. *Haliotis*. *Mem. Lpool mar. biol. Com.*, No. 29, 174 pp.

CUSHING, D. H., 1957. The number of Pilchards in the Channel. *Fish. Invest., Lond.*, ser. 2, Vol. 21, No. 5, 27 pp.

DARWIN, C., 1854. *A Monograph on the Sub-Class Cirripedia: Balanidae, Verrucidae, etc.* London: Ray Soc.

—— 1872. *The Origin of Species by Means of Natural Selection*, 6th ed., ch. 3. London: John Murray.

DIZERBO, A. H., 1947. La répartition et la zône de végétation dans le massif armoricaine d'*Alaria esculenta* (L.) Grev. *Bull. Soc. sci. Bretagne*, T. 22, pp. 113–17.

DODD, J. M., 1957. Artificial fertilisation, larval development and metamorphosis in *Patella vulgata* L. and *Patella caerulea* L. *Pubbl. Staz. zool. Napoli*, Vol. 29, pp. 172–86.

EVANS, R. G., 1953. Studies on the biology of British limpets—the genus *Patella* on the south coast of England. *Proc. zool. Soc., Lond.*, Vol. 132, pp. 357–76.

FISCHER, E., 1928. Sur la distribution géographique de quelques organismes de rocher le long des côtes de la Manche. *Trav. Lab. Mus. Hist. nat. St-Servan*, T. 2, pp. 1–16.

FISCHER-PIETTE, E., 1932. Répartition des principales espèces fixées sur les rochers battus des côtes et des îles de la Manche, de Lannion à Fécamp. *Ann. Inst. océanogr. Monaco*, T. 12, pp. 107–213.

—— 1935. Systematique et biogéographie. Les Patelles d'Europe et d'Afrique du Nord. *J. Conchyliol.*, T. 79, pp. 5–66.

—— 1936. Études sur la biogéographie intercotidale des deux rives de la Manche. *J. Linn. Soc. (Zool.)*, Vol. 40, pp. 181–272.

—— 1955. Répartition, le long des côtes septentrionales de l'Espagne, des principales espèces peuplant les rochers intercotidaux. *Ann. Inst. océanogr. Monaco*, T. 31, pp. 37–124.

FISCHER-PIETTE, E. & PRENANT, M., 1956. Distribution des cirripèdes intercotidaux d'Espagne septentrionale. *Bull. Cent. Rech. sci. Biarritz*, T. 1, pp. 7–19.

FORBES, E. & HANLEY, S., 1853. *A History of British Mollusca*. Vols. 2 & 3. London: Van Voorst.

GAILLARD, J. M., 1954. Revision des espèces des côtes de France du genre *Gibbula* Risso (Mollusque Prosobranche). *Bull. Mus. Hist. nat., Paris*, T. 26, pp. 238–43.

HAMEL, G., 1931–39. *Phéophycées de France*. Paris.

HARTOG, C. DEN, 1956. Speculations on the immigration of the barnacle *Elminius modestus* in France. *Beaufortia*, Vol. 5, p. 141.

HARVEY, H. W., 1930. Hydrography of the mouth of the English Channel 1925–1928. *J. mar. biol. Ass. U.K.*, Vol. 16, pp. 791–820.

HAWKSHAW, C., 1878. On the action of limpets in sucking pits in and eroding the surface of chalk at Dover. *J. Linn. Soc. (Zool.)*, Vol. 14, pp. 406–11.

KALLE, K., 1937. Nährstoff-Untersuchungen als Hydrographisches Hilfsmittel zur Unterscheidung von Wasserkörpern. *Ann. Hydrogr. Berl.*, Jahrg. 65, pp. 1–18.

KOEHLER, R., 1884. Recherches sur la faune marine des îles Anglo-Normandes. *Bull. Soc. Sci. Nancy*, 1884, pp. 51–99.

LAMI, R., 1941. Notules d'algologie marine (suite). III. Sur l'écologie de *Bifurcaria tuberculata* dans la région malouine. *Bull. Lab. marit. Dinard*, F. 23, pp. 24–29.

—— 1943. Notules d'algologie marine (suite). IX. Sur l'écologie et la répartition dans la Manche de *Laminaria ochroleuca* De La Pylaie. *Bull. Lab. marit. Dinard*, F. 25, pp. 75–90.

—— 1954. Une station normande de *Laminaria ochroleuca* de la Pylaie. *Trav. algol.*, N.S., T. 1, pp. 44–45.

LEBOUR, M. V., 1937. The eggs and larvae of the British prosobranchs with special reference to those living in the plankton. *J. mar. biol. Ass. U.K.*, Vol. 22, pp. 105–66.

LILLY, S. J., SLOANE, J. F., BASSINDALE, R., EBLING, F. J. & KITCHING, J. A., 1953. The ecology of Lough Ine rapids with special reference to water currents. IV. The sedentary fauna of sub-littoral boulders. *J. Anim. Ecol.*, Vol. 22, pp. 87–122.

LUMBY, J. R., 1935. Salinity and temperature of the English Channel. Atlas of charts. *Fish. Invest., Lond.*, Ser. 2, Vol. 14, No. 3.

LYSAGHT, A. M., 1941. The biology and trematode parasites of the gastropod *Littorina neritoides* (L.) on the Plymouth Breakwater. *J. mar. biol. Ass. U.K.*, Vol. 25, pp. 41–67.

MAGHRABY, A. M. EL & PERKINS, E. J., 1956. Additions to the marine fauna of Whitstable. *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 9, pp. 481–96.

MANSELL-PLEYDELL, J. C., 1898. *The Mollusca of Dorsetshire*. Dorchester: Dorset County Chronicle.

MARINE BIOLOGICAL ASSOCIATION, 1957. *Plymouth Marine Fauna*, 3rd ed. Plymouth.

MARTONNE, E. DE, 1935. *Traité de Géographie Physique*. Paris.

MOORE, H. B., 1935. The biology of *Balanus balanoides*. IV. Relation to environmental factors. *J. mar. biol. Ass. U.K.*, Vol. 20, pp. 273–307.

MOORE, H. B. & KITCHING, J. A., 1939. The biology of *Chthamalus stellatus* (Poli). *J. mar. biol. Ass. U.K.*, Vol. 23, pp. 521–41.

MORTENSEN, T., 1927. *Handbook of the Echinoderms of the British Isles*. Oxford University Press.

NOBRE, A., 1932. *Moluscos Marinhos de Portugal*. Universidade do Pôrto, Instituto de Zoologia.

NORRIS, E. & CRISP, D. J., 1953. The distribution and planktonic stages of the cirripede *Balanus perforatus* Brugière. *Proc. zool. Soc., Lond.*, Vol. 123, pp. 393–409.

OFFICE NATIONALE MÉTÉOROLOGIQUE, 1940. *Étude climatologique de la Bretagne.* Paris.

—— 1942*a*. *Étude climatologique de la Normandie.* Paris.

—— 1942*b*. *Étude climatologique de la région Flandre-Artois-Picardie.* Paris.

ORTON, J. H., 1920. Sea temperature, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 339–66.

ORTON, J. H., SOUTHWARD, A. J. & DODD, J. M., 1956. Studies on the biology of limpets. II. The breeding of *Patella vulgata* L. in Britain. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 149–76.

PARKE, M., 1948. *Laminaria ochroleuca* de la Pylaie growing on the coast of Britain. *Nature, Lond.*, Vol. 162, p. 295.

PEREZ, C., 1923. Sur la spécificité du parasitisme des *Hemioniscus*. *Bull. Soc. zool. Fr.*, T. 48, pp. 375–6.

PILSBRY, H. A., 1916. The sessile barnacles (Cirripedia) contained in the collections of the U.S. National Museum. *Bull. U.S. nat. Mus.*, Vol. 93, 366 pp.

PRENANT, M., 1929. Sur l'extension actuelle de *Pachygrapsus marmoratus* (Fabr.). *Bull. Lab. marit. Dinard*, F. 4, pp. 3–4.

REES, W. J., 1950. The distribution of *Octopus vulgaris* Lamarck in British waters. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 361–78.

REES, W. J. & LUMBY, J. R., 1954. The abundance of *Octopus* in the English Channel. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 515–36.

RUNNSTRÖM, S., 1929. Weitere Studien über Temperaturanpassung der Fortpflanzung und Entwicklung mariner Tiere. *Bergens Mus. Aarb.*, 1929, No. 10, 46 pp.

RUSSELL, F. S., 1935. On the value of certain plankton animals as indicators of water movements in the English Channel and North Sea. *J. mar. biol. Ass. U.K.*, Vol. 20, 309–32.

—— 1953. The English Channel. *Rep. Trans. Dev. Ass. Adv. Sci.*, Vol. 85, pp. 1–17.

SARS, G. O., 1878. *Bidrag til Kundskaben on Norges Arktiske Fauna. I. Mollusca.* Christiana.

SINEL, J., 1906. *An Outline of the Natural History of our Shores.* London: Swan Sonnenschein and Co.

SMITH, J. E., 1953. Maintenance and spread of sea shore faunas. *Advanc. Sci., Lond.*, Ser. 10, No. 38, pp. 145–56.

SOUTHWARD, A. J., 1950. Occurrence of *Chthamalus stellatus* in the Isle of Man. *Nature, Lond.*, Vol. 165, p. 408.

—— 1951. The distribution of *Chthamalus stellatus* in the Irish Sea. *Nature, Lond.*, Vol. 167, p. 410.

—— 1953. The ecology of some rocky shores in the south of the Isle of Man. *Proc. Lpool biol. Soc.*, Vol. 59, pp. 1–50.

—— 1955*a*. Feeding of barnacles. *Nature, Lond.*, Vol. 175, p. 1124.

—— 1955*b*. On the behaviour of barnacles. I. The relation of cirral and other activities to temperature. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 403–22.

—— 1955*c*. On the behaviour of barnacles. II. The influence of habitat and tide-level on cirral activity. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 423–33.

—— 1957. On the behaviour of barnacles. III. Further observations on the influence of temperature and age on cirral activity. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 323–34.

—— 1958. Notes on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 49–66.

SOUTHWARD, A. J. & CRISP, D. J., 1952. Changes in the distribution of the intertidal barnacles in relation to the environment. *Nature, Lond.*, Vol. 170, pp. 416–17.

SOUTHWARD, A. J. & CRISP, D. J., 1954*a*. Changes in the distribution of the intertidal barnacles *Chthamalus stellatus* Poli and *Balanus balanoides* L. in the British Isles. *J. Anim. Ecol.*, Vol. 23, pp. 163–77.

—— —— 1954*b*. The distribution of certain intertidal animals around the Irish coast. *Proc. R. Irish Acad.*, Vol. 57 (B), No. 1, 29 pp.

—— —— 1956. Fluctuations in the distribution and abundance of intertidal barnacles. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 211–29.

SOUTHWARD, A. J. & ORTON, J. H., 1954. The effects of wave-action on the distribution and numbers of the commoner plants and animals living on the Plymouth breakwater. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 1–19.

SPOONER, G. M., 1950. Additional records of *Laminaria ochroleuca* de la Pylaie. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 261–2.

STATION BIOLOGIQUE DE ROSCOFF, 1951*a*. Inventaire de la faune marine de Roscoff. Bryozoaires—Echinodermes. *Trav. Sta. biol. Roscoff*, Suppl. 4, 53 pp.

—— 1951*b*. Inventaire de la faune marine de Roscoff. Mollusques. *Trav. Sta. biol. Roscoff*, Suppl. 5, pp. 1–80.

—— 1954. Inventaire de la flore marine de Roscoff. Algues, Champignons, Lichens et Spermatophytes. *Trav. Sta. biol. Roscoff*, Suppl. 6, 152 pp.

STEPHENSEN, K., 1938. Cirripedia (incl. Rhizocephala). *Zool. Iceland*, Vol. 3, Pt. 30–31, 11 pp.

STEPHENSON, T. A., 1924. Notes on *Haliotis tuberculata*. *J. mar. biol. Ass. U.K.*, Vol. 13, pp. 480–95.

—— 1935. *The British Sea Anemones*, Vol. 2. London: Ray Soc.

THORSON, G., 1941. Marine Gastropoda Prosobranchiata. *Zool. Iceland*, Vol. 4, Pt. 60, 150 pp.

—— 1946. Reproduction and larval development of Danish marine bottom invertebrates. *Medd. Komm. Havundersøg., Kbh.*, Ser. Plankton, Vol. 4, 423 pp.

TOMLIN, J. R. LE B., 1937. Report of the conchological section, 1936. *Rep. Soc. guérnesiaise*, Vol. 12, pp. 454–5.

VAN VEEN, J., 1936. *Onderzoekingen in de Hoofden in verband met de gestelheid der Nederlandsche Kust*. S'Gravenhage.

WEGMANN, H., 1884. Contributions à l'histoire naturelle des Haliotides. *Arch. Zool. exp. gén.*, T. 2, pp. 289–378.

WOODWARD, S. P., 1880. *A Manual of the Mollusca*, 4th ed. London: Crosby Lockwood and Co.

APPENDIX

TABLE 7. THE DETAILED DISTRIBUTION OF THE COMMONER INTERTIDAL ANIMALS IN THE CHANNEL

The table summarizes observations made over a number of years. During the course of the work our methods improved and the number of species under investigation was increased. As it was not possible to revisit all stations some areas will be found to have less detailed records than others.

The place names are taken from the largest scale map of the region available at the time; where only 1:250,000 maps were used, the nearest place name was taken. The survey of the English coast was spread over 1949–57: where it was possible to choose between counts or observations at a station, those made nearest to the date of the French parts of the survey have been tabulated. Dates of observations not used to complete numerical details of abundance are shown in brackets.

The type of shore at each station has been tabulated from the point of view of the relative hardness and extent of the rocky substratum, the angle of slope and the degree of shelter provided by offshore reefs. The letters used have the following meanings: R, rocks other than chalk; C, chalk rocks; B, boulders (over 10 cm diameter); G, gravel (10 cm to 2 mm diameter); S, sand (2 mm to 0·02 mm diameter); M, silt and clay (under 0·02 mm); P, piers, breakwaters, wharves and other artificial substrata. To the letters R and C are suffixed other letters thus: S, steep slopes (more than 25° from horizontal); F, flat rocks (e.g. Limestone and Sandstone flags); I, broken surfaces, all angles; E, reefs extending well out from the shore. The meanings of the symbols used to denote the abundance of the animals will be found in Table 2.

No.	Station	Description of shore	Dates of survey	*Anemonia sulcata*	*Actinia equina*	*Chthamalus stellatus* H.W.	*Chthamalus stellatus* M.T.	*Chthamalus stellatus* L.W.	*Balanus balanoides* H.W.	*Balanus balanoides* M.T.	*Balanus balanoides* L.W.	*Balanus crenatus*	*B. improvisus*	*B. perforatus*	*Elminius modestus*	*Verruca stroemia*	*Hemiosicus balani*	*Patella aspera*	*P. depressa*	*P. vulgata*	*Gibbula cineraria*	*G. pennanti*	*G. umbilicalis*	*Monodonta lineata*	*Calliostoma zizyphinum*	*Littorina littorea*	*L. neritoides*	*L. obtusata*	*L. saxatilis*	*Paracentrotus lividus*
Isles of Scilly																														
1.	Shipman Hd., Bryher	R, S	May 55	.	A	3·6	3·0	2·2	N	N	N	.	.	.	.	.	.	90 %	N	C	.	.	.	.	.	.	A	.	.	N
2.	Hugh Town, St Mary's	P, B, S	May 55	A	.	0·4	.	.	N	N	.	.	.	C	N	.	.	.	N	A	.	.	.	.	.	.	A	C	.	N
3.	Peninnis Hd., St Mary's	RI, B	May 55	.	.	A	A	.	N	N	.	.	.	F	N	.	.	.	N	.	.	.	.	.	.	.	A	C	.	N
4.	Porth Minick and Porth Hellick	B, G, S	May 55	.	.	2·6	2·0	1·6	N	N	N	.	.	A	N	C	.	.	.	.	.	A	A	.	.	N	A	C	C	N
5.	Darrity's Hole	R, S	May 55	.	.	1·8	4·8	1·7	N	N	N	.	.	C	N	.	.	.	.	.	.	.	.	.	.	.	A	.	.	N
6.	Blockhouse Pt.	RI, B	May 55	.	.	4·8	4·0	1·2	N	N	N	.	.	A	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
Cornwall																														
7.	Cape Cornwall	RI, E	May 55, April 56	C	.	4·0	3·2	1·8	N	0·004	0·002	.	.	C	N	.	.	A	10 %	A	.	N	A	A	.	.	A	.	.	N
8.	Sennen Cove	B, S, P	May 55, April 56	C	C	2·8	3·2	2·3	N	0·01	0·03	.	.	A	N	.	0 %	A	20 %	A	.	N	A	C	.	.	C	.	.	N
9.	Nanjizal	RI, B	May 55	.	.	2·4	4·0	2·4	N	0·0002		.	.	A	N	.	.	A	22 %	A	.	N	C	C	.	.	A	.	.	N
10.	Porthgwrra	RI, B	May 55	.	.	3·2	2·4	1·4	N	N	N	.	.	A	N	.	.	.	N	.	.	N	C	C	.	.	A	.	.	N
11.	Logan Rock, Treen	RI, B, S	May 55	.	.	4·0	1·8	0·8	N	N	N	.	.	A	N	.	.	.	10 %	.	.	N	O	O	.	.	A	.	.	N
12.	Lamorna Cove	R, S	May 55	.	.	3·4	5·6	2·4	N	N	N	.	.	A	N	.	.	.	20 %	.	.	N	.	.	.	.	A	.	.	N
13.	Mousehole	B, S, P	May 55	R	.	4·8	2·4	0·6	N	N	N	.	.	C	N	.	.	.	20 %	.	.	N	A	A	.	.	A	.	.	N
14.	Penzance Harbour	M, P	May 50	.	.	A	.	.	N	R	.	.	.	C	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14.	Penzance Harbour	M, P	April 56, April 57	.	.	A	.	.	.	R	R	.	.	A	0·01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15.	Rinsey Head	RI, B	April 56, April 57	.	.	A	.	.	N	.	.	.	.	A	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16.	Porthleven Harbour	B, G, P	April 55, April 56	.	.	A	.	.	N	R	R	A	.	F	N	C	.	.	F	.	.	.	.	C	.	.	.	.	.	.
17.	Poldhu Cove	RI, E, B, S	May 51, April 55	.	A	4·9	5·4	3·1	N	N	N	.	.	C	N	.	.	.	40 %	.	.	N	A	A	.	.	A	.	.	N
18.	Porth Mellin	P	April 55	.	.	A	.	.	N	N	N	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
19.	Lizard Pt.	RI, E	May 51	.	.	A	.	.	N	.	.	.	.	N	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
19.	Lizard Pt.	RI, E	April 55, April 56	F	.	4·8	4·5	4·8	N	N	N	.	.	C	N	.	.	A	27 %	A	.	.	A	A	.	.	A	.	.	N
20.	Cadgwith	RI	April 55	.	.	A	.	.	.	N	.	.	.	C	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
21.	Gweek	M, P	May 50, April 55	.	.	N	N	N	N	N	N	N	N	N	4·0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
22.	Porth Navas	M, P	May 50, April 55, April 57	.	.	0·6	.	.	N	.	.	F	.	A	0·5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
23	Helford Passage	RI, G	May 50, April 55, April 57	.	.	A	.	.	N	N	.	.	.	A	R	.	.	.	.	.	.	.	A	A	.	.	.	.	.	.
24.	Durgan	RI, G	May 50, April 56	.	.	A	.	.	N	N	.	.	.	A	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
25.	Maenporth	RI, S	May 50, April 56	.	.	A	.	.	N	N	.	.	.	A	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
26.	Pendennis Pt.	RI	April 56	.	.	3·2	1·7	1·7	N	N	N	.	.	A	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

27.	Flushing	G, M, P	April 56	.	.	A	A	A	N	N	N	.	.	A	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
28.	Mevagissey	RI, P	April 56	.	.	A	A	.	N	N	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
29.	Charlestown	P	April 56	.	.	4·0	3·2	.	N	0·06	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
30.	Par	P	Dec. 50	.	.	A	A	A	N	N	O	.	.	C	O	.	94%	.	.	.	.	.	.	.	.	.	.	.	.	.
31.	Polkerris	RI, B	April 55	.	A	4·0	3·2	2·3	N	N	R	.	.	A	0·01	.	72%	A	40%	A	.	N	A	C	.	.	A	.	.	N
32.	Fowey	P, G	Dec. 50, April 56	.	.	3·2	2·9	4·8	N	R	N	O	.	A	0·16	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
33.	Llansallos	RS	May 54	A	A	A	A	.	N	N	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	A	.	.	N
34.	Nailzee Pt., Looe	RI, G, S, E	(Sept. 49), April 56	.	A	4·8	3·0	1·0	N	R	O–R	.	.	.	N	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
35.	Looe Harbour	R, B, P	(Sept. 49), April 56	.	.	A	A	N	R	O	N	O	.	C	0·2	.	50%	.	.	.	.	.	.	.	.	.	.	.	.	.
36.	Whitsand Bay	RS, S	Oct. 55	.	A	5·0	3·6	2·4	0·2	2·1	1·2	.	.	A	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	N
37.	Amory Bight	RS	Dec. 54	.	.	7·5	4·4	1·6	0·01	0·16	0·05	.	.	A	0·08	.	.	.	.	.	.	.	.	.	.	.	A	.	.	N
38.	Hen Pt.	B, M	Dec. 54	.	.	R	N	N	N	R	R	C	F	N	2·0	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	S. Devon																													
39.	Weirquay	B, M, P	Jan. 55	.	.	N	N	N	N	N	N	F	C	N	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
40.	Tinside, Plymouth	RS, B	(Aug. 50), Dec. 54	.	.	3·4	2·4	0·3	N	R	N	.	.	C	C–F	.	10–60%	.	.	.	.	.	.	.	.	.	C	.	.	N
41.	Laira, R. Plym	B, M, P	Mar. 51, Jan. 56	.	A	C	F	O	O–R	O–R	F–R	C	.	C	C–A	.	28%	.	.	.	.	.	.	.	.	.	.	.	.	.
42.	Wembury	RSI, E	Feb. 55	A	A	5·2	2·8	0·4	0·2	1·1	0·3	.	.	A	R	F	20%	C	C	A	A	N	A	A	+	C	A	C	A	N
43.	Hope Cove	RS, B, S, E	April 55	N	.	5·8	2·8	1·6	N	0·2	0·3	.	.	C	N	.	7%	.	50%	.	.	.	A	C	.	.	C	.	.	N
44.	Salcombe, South sands	RS, S	Feb. 51, April 55, April 57	C	A	3·7	2·2	0·3	0·05	1·1	1·9	C	O	A	O	O	8%	F	17%	A	C	.	C	C	+	F	C	C	C	N
45.	Salcombe, Charlton bridge	P, G, M	April 57	.	.	.	.	.	.	0·02	.	R	.	O	A	.	.	.	.	.	.	.	N	N	.	C	.	.	.	.
46.	East Prawle	RI, E	April 55, April 56	.	.	4·8	3·2	1·3	0·04	0·6	0·4	.	.	A	N	.	.	.	15%	.	.	.	A	A	.	.	.	.	.	N
47.	Start Pt.	RSI	April 57	.	C	2·5	2·7	2·0	N	0·03	0·1	.	.	F	.	.	0%	C	62%	A	.	.	.	.	.	.	A	.	F	N
48.	Torcross	RF, G	April 50, April 57	.	C	C	.	.	.	O	.	.	.	O	N	.	.	C	15%	.	.	.	C	.	.	.	.	.	.	.
49.	Blackpool	R, S, G	April 50, April 57	.	.	A	.	.	.	O	.	.	.	C	N	.	.	.	37%	.	.	.	.	.	.	.	A	.	F	.
50.	Dartmouth	P, G, M	April 50, April 57	.	.	C	O	N	C	A	A	.	.	.	O	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.
51.	Greenway	B, P, M	Feb. 51, April 57	.	.	F	R	N	C	A	C	C	O	O	C	F–C	.	.	.	C	.	.	.	.	.	C	N	.	C	.
52.	Stoke Gabriel	B, M	Feb. 51	.	.	N	N	N	C	C	O	O	C	N	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
53.	Brixham	RI, B	May 53	C	C	9·5	6·8	4·8	0·6	1·1	2·3	F	R	C	R	C	20%	A	30%	A	C	N	A	A	+	F	C	C	A	N
54.	Torquay	RI, S	May 53	C	C	.	.	.	.	.	.	F	N	A	R	.	.	A	80%	A	C	.	A	A	+	F	C	C	C	N
55.	Teignmouth	B, P, S	May 53	F	C	A	A	A	R	C	C	O	O	C	F	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
56.	Dawlish	RF, P, S	May 53	F	C	5·5	3·5	1·5	N	2·0	5·0	O	.	C	R	.	.	C	50%	A	.	N	C	C	.	.	A	.	.	.
57.	Starcross	B, P, M	July 54	.	.	0·005	N	N	2·5	3·5	4·5	O	.	F	F	.	.	N	N	F	.	.	.	.	.	C	N	F	C	.
58.	Budleigh Salterton	RI, G	July 54	.	C	2·9	1·5	3·1	2·5	2·2	4·2	.	.	A	N	.	.	.	C	C	C	.	A	F	.	.	C	.	.	.
59.	Sidmouth	RF, G, S	July 54	.	.	.	.	.	.	.	.	.	.	C	N	.	.	.	C	C	.	.	C	N	.	.	C	.	.	.
60.	Branscombe	RF, G	July 54	F	C	C	O	N	C	A	A	.	.	F	N	.	.	.	F	C	.	.	C	C	.	.	F	.	F	.
61.	Seaton	B, G	July 54	.	.	A	F	R	C	C	A	.	.	F	N	.	3%	.	30%	C	.	.	F	A	.	.	C	.	.	.
	Dorset																													
62.	Lyme Regis	RF, B, S, E	Aug. 52, Aug. 56	C	A	A	A	C	C	A	C	F	.	C	N	A	.	C	C	A	A	N	A	A	+	.	F	.	.	N
63.	West Bay	RFI, G	July 54, April 57	.	.	3·5	3·5	0·8	0·5	1·0	1·3	.	.	A	N	.	0%	10%	70%	C	F	N	C	N	.	F	N	.	F	N
64.	Portland Bill	RF	July 53	.	A	4·5	3·5	0·6	N	0·15	2·9	.	.	A	N	.	.	C	50%	A	.	.	C	N	.	.	A	.	.	N
64.	Portland Bill	RF	April 56, April 57	.	.	1·2	1·2	.	N	0·34	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
65.	Weymouth	S, P	July 53	.	.	O	.	.	A	.	.	.	.	R	F	.	0%	N	N	F	.	.	N	N	.	.	N	.	.	.
66.	Osmington Mills	B, G	July 53	.	.	5·0	2·0	2·5	N	0·2	0·1	.	.	R	O	.	.	.	50%	C	.	.	A	N	.	.	F	.	.	.
67.	Lulworth Cove	C, B, G	July 53	.	.	0·05	0·1	0·0003	0·02	0·05	0·001	.	.	N	R	.	.	.	20%	F	.	.	C	N	.	.	.	.	.	.
68.	Kimmeridge	RF, B, G, E	July 53	.	C	4·0	5·0	3·0	0·01	0·01	0·2	.	.	N	F	.	.	.	25%	C	.	.	A	N	.	.	C	.	.	.
69.	Peveril Pt., Swanage	RSI, B, S	June 49, July 53	C	A	0·16	0·2	0·0005	0·03	0·08	0·25	.	.	A–C	R	.	.	A	30%	A	.	.	C	N	.	.	C	.	.	N
70.	Swanage Bay	B, G, S, P	June 49, July 53	.	.	.	.	.	.	.	.	.	.	N	C	.	6%	N	N	C	C	.	R	N	.	.	N	.	.	.
71.	Studland	RF, S	June 49	.	.	N	N	N	A	.	.	.	.	C	C	.	.	.	.	.	.	.	N	N	.	.	R	.	.	.
72.	Sandbanks	B, M, P	Mar. 48	.	.	N	N	N	A	.	.	C	C	F	F	.	.	.	.	.	.	.	.	.	.	.	N	.	.	.
	Hampshire																													
73.	Bournemouth	S, P	June 49	.	.	N	N	N	.	.	.	.	.	F	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
74.	Hengistbury Head	B, G	July 49	.	.	R	N	N	F	F	C	O	.	O	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
75.	Christchurch	G, M, P	July 49	.	.	N	N	N	A	A	A	.	.	N	A	.	1%	N	N	F	.	.	N	N	.	C	N	F	C	.
76.	Mudeford	G, M, P	April 57	.	.	R	N	N	0·24	.	.	.	.	N	A	.	.	.	.	.	.	.	.	N	.	.	N	.	.	.
77.	Southsea	B, S, P	Mar. 49, July 53, April 56	N	F	N	N	N	A	A	A	.	.	N	A	.	.	N	N	A	.	N	N	N	.	C	N	.	.	C

TABLE 7 (*continued*)

No.	Station	Description of shore	Dates of survey	*Anemonia sulcata*	*Actinia equina*	*Chthamalus stellatus* H.W.	*Chthamalus stellatus* M.T.	*Chthamalus stellatus* L.W.	*Balanus balanoides* H.W.	*Balanus balanoides* M.T.	*Balanus balanoides* L.W.	*Balanus crenatus*	*B. improvisus*	*B. perforatus*	*Elminius modestus*	*Verruca stroemia*	*Hemioniscus balani*	*Patella aspera*	*P. depressa*	*P. vulgata*	*Gibbula cineraria*	*G. pennanti*	*G. umbilicalis*	*Monodonta lineata*	*Calliostoma zizyphinum*	*Littorina littorea*	*L. neritoides*	*L. obtusata*	*L. saxatilis*	*Paracentrotus lividus*
Isle of Wight																														
78.	Alum Bay	RI, S	July 53	.	.	.	.	.	.	.	.	.	.	O	.	O	.	4%	12%	C	.	.	N	N	.	.	C	F	.	C	N
79.	Freshwater Bay	RFI	July 53	.	C	0·1	N	N	0·03	0·5	0·2	.	.	R	.	.	.	15%	30%	C	.	.	N	N	.	.	C	F	.	C	.
80.	Brook	RF, S	July 53	C	C	0·004	0·5	N	0·005	1·5	3·0	.	.	N	.	.	0%	.	40%	C	.	.	N	N	.	.	C	F	.	C	.
81.	Blackgang	RS, G	July 53	.	.	.	.	.	.	.	.	.	.	N	.	.	.	15%	30%	C	O	.	N	N	.	.	C	F	.	C	.
82.	St Catherine's Pt.	RS, B	July 53	.	F	0·003	N	N	0·2	0·4	1·0	.	.	N	.	.	.	3%	10%	C	.	.	N	N	.	.	C	F	.	C	.
83.	Ventnor	B, S	July 53	.	.	R	N	N	1·5	2·5	4·0	.	.	N	F	.	.	17%	3%	C	.	.	N	N	.	.	C	F	.	C	.
84.	Shanklin	S, P	July 53	.	.	N	N	N	A	A	A	C	.	N	.	.	.	N	N	C	.	.	N	N	.	.	C	F	.	C	.
85.	Sandown	S, P	July 53	.	R	N	N	N	4·0	6·0	6·0	.	.	N	.	.	.	N	N	C	.	.	N	N	.	.	C	F	.	C	.
86.	Lane end, Bembridge	RF, S, P	July 53	N	C	N	N	N	1·7	4·0	6·5	C	.	N	.	O	.	N	N	C	A	.	R	N	.	.	C	F	.	C	.
87.	Ryde	S, P	July 53	.	.	N	N	A	A	A	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
88.	Cowes	B, S, P	July 53	.	.	N	N	N	A	A	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
89.	Yarmouth	G, S, P	July 53	.	.	R	N	N	A	A	A	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.
90.	Coldwell and Totland Bay	RFI, S	July 53	C	.	0·003	N	N	0·2	0·4	1·0	.	.	O	.	.	0%	10%	7%	.	.	.	R	N	.	.	C	C	.	C	.
Sussex																														
91.	Selsey Bill	G, S, P	April 56	N	N	N	N	N	A	.	.	.	.	N	A	.	.	N	N	F	.	.	N	N	.	.	F	F	.	F	.
92.	Bognor Regis	S, P	June 53	N	.	N	N	N	A	A	A	.	.	N	C	.	.	.	.	O	.	.	N	N	.	.	F	F	.	F	.
93.	Littlehampton	S, P	June 53	N	.	N	N	N	C	C	C	.	.	N	A	.	.	N	N	F	.	.	N	N	.	.	F	F	.	C	.
94.	Worthing	S, P	July 53	.	.	N	N	N	C	C	A	.	N	N	F	.	0%	.	.	O	.	.	N	N	.	.	F	F	.	C	.
95.	Shoreham	B, M, P	July 53	.	.	N	N	N	A	A	A	C	O	N	C	.	.	N	N	.	.	.	N	N	.	.	F	F	.	C	.
96.	Portslade	S, P	July 53	.	.	N	N	N	A	A	A	.	C	N	A	.	2%	.	.	O	.	.	N	N	.	.	F	F	.	C	.
97.	Brighton	G, P	July 53	.	.	N	N	N	A	A	A	C	.	N	F	.	1%	.	.	O	.	.	N	N	.	.	F	F	.	C	.
98.	Black Rock, Brighton	C, G, P	Aug. 56	R	C	N	N	N	C	F	A	.	.	N	F	.	.	.	.	O	.	.	N	N	.	.	F	F	.	C	.
99.	Rottingdean	C, G	Aug. 56	.	A	N	N	N	F	F	F	.	.	N	C	.	2%	N	N	F	A	.	N	N	.	.	F	F	.	C	.
100.	Seaford	CI, B, G	July 53	.	.	N	N	N	A	A	A	.	.	N	O	.	.	N	N	F	O	.	N	N	.	.	F	F	.	C	.
101.	Eastbourne, Beachy Head	CI, B, G, S	July 53	.	.	N	N	N	C	C	A	.	.	N	C	O	.	N	N	F	O	.	N	N	.	.	F	F	.	C	N
102.	Bexhill	S, P	July 53	.	.	N	N	N	A	A	A	.	.	N	O	.	0%	N	N	F	O	.	N	N	.	.	F	F	.	C	.
103.	Hastings	S, P	July 53	.	.	N	N	N	A	A	A	.	.	N	F	.	.	.	.	C	.	.	N	N	.	.	F	F	.	C	.
104.	Fairlight	RF, B, S	July 53	N	F	N	N	N	A	A	A	C	.	N	F	R	0%	N	N	C	N	.	N	N	.	.	F	F	.	C	.
Kent																														
105.	Hythe	G, P	Aug. 56	.	.	N	N	N	C	A	C	A	.	N	O	.	.	N	N	N	C	.	N	N	.	.	N	F	.	F	.
106.	Folkestone	B, S	Aug. 56	.	.	N	N	N	A	A	A	C	.	N	F	.	0%	.	.	C	.	.	N	N	.	.	N	F	.	F	.
107.	Dover	B, S, P	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	C	.	0%	N	N	F	.	.	N	N	.	.	N	F	.	F	.
108.	Ramsgate	B, G	April 51	.	.	N	N	N	C	C	C	.	.	N	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
109.	North Foreland	CI, B, G	April 51	.	.	N	N	N	C	C	C	.	.	N	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pas de Calais																														
110.	Calais	S, P	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	C	.	0%	.	.	.	.	.	N	N	.	.	N	C	.	N	.
111.	Cap Blanc Nez	CF	Sept. 54	.	.	N	N	N	F	A	A	.	.	N	R	.	.	N	.	N	C	.	N	N	.	.	N	C	.	N	.
112.	Audresselles	R, B, S	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	N	.	0%	N	N	A	.	.	R	N	.	.	R	N	.	N	.
113.	Wimereux	RI, G	Sept. 54	N	C	N	N	N	A	A	A	C	.	N	F	.	0%	N	N	A	O	.	C	N	.	.	N	C	.	N	.
114.	Boulogne	M, P	Sept. 54	.	.	.	.	.	.	.	.	O	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
115.	Berck-s.-mer	S	Sept. 54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Seine Inférieure																														
116.	Le Tréport	C, G	Sept. 54	.	C	.	.	.	.	.	.	.	.	N	F	.	.	.	.	C	.	N	N	N	.	.	C	F	.	C	.
117.	Dieppe	C, B, G	Sept. 54, Aug. 57	.	C	N	N	N	A	A	A	C	.	N	C	.	0%	N	N	F	O	N	F–C	N	.	.	C	F	.	C	.
118.	St Valéry-en-Caux	C, S	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	F	.	0%	.	.	C	.	.	O	O	.	.	.	F	.	F	.
119.	Fécamp	C, B, G	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	F	.	0%	.	.	C	.	.	.	O	.	.	N	N	.	N	.
120.	Étretat	C, B, G	Sept. 54	.	.	N	N	N	A	A	A	.	.	N	F	.	0%	.	.	C	.	.	.	O	.	.	N	N	.	N	.
121.	Le Havre	M, P	Sept. 54	.	.	N	N	.	A	A	.	.	.	N	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.

Calvados																												
122. Honfleur	M, P	Sept. 54	.	.	N	N	.	C	C	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
123. Deauville	C, B, S	Sept. 54	N	F	N	N	N	C	C	C	.	.	N	A	.	0%	.	.	.	.	.	N	.	.	C	N	.	C	.
124. Cabourg	G, S	Sept. 54	.	.	.	N	.	.	N	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
125. Longués	RF, B, G	Sept. 54	N	C	N	N	N	.	.	.	.	.	N	O	.	.	.	N	F	.	.	C	N	.	.	F	.	A	.
126. Port en Bessin	CF, B, S	Aug. 57	.	.	.	.	.	.	.	.	.	.	.	C	.	2%	N	N	C	O	N	A	N	.	F	R	F	C	.
127. West of Vierville-s.-mer	B, G	Sept. 54	N	C	N	N	N	A	A	A	.	.	N	F	.	.	.	N	F	.	.	O	.	.	.	N	C	A	.
128. Pointe de Hoc	RS	Sept. 54, Aug. 57	N	A	N	N	N	A	A	A	.	.	N	C	.	0%	N	N	C	O	N	A	R	.	F	N	.	C	.
129. Grandcamp-les-Bains	RF, B, G, P	Sept. 54, Aug. 57	R	F	.	.	.	.	.	.	F	.	N	C	.	0%	.	.	.	C	N	C	N	.	F	N	C	F	.
Manche																												
130. Quineville	G, S	Sept. 54	.	.	N	N	N	C	C	C	.	.	.	A	.	.	N	N	N	.	.	N	N	.	.	N	N	F	.
131. St Vaast-la-Hougue	B, M, P	Sept. 54, Aug. 57	O	A	N	N	N	C	C	C	.	.	R	C	.	6%	N	N	C	C	N	C	N	.	C	N	O	C	.
132. Barfleur	RI, S, M	Sept. 54	C	.	.	.	.	.	.	.	.	.	N	F	.	0%	.	N	O	C	C	C	C	O	.	.	.	.	.
133. Pte. de Barfleur	RF, E	Sept. 54, Aug. 57	C	A	N	N	N	A	A	A	.	.	C	.	.	0%	C	23%	A	F	F	C	R	O	.	R	C	C	N
134. Cap Lévy	RF, B	Sept. 54, Aug. 57	A	F	N	N	N	A	A	A	C	.	C	.	.	4%	20%	35%	A	C	A	C	R	+	.	O	F	F	.
135. Bretteville	B, G	Sept. 54	.	C	N	N	N	A	A	A	.	.	R	O	.	.	C	40%	C	.	.	C	R	.	.	.	.	.	.
136. Cherbourg Harbour	M, P	Sept. 54	.	.	N	N	N	C	C	C	.	.	.	C	.	50%	.	.	.	.	.	.	.	.	.	N	.	.	.
137. Nacqueville	RI, S	Sept. 54	C	C	.	N	N	.	C	A	.	.	O	O	.	10%	F	3%	C	F	.	C	R	.	.	F	.	C	.
138. Rocher du Castel Vendon	RS	Sept. 54	.	.	N	N	N	A	A	A	.	.	.	.	.	.	.	F	C	.	.	C	C	.	.	F	.	.	N
139. Omonville-la-Rogue	R, B, G	Sept. 54	.	C	N	N	N	A	A	A	.	.	.	R	.	.	.	60%	C	.	.	.	.	.	.	C	.	.	.
140. Cap de la Hague	RF, E	Sept. 54	C	.	N	N	N	A	A	A	.	.	.	O	.	.	.	C	C	.	.	C	A	.	.	F	.	.	N
141. Vauville	R, S	Sept. 54	.	.	0·0005	N	N	A	A	A	.	.	.	O	.	10%	.	40%	C	.	.	.	C	.	.	C	.	.	.
142. Cap de Carteret	RI, S	Sept. 54	N	A	0·0005	N	N	5·6	4·5	5·0	.	.	O	.	.	.	C	.	.	.	.	O	O	.	F	C	.	C	N
143. Carteret	S, P	Sept. 54	.	.	0·01	.	.	3·8	.	.	.	.	.	.	.	0%	.	20%	C	.	.	.	.	.	.	C	.	.	.
144. Gouville	RI, G, S, E	Sept. 54	F	C	N	N	0·001	3·5	3·5	4·0	.	.	N	R	.	20%	O	N	F	F	.	C	C	.	A	N	F	F	.
145. Granville	R, G, S	Sept. 54, Aug. 57	C	A	1·5	N	N	2·0	2·9	3·3	.	.	F–C	.	.	4%	C	N	A	.	C	A	C	.	F	F	C	C	.
146. Carolles	RF, B, S	Aug. 57	F	A	0·25	N	N	C	5·2	C	F	.	R	N	.	0%	N	N	C	O	N	A	A	.	C	N	.	F	.
147. St Jean le Thomas	B, S	Sept. 54	.	.	0·5	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F	R	F	A	.
148. Mont St Michel	RS, M	Aug. 57	.	.	0·002	.	.	0·05	.	.	.	.	.	.	.	3%	.	.	.	.	.	.	.	.	.	.	.	.	.
Channel Islands: Alderney																												
149. Clonque Fort (sheltered)	RI, B, S, E	Sept. 56	A	A	0·06	0·07	0·02	0·35	2·7	2·5	.	.	.	N	.	.	.	.	.	C	A	A	A	.	N	A	.	.	N
150. Clonque Fort (outer reef)	RS	Sept. 56	.	A	0·09	0·25	0·5	1·9	1·8	1·8	.	.	.	N	A	0%	A	65%	A	.	.	.	.	+	.	.	.	.	N
151. Pt. Grosnez	RIS	Sept. 56	A	A	0·03	0·23	0·02	2·2	2·7	3·1	.	.	A	N	.	.	.	.	.	.	A	A	A	.	.	.	.	.	.
152. Braye Harbour and beach	B, S, P	Sept. 56	.	.	0·025	.	.	.	.	.	.	.	F	N	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.
153. Pt. Quesnard	RSF, E	Sept. 56	.	.	0·004	0·0002	N	3·6	2·8	2·8	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
154. Longy Bay	B, G, S	Sept. 56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	A	.	N	.	.	.	.
155. Telegraph Bay	RI, B, S	Sept. 56	A	A	0·025	0·01	0·005	1·8	4·0	2·4	.	.	A	.	.	10%	.	.	.	A	A	A	A	.	.	A	.	.	N
Guernsey																												
156. St Peter Port	RI	Sept. 56	.	C	0·0004	N	N	A	A	A	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
157. Bordeaux Harbour	B, S	Sept. 56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	C	C	.	.	.	.	.	.
158. Fort Doyle	RI	Sept. 56	A	A	0·01	0·02	N	3·0	3·2	2·5	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
159. Grand Camp	RI, B, E	Sept. 56	.	.	0·05	0·06	0·24	1·6	2·7	1·8	.	.	A	.	.	35%	.	60%	.	C	C	C	C	.	.	.	.	.	N
160. Isle Lihou	RI, B, E	Sept. 56	C	C	0·04	0·05	0·08	2·5	3·2	1·9	.	.	.	.	.	44%	.	85%	.	.	A	A	A	.	.	A	.	.	N
161. La Moye Pt.	RS	Sept. 56	.	A	0·36	0·30	0·005	2·2	3·5	1·6	.	.	A	.	.	.	.	90%	.	.	.	.	.	.	.	.	.	.	N
162. Jerbourg Pt.	RS	Sept. 56	A	A	0·015	N	N	3·3	4·4	2·0	.	.	A	.	.	.	.	.	.	A	A	A	A	.	.	A	.	.	N
Jersey																												
163. Montorgueil Castle	RS, G, S	Sept. 56	A	A	0·01	0·001	N	2·8	3·6	1·6	.	.	R	.	.	.	.	.	.	.	C	A	A	.	F	.	.	.	N
164. Elizabeth Castle	RI, S, E	Sept. 56	A	A	0·02	N	N	4·8	4·8	4·9	.	.	N	.	.	.	.	.	.	A	C	A	A	.	.	.	.	.	.
165. Fremont Pt.	RI	Sept. 56	.	.	0·045	0·001	.	2·7	2·2	.	.	.	N	.	.	25%	.	90%	.	.	O	.	A	.	.	A	.	.	.
166. Plemont Pt.	RS	Sept. 56	.	.	0·02	0·04	N	3·4	6·0	4·8	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N
167. La Corbière	RI, B, E	Sept. 56	A	A	0·007	0·015	N	7·0	2·4	4·0	.	.	.	.	.	0%	.	.	.	.	C	.	.	+	.	A	.	.	N
Ille-et-Vilaine																												
168. Cancale	RS, B	Sept. 54, Aug. 57	A	C	1·9	2·8	.	0·9	1·4	.	.	.	.	.	.	20%	.	38%	C	.	C	C	A	+	C	R	.	C	.
169. Pte. du Grouin	RS	Aug. 57	A	F	2·8	2·0	0·7	0·3	1·2	2·2	.	.	C	N	.	.	C	90%	F	.	C	F	C	+	O	R	.	A	N
170. Rothéneuf	RS	Sept. 54	C	C	4·1	1·8	.	0·3	2·4	.	.	.	.	.	.	.	F	70%	A	.	.	C	C	.	.	R	.	A	.
171. St Malo	RI, S, B	Aug. 57	A	.	2·3	1·7	.	0·5	1·8	.	.	.	C	.	.	.	C	90%	C	.	A	A	C	+	C	O	A	C	.

TABLE 7 (*continued*)

No.	Station	Description of shore	Dates of survey	*Anemonia sulcata*	*Actinia equina*	*Chthamalus stellatus* H.W.	*Chthamalus stellatus* M.T.	*Chthamalus stellatus* L.W.	*Balanus balanoides* H.W.	*Balanus balanoides* M.T.	*Balanus balanoides* L.W.	*Balanus crenatus*	*B. improvisus*	*B. perforatus*	*Elminius modestus*	*Verruca stroemia*	*Hemioniscus balani*	*Patella aspera*	*P. depressa*	*P. vulgata*	*Gibbula cineraria*	*G. pennanti*	*G. umbilicalis*	*Monodonta lineata*	*Callistoma zizyphinum*	*Littorina littorea*	*L. neritoides*	*L. obtusata*	*L. saxatilis*	*Paracentrotus lividus*
Côtes-du-Nord																														
172.	Dinard	RI, S	Sept. 54, Aug. 57	C	F	2·7	2·5	0·6	1·5	2·0	2·9	.	.	C	N	.	.	C	80 %	C	.	C	A	C	.	C	.	.	A	.
173.	Notre-Dame-du-Guildo	S, M, P	Sept. 54	.	.	C	O	.	C	A	.	.	.	A	N	.	30 %	C	.	C	.	C	A	O	.	O	.	.	F	+
174.	St Cast	RI, E	Aug. 57	A	O	4·1	2·5	1·5	R	1·8	3·1	.	.	A	N	.	17 %	C	67 %	C	.	F	C	A	.	C	.	.	A	N
175.	Cap Fréhel	RS	Sept. 54, Aug. 57	R	O	2·9	2·5	1·5	1·0	2·5	2·4	.	.	A	N	.	.	C	70 %	C	.	F	C	C	.	C	.	.	C	+
176.	Cap d'Erquy	RS, B	Aug. 57	O	O	2·7	1·2	3·3	N	1·4	0·1	.	.	A	N	.	.	C	75 %	C	.	F	F	A	.	C	.	.	A	N
177.	Pte. de Pléneuf	RSI	Sept. 54	C	C	6·0	3·6	2·5	0·1	1·8	2·3	.	.	A	N	.	.	C	80 %	C	.	C	C	C	.	C	.	.	C	N
178.	le Val André	M, P	Sept. 54	.	.	0·005	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
179.	Pte. de Cesson	G, M, P	Sept. 54	.	.	0·01	.	.	N	.	.	.	.	.	.	.	10 %	.	.	.	.	.	A	C	.	C	.	.	.	.
180.	Pte. du Roselier	RS, G	Sept. 54	.	C	4·5	2·1	1·1	0·2	1·5	2·3	.	.	.	.	.	.	.	90 %	.	.	.	.	.	.	F	.	.	.	.
181.	Pte. de Pordic	RS, G	Aug. 57	O	A	3·4	2·8	1·0	0·2	3·4	0·5	.	.	C	N	.	.	A	91 %	C	C	N	C	C	.	O	.	.	.	.
182.	Binic	P, M	Aug. 57	.	.	.	2·0	.	.	2·0	.	.	.	.	.	.	26 %	.	O	.	.	.	.	.	.	A	.	.	.	.
183.	St Quay—Portrieux	RSI, S	Sept. 54, Aug. 57	F	C	3·5	1·6	1·0	1·6	3·4	2·5	.	.	A	N	.	38 %	F	81 %	A	C	C	C	A	.	C	.	.	.	.
184.	Lèzardrieux	B, M, P	Sept. 54	.	.	O	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
185.	Sillon de Talbert	RI, G, E	Sept. 54	.	.	2·2	0·5	.	1·0	1·5	.	.	.	.	.	.	10 %	.	.	.	.	.	A	C	.	C	.	.	.	.
186.	Tréguier	B, M, P	Sept. 54	.	.	1·5	2·0	0·3	0·2	1·2	2·8	F	C	C	N	.	11 %	.	25 %	C	.	.	A	C	.	C	.	.	.	.
187.	Trestrignel	RI, B, S	Aug. 57	A	A	2·2	2·7	.	0·3	1·3	.	.	.	.	.	.	.	C	5 %	A	.	.	A	C	.	C	.	.	.	.
188.	Trégastel-Plage	RI, B, S	Sept. 54	C	C	2·0	2·0	2·0	.	.	.	.	.	.	.	.	.	.	0 %	A	.	.	A	C	.	C	.	.	.	.
189.	Pte. de Bihit	RI B, S	Aug. 57	.	.	.	2·2	1·5	.	1·9	1·6	.	.	O	.	.	.	C	70 %	A	.	.	A	C	.	C	.	.	.	.
190.	Pte. de Dourvin	RI, E	Aug. 57	C	O	A	.	C	N	.	O	C	.	C	.	O	.	N	.	.	.	.	.	.	.	.	.	.	.	.
191.	Pte. de l'Armorique	RI	Sept. 54	C	C	.	.	.	.	.	.	.	.	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Finistère																														
192.	Pte. de Locquirec	RF, S	Aug. 57	C	C	4·1	3·7	1·6	N	0·05	0·1	.	.	A	.	C	.	C	67 %	C	.	A	C	A	.	C	.	.	F	+
193.	Primel-Trégastel	R, B	Sept. 54	.	.	.	.	.	O	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	F	N
194.	Pte. de Primel	RSI	Sept. 54	C	.	.	.	.	.	.	.	.	.	.	O	.	.	.	60 %	C	.	C	.	C	.	A	.	.	.	+
195.	Morlaix	M, P	Sept. 54	.	.	.	.	.	.	.	.	.	C	F	A	F	.	.	.	.	.	.	.	.	.	.	.	.	.	N
196.	Penzé	P, M	Aug. 57	.	.	.	.	.	.	.	.	.	.	.	.	.	33 %	.	.	.	.	.	.	.	.	.	.	.	.	+
197.	Pte. de Bloscon, Roscoff	RI, B	Aug. 57	F	O	3·2	2·5	1·8	R	0·2	0·002	.	.	A	.	C	.	C	17 %	C	.	C	C	A	.	C	.	.	.	.
198.	Grève de Poulfoën	RF, B, S, E	Aug. 57	F	.	0·8	3·1	0·9	0·3	0·15	0·01	.	.	A	.	A	0 %	A	73 %	C	.	C	C	A	.	F	.	.	.	.
199.	Pte. de Pontusval	R, B, S	Sept. 54, Aug. 57	R	.	C	0·2	.	O	0·5	.	.	.	C	.	.	.	.	60 %	C	.	C	C	A	.	A	.	.	.	.
200.	Grèves du Vougot	RFI, S, E	Sept. 54 Aug. 57	C	.	A	.	.	N	.	.	.	.	C	R	.	.	.	60 %	C	.	O	A	A	.	C	.	.	.	.
201.	Grève de Lilia	RS, B	Aug. 57	O	F	1·2	.	.	0·3	.	.	.	.	.	O	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
202.	L'Aber Wrac'h	B, M, P	Sept. 54	.	.	.	.	.	.	.	.	.	F	O	R	.	5 %	C	R	C	.	O	F	C	.	F	.	.	.	N
203.	Tréglonou	B, S, P	Sept. 54	.	.	.	.	.	O	.	.	.	.	R	N	.	.	C	R	C	.	O	A	A	.	+	.	.	.	.
204.	Trémazan	RSI, B	Sept. 54, Aug. 57	F	C	1·8	3·0	2·1	N	N	0·08	.	.	.	C	.	5 %	C	60 %	C	.	O	A	A	.	.	.	.	.	.
205.	Melon	RF, B	Aug. 57	C	F	1·9	A	2·2	0·02	O	0·04	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
206.	L'Aber Ildut	B, M	Aug. 57	.	.	.	O	.	N	N	N	.	.	.	O	.	5 %	C	60 %	C	.	O	A	A	.	.	.	.	.	.
207.	Le Conquet	RSI, B	Sept. 54, Aug. 57	A	C	4·3	4·9	2·2	R	0·03	0·02	C	.	A	R	.	0 %	A	.	.	.	.	.	.	.	.	.	.	.	.
Ile d'Ouessant																														
208.	Baie du Stiff	RI, B, S	Aug. 57	O	A	1·8	2·5	0·4	0·2	0·2	0·0005	.	.	R	N	.	0 %	C	N	A	.	C	C	C	.	A	.	.	.	.
209.	Pte. du Stiff	RS, I	Aug. 57	.	A	1·0	2·0	.	N	0·05	.	.	.	R	N	.	.	C	R	F	.	C	.	N	.	C	.	.	.	.
210.	S.E. point of island	RI, B	Aug. 57	C	A	1·4	R	O	N	N	N	.	.	R	N	.	.	C	3 %	C	.	C	.	O	.	C	.	.	.	.
211.	Baie de Lampaul	RS	Aug. 57	O	C	C	C	C	N	0·0001	N	.	.	R	N	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
212.	Lampaul	M, P	Aug. 57	.	.	0·3	.	.	.	0·02	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
213.	Pte. de Pern	RI, E	Aug. 57	O	A	1·1	0·9	0·8	N	N	0·01	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
214.	Phare de Créac'h	RS	Aug. 57	.	C	.	.	.	N	N	N	.	.	.	N	.	.	A	R	.	.	.	.	.	.	.	.	.	.	.

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STUDIES ON MARINE FLAGELLATES

IV. MORPHOLOGY AND MICROANATOMY OF A NEW SPECIES OF *CHRYSOCHROMULINA*

By MARY PARKE
The Plymouth Laboratory

IRENE MANTON AND B. CLARKE
Botany Department, Leeds University

(With total of 37 Figures in text and on Plates I–X)

CONTENTS

INTRODUCTION

In our previous descriptions of new species of flagellates provisionally referred to the genus *Chrysochromulina* (Parke, Manton & Clarke, 1955, 1956) the electron microscope has been used to amplify knowledge of the external morphology but not of the internal structure. This limitation was known to be temporary, but considerable effort has had to be expended on perfecting methods of handling these very delicate organisms before it could be overcome. That to some extent this has now been achieved is due to two independent circumstances, on the one hand a fairly large and relatively resistant organism with some specially favourable features about its external appendages, and on the other hand the opportunity of examining good sections with a more powerful microscope than that normally available to us in Leeds.

The optical requirements for the electron microscopy of thin sections of biological material are far more stringent than for shadow-cast whole mounts

and without the RCA microscope at the Rockefeller Institute, or some equivalent instrument, the sections reproduced here could not have been effectively studied. That this microscope was made available to one of us (I. M.) during a 2 weeks' visit to New York at Christmas 1956 is a matter for very lively personal gratitude to Dr K. R. Porter and other members of the staff of the Cytology Section who were at hand at a holiday period. As a result, although several other problems were studied during this visit, the work on the new flagellate advanced sufficiently far to reach a state suitable for publication with only 4 days' further work on a high-resolution microscope in England (the Siemens microscope at the Aeon Laboratories at Egham, Surrey).

It should perhaps be explained, however, that in a study of this kind, even without the present limitation of access to suitable microscopes, completeness in the description of fine structure is rarely a practicable objective at the first attempt. With every new type of cell it is a sufficient undertaking to identify the various organs present and determine their salient structural features. This is all that we are attempting here, but among the organs known to be present two stand out as of special interest in the present context, namely, the scales and the haptonema. Without the sections of scales the taxonomic description of this particular species could not have been completed, and special attention has necessarily been devoted to them for this purpose. The haptonema, on the other hand, is an organ of general interest which characterizes the whole genus but about which nothing is known anatomically. Though much still remains unknown we have succeeded in revealing at least some of the more obvious structural differences which distinguish a haptonema from the flagella with which it was formerly confused (Lackey, 1939), and since it will in any case be necessary to examine this unfamiliar organ in more than one species we may hope for future opportunities to extend our present limited knowledge. The other body organs have been examined only incidentally. Most, and probably all, of those detected with the light microscope have been found with the electron microscope and a few others have been added. But in all, our study of structure has been limited to the necessary minimum required for identification of the organ, since space as well as time would have precluded more detailed analysis.

MATERIALS AND METHODS

The material came from three separate isolates originally numbered 146, 149 and 150 in the Plymouth collection. All three have been used for external morphology, but only culture 146 was embedded. As on previous occasions each culture was unialgal though not bacteria-free.

The methods used for visual microscopy and for external morphology with the electron microscope have been exactly as in our previous papers. They are described in Parke *et al.* (1955), and therefore the only new details to mention

are those concerned with the production of thin sections for the electron microscope.

Before embedding it is necessary to concentrate the cells by 10–15 min centrifugation at a speed which has to be determined by experiment. The test for successful centrifugation is that there should be sufficient cells in the concentrate and that these should still be swimming when examined under the light microscope; it is not necessary to remove all cells from the supernatant. The fixative (1% osmium tetroxide buffered to pH 7 with acetate veronal in distilled water) is then poured on after removal of the supernatant and the tube is left undisturbed in a domestic refrigerator for 1 h. Alternatively, the concentrated cells may be sucked up with a pipette and squirted into the fixative before being again spun down for ease of handling.

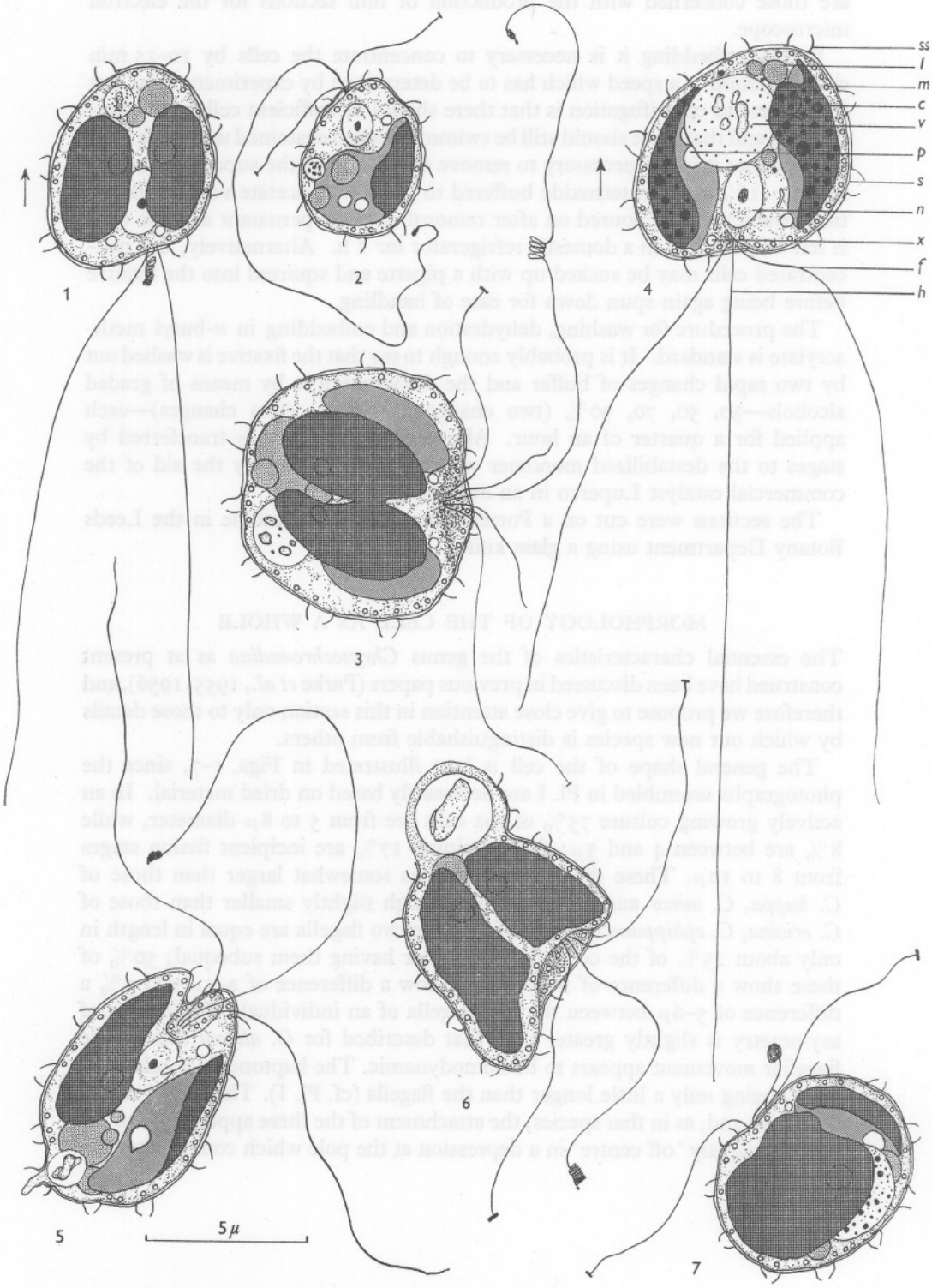
The procedure for washing, dehydration and embedding in *n*-butyl methacrylate is standard. It is probably enough to say that the fixative is washed out by two rapid changes of buffer and the dehydration is by means of graded alcohols—30, 50, 70, 90% (two changes), absolute (two changes)—each applied for a quarter of an hour. After this the material is transferred by stages to the destabilized monomer which is polymerized by the aid of the commercial catalyst Luperco in an oven at 48° C.

The sections were cut on a Porter-Blum Serval microtome in the Leeds Botany Department using a glass knife.

MORPHOLOGY OF THE CELL AS A WHOLE

The essential characteristics of the genus *Chrysochromulina* as at present construed have been discussed in previous papers (Parke *et al.*, 1955, 1956), and therefore we propose to give close attention in this section only to those details by which our new species is distinguishable from others.

The general shape of the cell is best illustrated in Figs. 1–7, since the photographs assembled in Pl. I are necessarily based on dried material. In an actively growing culture 75% of the cells are from 5 to 8 μ diameter, while 8% are between 4 and 5 μ; the remaining 17% are incipient fission stages from 8 to 10 μ. These dimensions are thus somewhat larger than those of *C. kappa*, *C. minor* and *C. brevifilum*, though slightly smaller than those of *C. ericina*, *C. ephippium* and *C. alifera*. The two flagella are equal in length in only about 25% of the cells, the remainder having them subequal; 50% of these show a difference of 1–2 μ, 45% show a difference of 2–4 μ and 5% a difference of 5–6 μ between the two flagella of an individual. This degree of asymmetry is slightly greater than that described for *C. alifera* though the flagellar movement appears to be homodynamic. The haptonema is relatively short, being only a little longer than the flagella (cf. Pl. I). This detail recalls *C. ericina* and, as in that species, the attachment of the three appendages to the body is slightly 'off centre' in a depression at the pole which commonly faces



Figs. 1–7. *Chrysochromulina chiton* sp.nov. (×5000)

backwards during swimming. Further details regarding the behaviour of the cell in life will be found on p. 222.

In spite of a superficial resemblance to some of our other species in various details of relative dimensions our new species is sharply distinguished from everything that we have previously described by its very remarkable and large scales. These are so big (up to nearly 3 μ in diameter) that they can be seen individually with the light microscope on a dried preparation (cf. Fig. 10, Pl. I), but even without drying they are liable to become detached from the surface of the body in sheets which stain readily with cresyl blue and are then clearly visible. In section they are equally distinctive in ways which will be described below. For these reasons we propose to name our new species *C. chiton* to draw attention to the remarkable scale characters.

THE SCALE CHARACTERS

The large size and spectacular appearance of the scales can be seen in Fig. 11, Pl. I, and in Pls. II and III. They are manifestly of two kinds, each with characteristic differences in the appearance of the two faces. There are smaller, roundish or oval scales, each with a rim and a radiating pattern of ridges on one face which is absent from the other (see specially Figs. 16 and 17). These represent a general type of scale which we have encountered not uncommonly (1955, 1956), often in association with others of markedly different appearance. The second type of scale and the one characteristic of our new species has not previously been encountered. These scales are much larger and manifestly bipartite, having a wide rim delimited from an oval centre by a ridge which suggests that these two parts cannot have been in the same plane in life, although in the flattened condition produced by drying it is not possible to deduce what their mutual relations may have been. Unconsciously one is influenced by their astonishing resemblance to straw hats. The pattern of

Legends to Text-figs. 1–7

Chrysochromulina chiton sp.nov. (× 5000)

Fig. 1. Individual swimming with flagella and haptonema behind body in position characteristic for the species during rapid swimming.

Fig. 2. Anchored individual with one chromatophore; haptonema nearly fully extended but bent.

Fig. 3. Early fission stage: four flagella, one haptonema, chromatophores dividing.

Fig. 4. Individual gliding without rotating, with flagella and haptonema behind body in position characteristic for the species during gliding movement. *c*, chromatophore containing saturated lipoid globules stained by Sudan Black; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid; *s*, smaller plate-shaped scale; *ss*, larger saucer-shaped scale; *v*, vacuole containing ingested bacteria; *x*, mitochondrion?

Fig. 5. Individual swinging round on partly extended, bent, anchored haptonema; recently ingested bacteria in vacuole at non-flagellar pole.

Fig. 6. Anchored individual with haptonema coiled at distal end; recently ingested *Monas* cell in vacuole at non-flagellar pole.

Fig. 7. Anchored individual with haptonema coiled; large vacuole containing granules showing Brownian movement near non-flagellar pole.

sculpturing which is partly responsible for this illusion is present on one face only, the other face being smooth (compare S 1 with S 2 in Fig. 15, Pl. II).

The true shape of both types of scale is at once made clear from a section, e.g. Fig. 30, Pl. IX. The large scales are not hat-shaped but saucer-shaped, and the small scales are plate-like with a rim sharply flexed towards the upper surface. Both kinds of scale become detached from the cells so easily that these are commonly naked when sectioned though scales may litter the field cut at all angles and without orientation to a surface (Pl. IV, etc.). The two characteristic types of profile are easily picked out. Occasionally, however, a favourable cell may retain its scales in position and two are illustrated here (Pl. VIII and Fig. 29, Pl. IX) from each of which the arrangement of the scales on the cell surface is obvious. They form a single layer with the two scale types so arranged that the small scales alternate with the large ones, occupying the interstices between them. In surface view this must mean that each large scale is surrounded by a ring of 5–6 small scales in a manner which is partly retained in the detached mat illustrated in Pl. III.

Apart from the evidence on scale arrangement, the cells of Figs. 28 and 29, Pls. VIII and IX, are important for providing information regarding the relative positions of the patterned versus patternless scale faces with respect to the cell surface. In both cases the scale shape in section combined with the information from shadowcast material makes it clear that the patterned surface lies against the cell body and the relatively patternless surface outwards. This is in exact agreement with the position deduced from the evidence of the spined scales in *C. ephippium* (1956, p. 402).

A fuller interpretation of the nature of the patterning on the scale faces cannot be arrived at without knowledge of their mode of growth. There is, however, no doubt that, at least for the larger scales, there are two quite distinct layers of material present which in section can be seen separately in the angle where the rim joins the central plate (Pl. VIII). Though the

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Explanation of Plates I–II

Chrysochromulina chiton sp.nov.

I

Figs. 8–10. Three dried cells photographed with the light microscope to show the flagella and haptonemata extended; detached scales visible near arrows in Fig. 10. × 1000.

Fig. 11. Low-power electron micrograph (M 489.10) of a cell with a coiled haptonema; the body scales very conspicuous. × 3000.

Fig. 12. Similar but with haptonema extended and scales not conspicuous. M 380.24, × 3000.

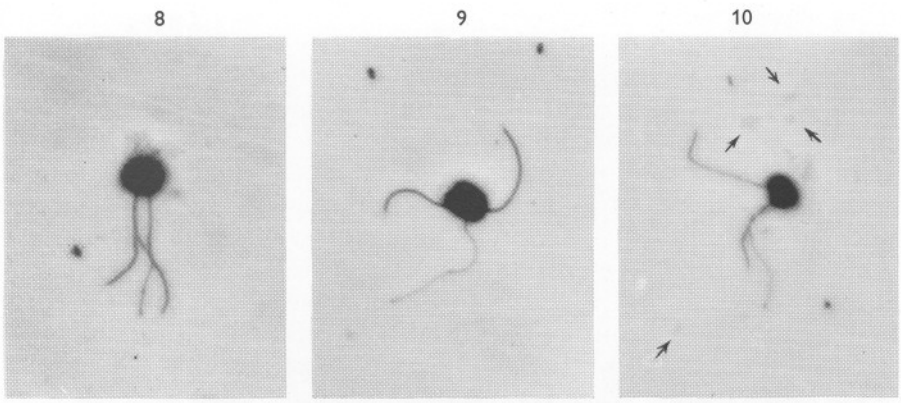
Fig. 13. Tip of the haptonema and one flagellum of Fig. 12 more highly magnified. M 380.15, × 10,000.

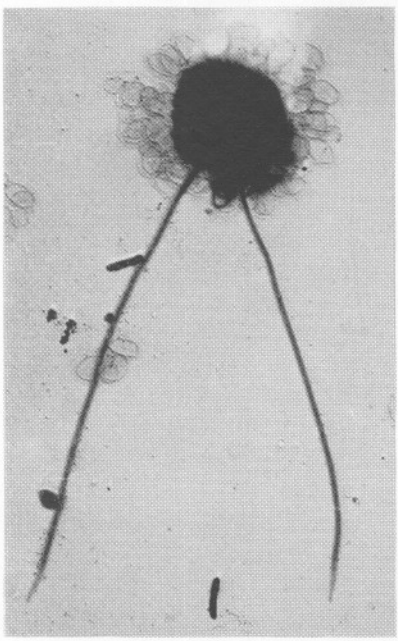
II

Fig. 14. Tip of a haptonema to show apical swelling and sheath. M 480.6, × 18,000.

Fig. 15. A cell with scales, S1 showing smooth outer face, S 2 showing patterned lower face. M 301.6, × 15,000.

Fig. 16. A field of detached scales more highly resolved. Mid 502.5, × 30,000.

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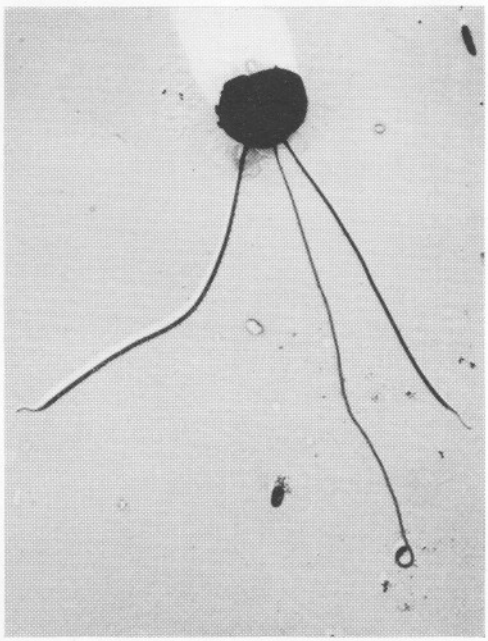
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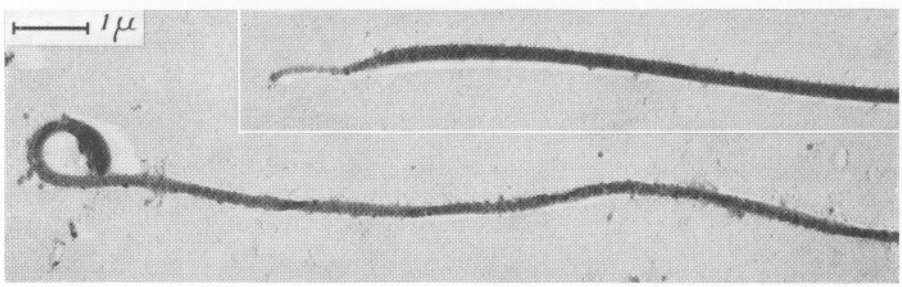

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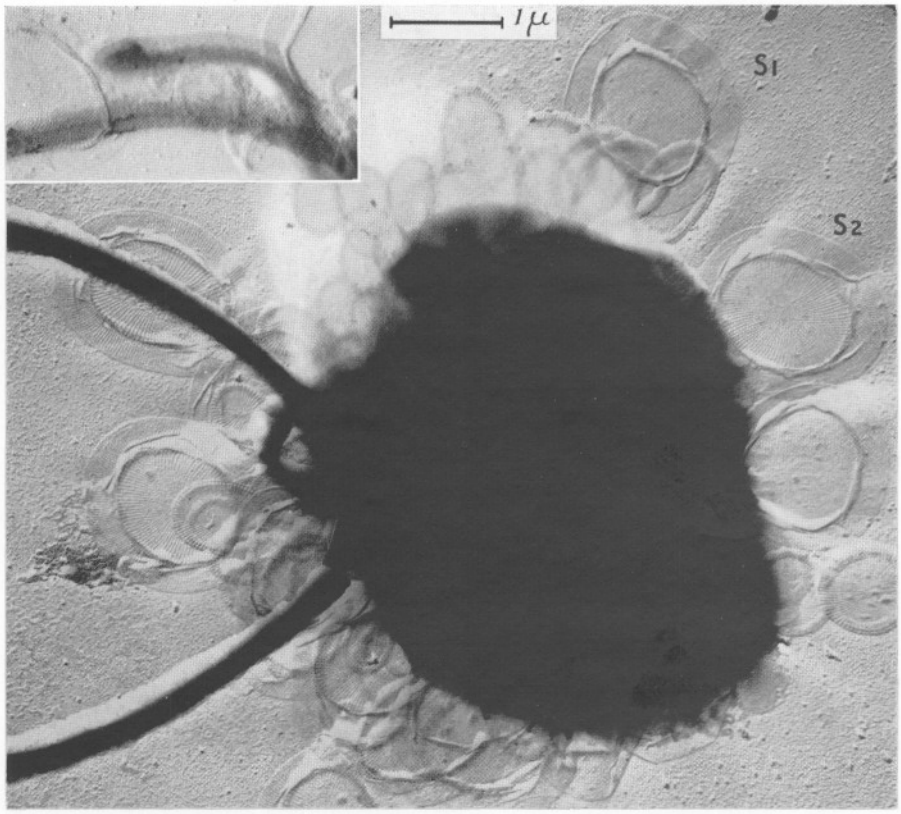

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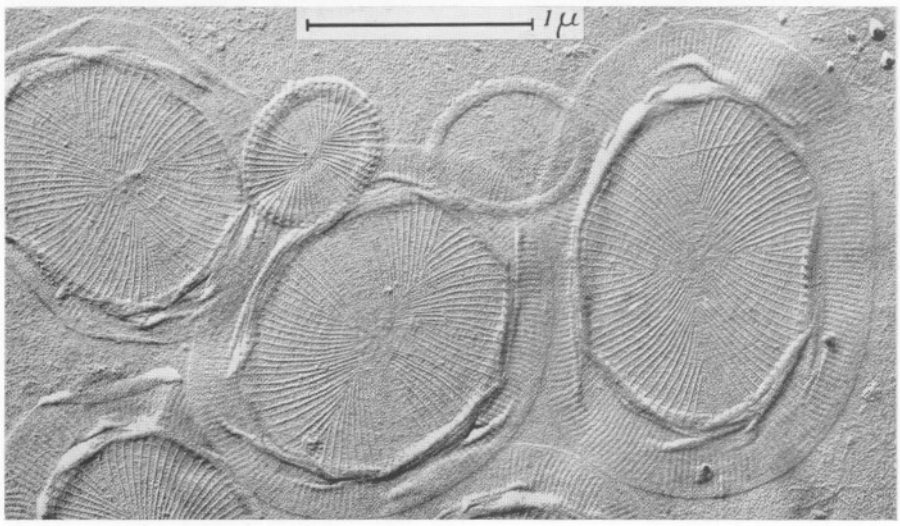

sculpturing is confined to the lower layer, it is probable that this layer is actually compound since our best resolved pictures of the patterned surface, Fig. 16, Pl. II, give a strong suggestion of interwoven fibres rather than sculpturing in the strict sense.

STRUCTURE OF THE FLAGELLA AND THE HAPTONEMA

The two types of filiform appendages are visible in Pl. I and part of Pl. II. The club-shaped tip of the haptonema contrasting with the attenuated knobbed apices of the flagella (Fig. 13) is a familiar feature from other species. That the axis of the haptonema is narrower than that of a flagellum is also familiar, as is the occasional appearance as of a loose sheath covering the haptonema in favourable preparations (Fig. 14).

The internal structure of flagella is now so well known that they can be recognized at sight in almost any type of section. In Fig. 30, Pl. IX, for example, there is a good transverse section showing the outer membrane, the ring of nine strands and the central pair, though the latter are not separately resolved. Other views will be found in several of the micrographs, but special attention can usefully be given to the one on the right of Fig. 21, Pl. V, since this, though somewhat flattened, shows the two central strands clearly and also traces of the split in some of the peripheral strands.

The other objects contained in the various micrographs on Pl. V are almost all parts of coiled haptonemata cut in various directions. In studying the internal structure of the haptonema the first and major difficulty is identifying it in section, since its attachment to the body cannot usually be traced. The coiled nature of the haptonema, however, in this species made identification possible, since in section the haptonema gave clusters of small profiles with an opaque micro-structure which could be distinguished from other small circular profiles (flagella, bacteria, and the debris from broken cells) which crowd the field. The clusters of small profiles are a sure indication of the presence of a haptonema once the general characteristics of flagella and bacteria, both of which are larger than haptonemata in section, have been recognized. A characteristic field containing a haptonema is reproduced at a low magnification in Pl. IV, and more highly magnified parts of four different specimens are included in Pl. V.

A very common fixation artifact which is unavoidably represented in parts of each of the four haptonemata reproduced in Pl. V is 'blistering'. This is almost certainly due to the presence of osmotically active materials beneath a relatively impermeable membrane, and the local distension of this membrane may cause not only a blister-like swelling of the surface but also distortion of the more resistant internal parts of the organ. In flagella, where this artifact is well known, the axis may become bent within the blister so that certain planes of section will suggest the presence of two or more axes within a

common skin. Something similar is occurring in parts of all the haptonemata illustrated in Pl. V and therefore not every profile can be interpreted as normal. Since, however, all sections in any one cluster belong to one and the same organ it is generally possible to distinguish undistorted sections as such when the plane of cutting is favourable.

The best transverse sections in Figs. 19–21 are indicated by arrows, and Figs. 21 *a* and *b* represent two successive sections reproduced side by side. Some other profiles in the various figures are oblique or longitudinal. Wherever the orientation is adequate, however, there is complete agreement in the numerical details displayed. In contrast to the flagella a haptonema possesses at least three concentric membranes surrounding a ring of seven strands, and the centre of the organ seems to be hollow. The sectional outline is probably circular except where flattening by pressure from the knife has occurred in cutting.

In many specimens, notably that of Fig. 20, the ring of seven strands (or tubes) is closely pressed to the inner side of the innermost membrane, but they are definitely not part of this membrane since they can become detached from it if blistering extends to the interior of the whole organ (Fig. 22). The outermost membrane in this specimen is greatly distended and within it the axis of the organ has been transversed three times. Two of these axial sections

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Explanation of Plates III–VI

Chrysochromulina chiton sp.nov.

III

Fig. 17. A field of scales, reversed print. Mid 301.18, × 20,000.

IV

Fig. 18. Section of a field containing a haptonema (arrow) between cells and debris. S 37, × 12,500.

V

Fig. 19. Another section of the haptonema contained in Pl. IV at a different angle on the page; parts of eight profiles through the organ represented, the best transverse section indicated by the arrow. S 42, × 40,000.

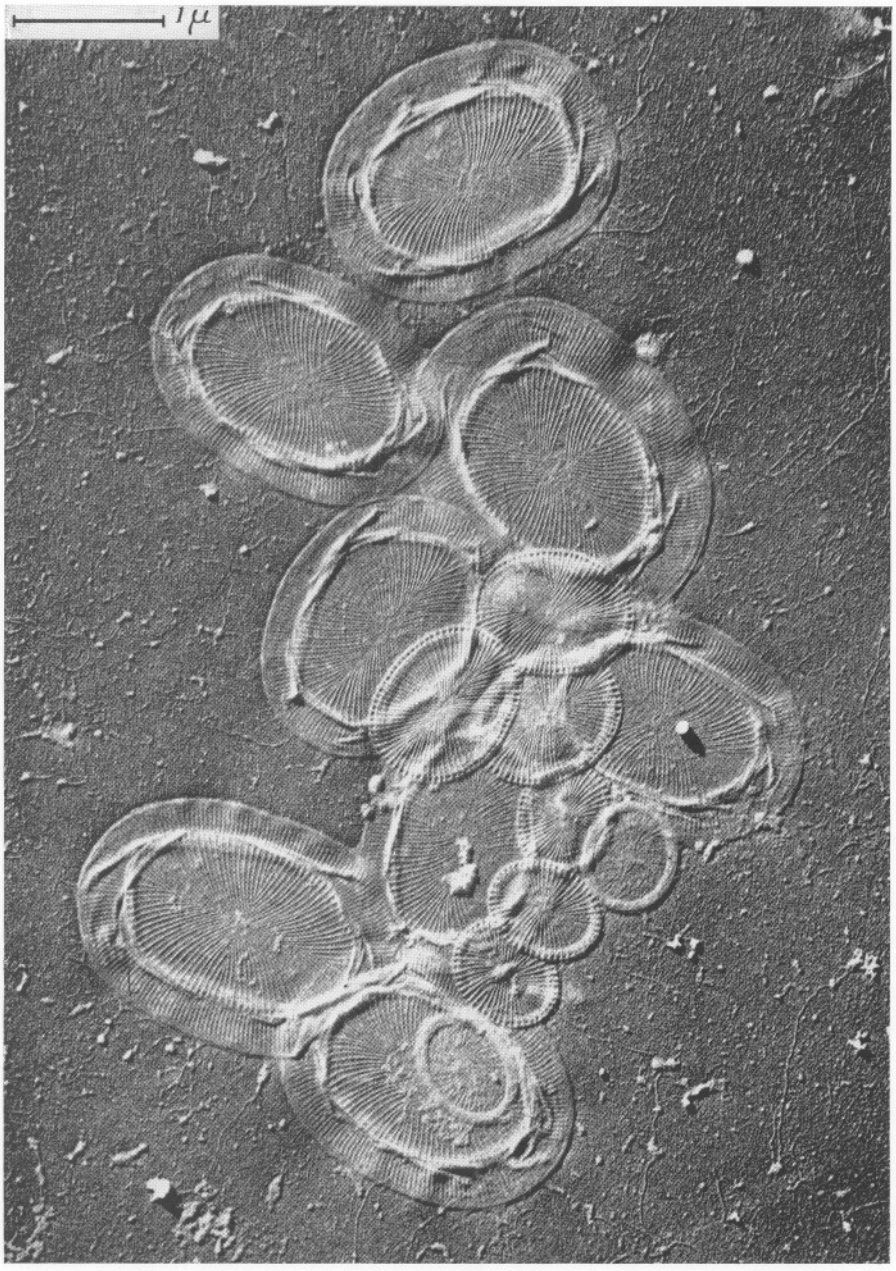
Fig. 20. Another haptonema showing parts of five sections through the axis, the best transverse section marked by an arrow. R 86e, × 40,000.

Fig. 21. Transverse section of a flagellum (right) and parts of three sections of a haptonema (left), the best transverse section in the top left-hand corner (*b*); another view of this from an adjacent section in *a*. S 74 and S 83, × 50,000.

Fig. 22. Transverse section of another haptonema showing advanced 'blistering'; the flexed axis within the distended outer sheath has been traversed three times and in two of these the seven central strands have become detached from the innermost membrane, which is also greatly distended. S 115, × 70,000.

VI

Figs. 23–25. Three successive sections to show details of attachment of two pyrenoids to neighbouring chromatophores; a third pyrenoid, part of the nucleus near the surface, a mitochondrion and other inclusions also visible; for further details see Pl. VII. Mid 502.11, S 97 and S 108, × 12,000.



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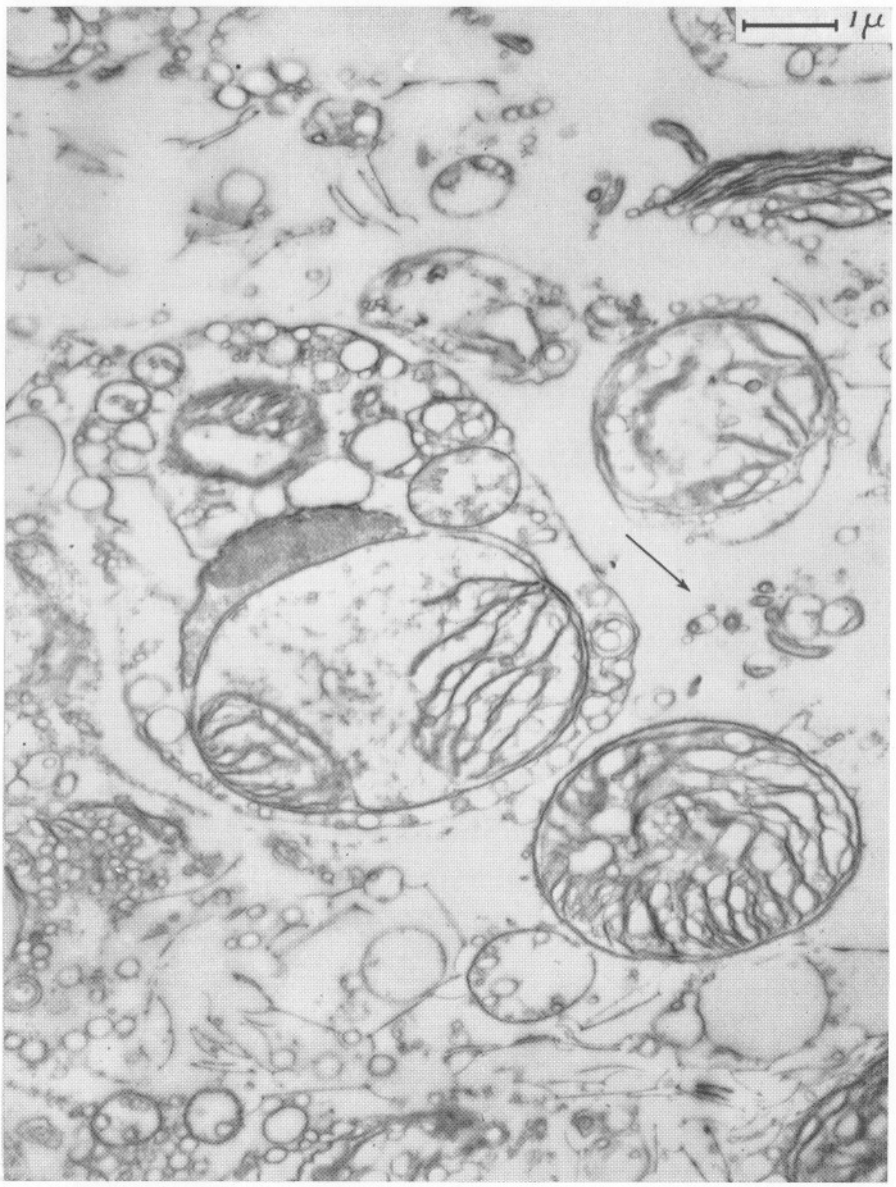

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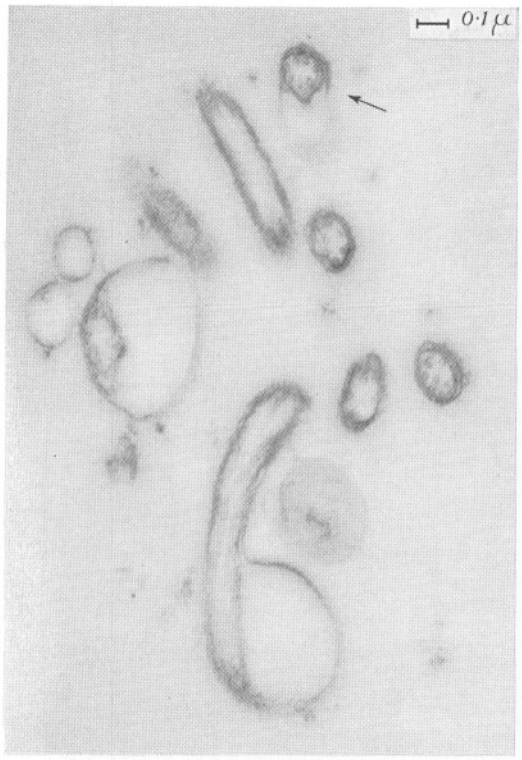

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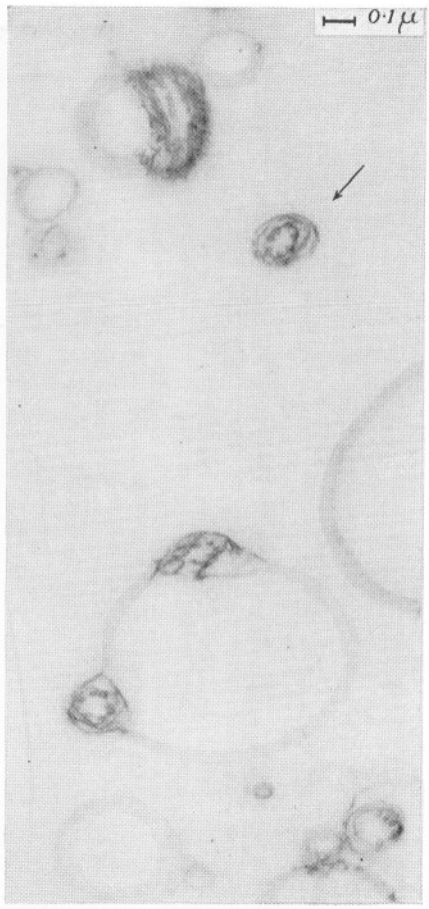

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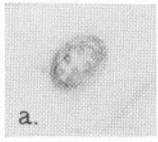

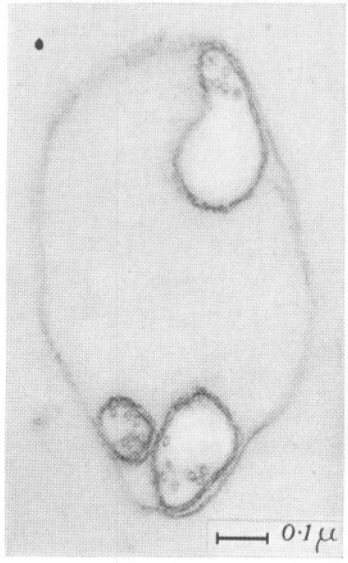

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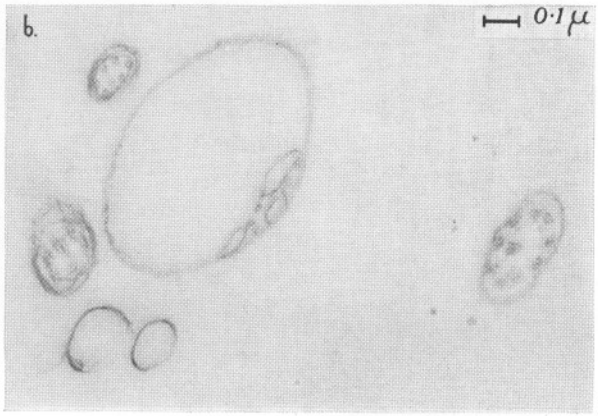

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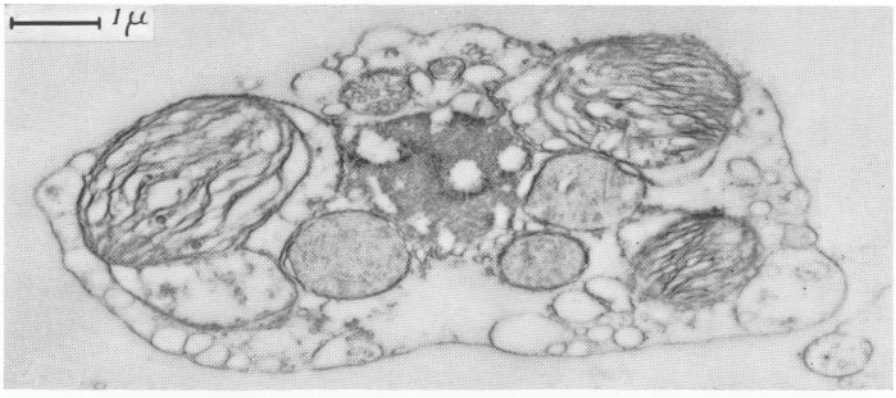

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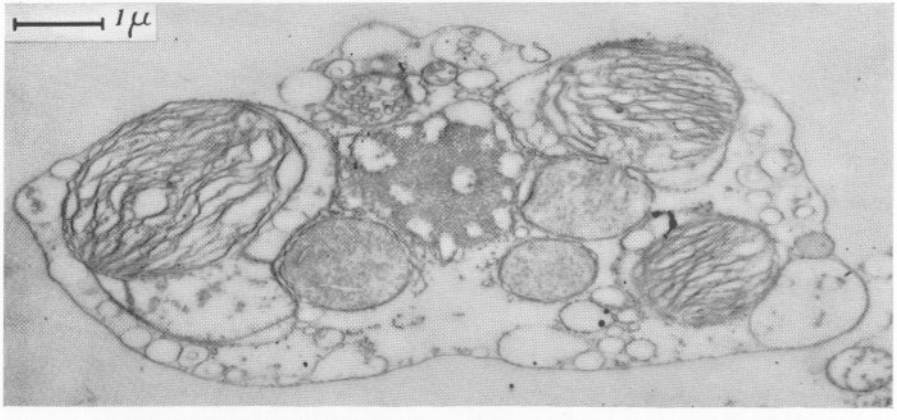

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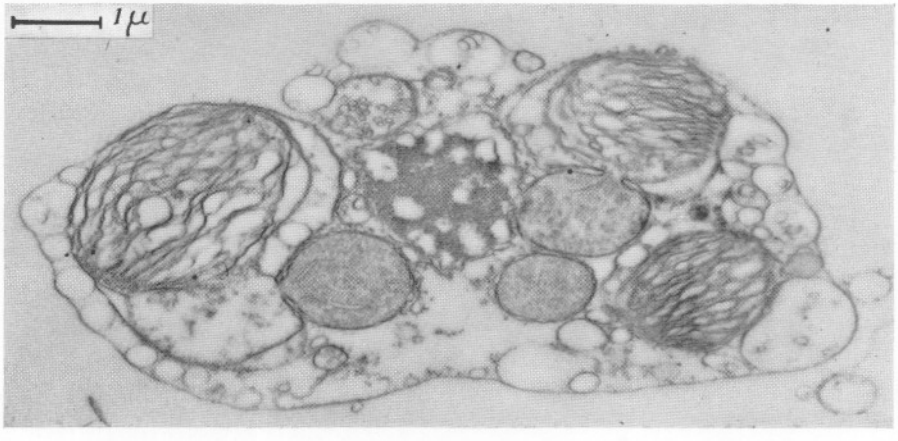


25

show distension of the remaining membranes also, and the fibres have become detached and are very clearly countable.

The longitudinal path of the internal haptonema fibres is visible in parts of Figs. 19 and 20. The strands run roughly parallel to each other, though Fig. 19 suggests a slightly spiral path. Minor undulations, never encountered in the peripheral fibres of flagella, are also detectable on individual fibres. There is therefore little doubt that the fibres themselves are different in character as well as in arrangement from those of the flagella.

Since not only the membranes but also the walls of the haptonema fibres are relatively thick, it is probable that both will reveal further microstructure when still higher resolution can be applied. This has not yet been seriously attempted. We likewise lack all anatomical information on the structure of the two extremities of the organ.

With regard to the details of attachment of the appendages to the cell we have some incidental observations for the flagella only. Two examples are illustrated in Figs. 33 and 34, Pl. IX. Fig. 33 is important for demonstrating the spatial relation of the flagellar bases to a group of characteristic vesicles immediately below them which will be described below under golgi material. Fig. 34 is chosen as the best example so far obtained of the structural details of two bases, one in transverse section and the other in longitudinal section, both of which are probably flagella and not haptonemata. If this identification is correct the flagellum seen in longitudinal section seems to lack several of the normal structural details of a basal body, since there is no transverse partition at the level of the cell surface and the interior, which in other cases is hollow, seems occupied here by amorphous dark contents. The transverse section visible (see arrow) beside the posterior end of the longitudinal section shows a stellate outline corresponding to the nine peripheral strands and the centre is similarly filled with dark material. It therefore seems probable that some new features may be expected to exist in the basal parts of the flagella in this organism when they can be further studied.

CONTENTS OF THE BODY AS SEEN WITH THE LIGHT MICROSCOPE

With the light microscope an extremely thin pectic layer covering the body can be distinguished after staining with Ruthenian Red. The principal objects detectable inside the cell are as follows. (i) There are usually two or four golden brown chromatophores, each with internal striations clearly visible from the outer face and probably representing lamellae, and with a variable number (1 to very numerous) of distinct vesicles (0·2–0·5 μ diameter) between the striations, which are soluble in alcohol and which stain intensely with Sudan black (Fig. 4) or Sudan IV especially when the latter is used as a vital stain, these reactions indicating saturated lipoid (cf. Doflein, 1922, for

Ochromonas). (ii) Against the inner face of each chromatophore, and probably attached to it, is a body of variable size (0·5–1·0 μ), usually non-refractile and not staining with Sudan IV, which is probably a pyrenoid (cf. Schwarz, 1932; Chadefaud & Feldmann, 1949; Magne, 1952). A pink coloration of the surface with Sudan IV suggests the possible presence of a lipoid envelope covering this body. (iii) The cell nucleus commonly lies between the chromatophores but is liable to be displaced towards the cell surface from time to time. (iv) Two other bodies (0·5–1·0 μ), one spherical and one rod-shaped, can sometimes be seen close to the nucleus. (v) One or more food vacuoles are often present which move about the body from the non-flagellar pole towards the region of the pyrenoids before their contents are digested. (vi) Usually two or three leucosin vesicles of small size, but sometimes as large as 3 μ, are confined to the non-flagellar half of the cell and stain rose-violet with cresyl blue, a reagent which also picks out (pale blue) the very small muciferous bodies scattered over the cell surface but especially numerous near the flagellar pole, and some tiny bright blue granules perhaps associated with them. (vii) Osmium tetroxide stains one or two bodies of unsaturated fat

Explanation of Plates VII–X

VII

Fig. 26. A further section from the series in Pl. VI, adjacent to Fig. 25. Mid 501.14, × 12,000.

Fig. 27. Enlarged detail from Fig. 26 to show the pyrenoid (*py*) and neighbouring structures more clearly, including part of nucleus (*N*) and a mitochondrion (*m*). Mid 501.15, × 20,000.

VIII

Fig. 28. Section of a cell with an undisturbed covering of scales and also showing the nucleus (*N*) and nucleolus; two obliquely cut flagella in the neighbourhood. S 71, × 26,000.

IX

Fig. 29. Section of a cell with an undisturbed covering of scales and also showing part of a leucosin cavity, muciferous bodies on the surface, two sections of flagella beneath the scales and therefore belonging to the cell, part of the nucleus, two fat bodies, mitochondria and other organelles also visible. S 104, × 16,000.

Fig. 30. Section of two detached scales and a flagellum to show relative sizes and shapes. S 21, × 30,000.

Figs. 31, 32. Two adjacent sections through a group of muciferous bodies. S 88 and S 78, magnification uncertain.

Fig. 33. Part of a section in the region of the flagellar bases. S197, × 20,000.

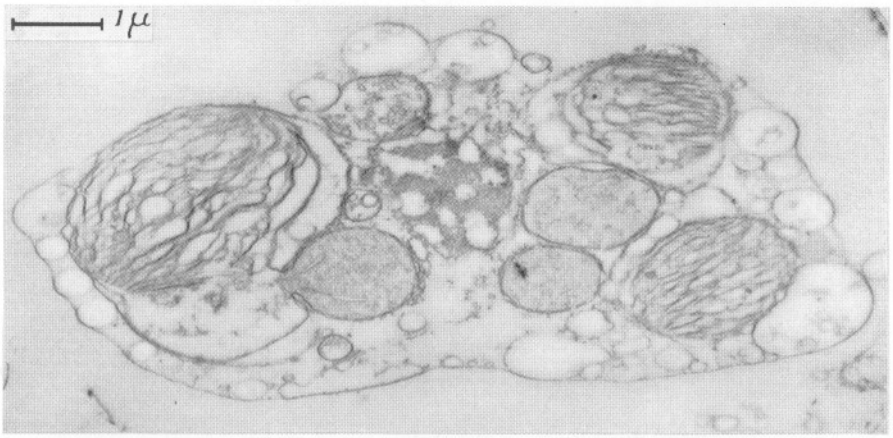
Fig. 34. Part of a section in the region of the flagellar bases, one in longitudinal section and the other in transverse section (arrow); adjacent organelles include leucosin cavity and muciferous bodies. S 202, × 28,000.

X

Fig. 35. A cell showing nucleus (*N*), two chromatophores, a food vacuole, a fat body and golgi vesicles. R 88c, × 10,000.

Fig. 36. A cell showing nucleus, parts of leucosin cavities, mitochondria, three chromatophores and golgi area. S 91, × 10,000.

Fig. 37. Details of the golgi area of Fig. 36 more highly magnified and placed at a different angle on the page; part of the nucleus (*N*) and two chromatophores (*c*) also present. S 92, × 30,000.



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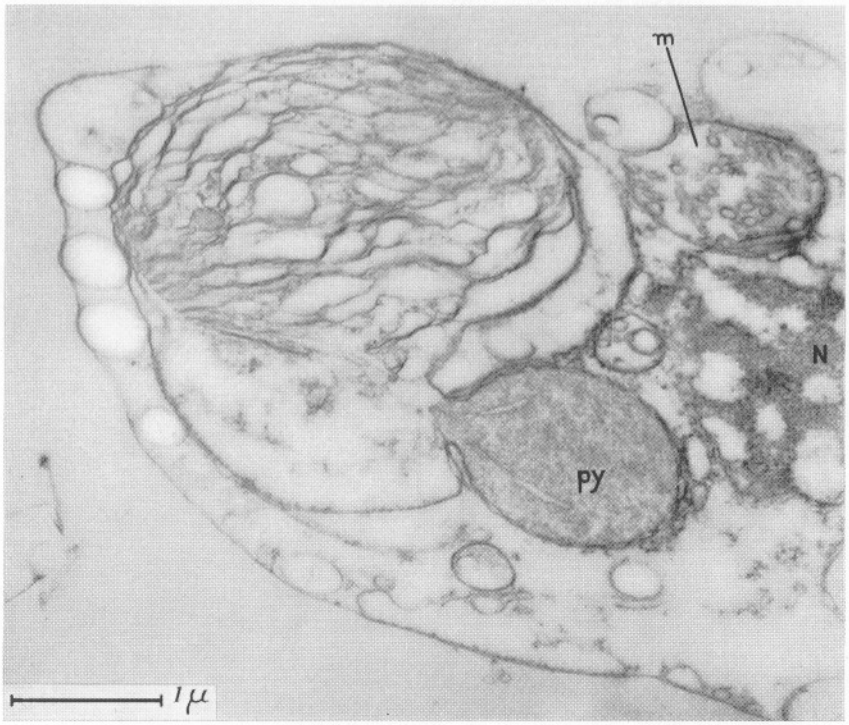

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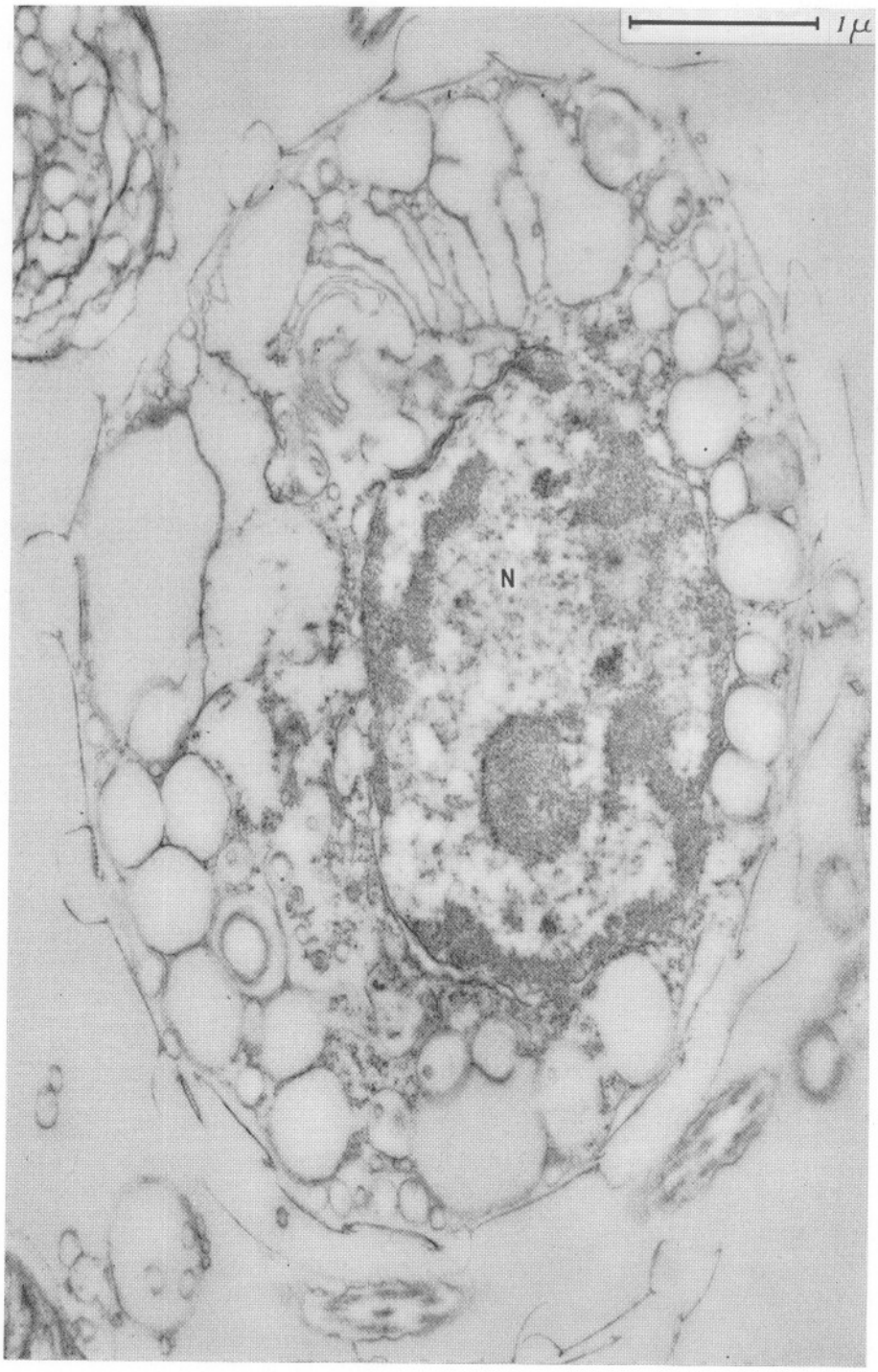

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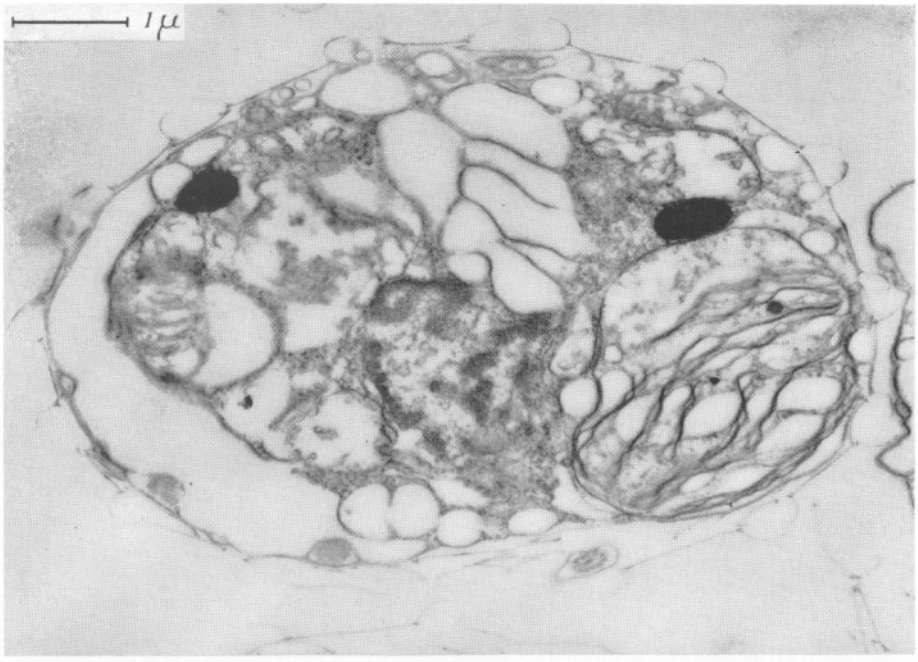

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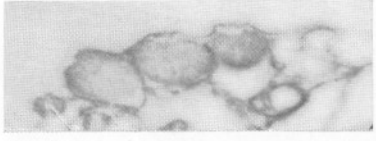

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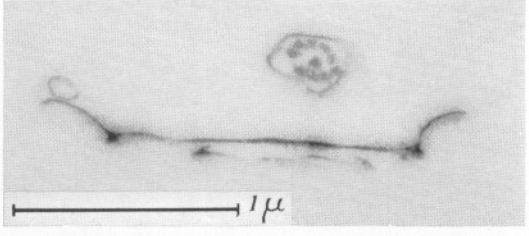

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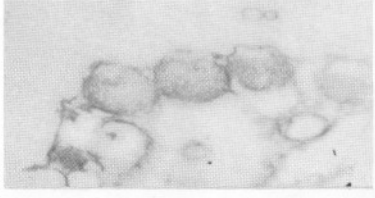

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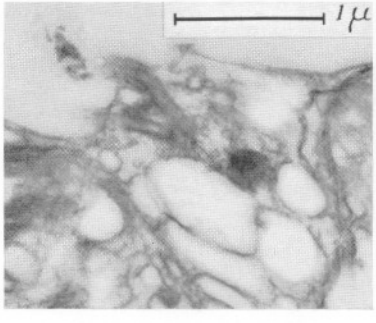

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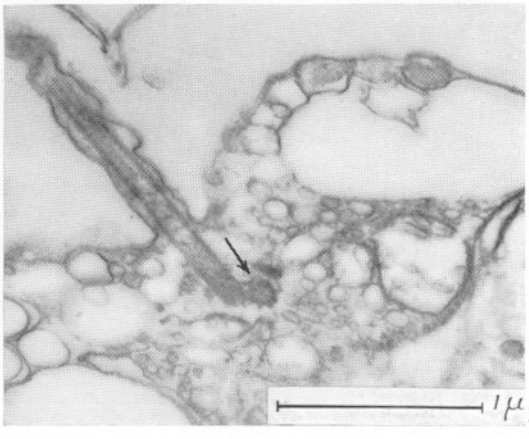

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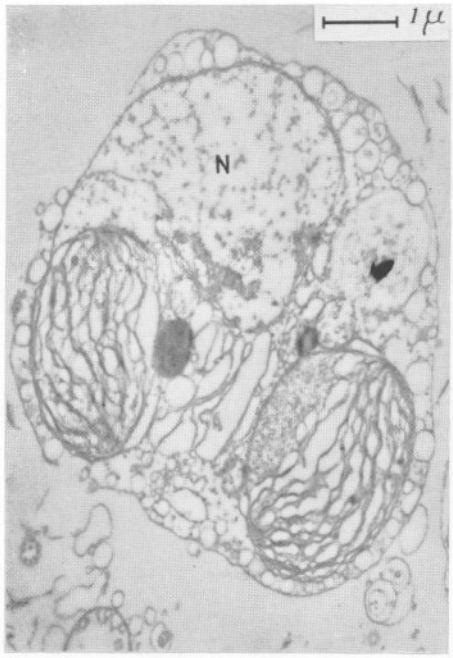

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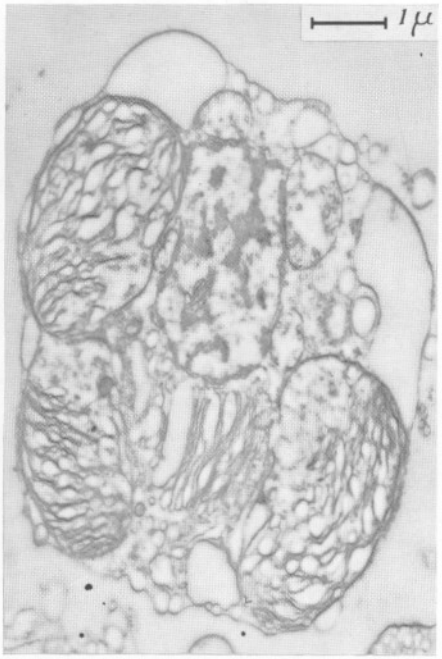

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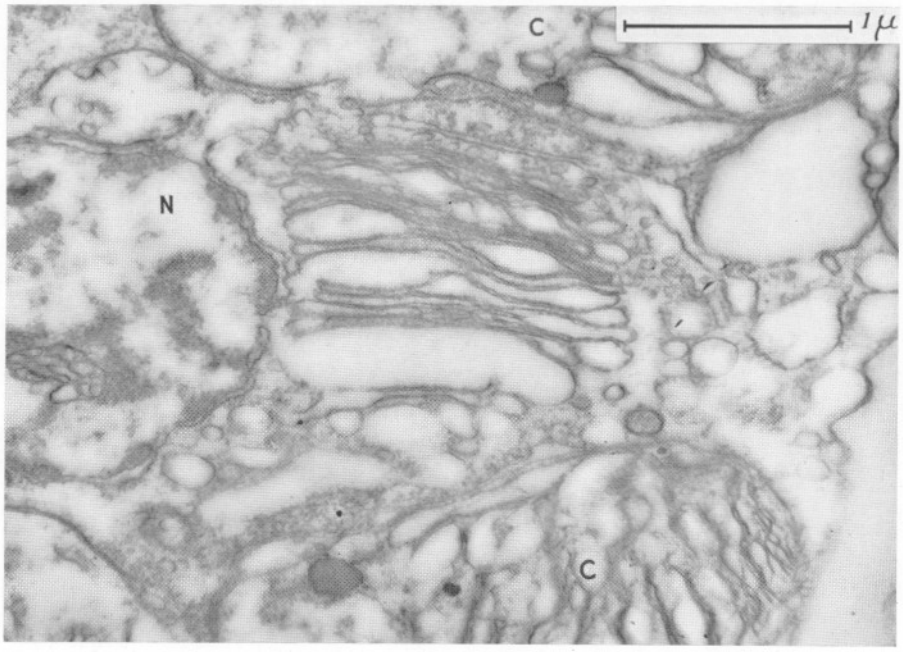

37

which occur in the cytoplasm. (viii) A number (of the order of 6–8) of granules of fairly uniform size (0·25 μ) stain with Janus green, which are probably mitochondria. (ix) Janus green also stains faintly one, or more rarely two, much larger bodies of the order of 1 μ in diameter situated near the flagellar pole (see above) and perhaps representing the golgi material identified with the electron microscope (see p. 221).

The positions of these various components in life are indicated in Figs. 1–7 (p. 212), though most of them can change with respect to the flagellar pole in the course of metaboly of the body.

CONTENTS OF THE BODY AS SEEN WITH THE ELECTRON MICROSCOPE

The criteria for recognizing the internal cell organs with the electron microscope are rather different from those available with the light microscope and many are much more precise. Observations on the living cells have, therefore, been of less help in the interpretation of the sections than might have been expected, but, conversely, it is probable that further study with the light microscope after the identity of the various components has been established might amplify knowledge of function and development. With our present interest concentrated on identification, the principal organs detected with the electron microscope will be briefly described.

The Nucleus

The nucleus is the most important organ to recognize at the outset since until this is done certain types of sections of it could easily be mistaken for those of other cell components. There is no such difficulty about a section such as that of Pl. VIII in which the nucleus is the only major organ visible. It contains a single nucleolus, and the rest of the nuclear volume is occupied by irregularly disposed light and dark masses. The darker material is usually aggregated near to the nuclear surface, sometimes very asymmetrically (cf. Fig. 35), and the size of the aggregates varies also, both types of difference being probably associated with different stages in the nuclear division cycle. Other, more confusing, differences are in the overall shape and position, which undoubtedly change with body metaboly. Thus in Fig. 35, Pl. X, the nucleus is very asymmetrical both in shape and position while that of Fig. 36 is of more normal appearance. Parts of tangential sections showing different views of the dense material in an undoubtedly resting nucleus are contained in Pls. VI and VII and in Fig. 29, Pl. IX.

A detail which cannot profitably be discussed at the present stage is that of the nuclear membrane. This is known to have a highly complex structure in all animals and plants in which it has been carefully examined. To study it effectively requires higher magnification than those used here, but attention

can be drawn to the part of the nuclear surface included in Fig. 37, Pl. X, and to the nucleus in Pl. VIII to substantiate the statement that in this as in other cells the interior of the nucleus is separated from the body of the cytoplasm not only by membranes but also by a translucent, apparently liquid, layer which, in any one section, appears to be discontinuous but which could equally be a single circum-nuclear vesicle ('cisterna' of Palade & Porter, 1954), or peri-nuclear space bridged at intervals.

Chromatophores

Larger than the nucleus and conspicuous in most sections are the chromatophores. These are very easily distinguished by their laminated structure, which is comparable to that of many other lower plants. As in *Synura* (Manton, 1955), Phaeophyceae (Leyon & von Wettstein, 1954; Manton, 1957*a*), etc., the laminae are compound, but they do not here fill the entire organ, much of which seems to consist of storage space. The nature of the storage product is unknown and only small traces of it are still present in most sections. Some granular material and a few osmiophilic vesicles are commonly present between the laminae (Fig. 27, Pl. VII), though the lipoid globules which stain so distinctly with Sudan IV on the fresh material are entirely dissolved by the reagents used for embedding and are only traceable as empty vesicles.

The pyrenoid

A very distinctive part of the chromatophore is the pyrenoid. When encountered in isolation a pyrenoid in the sense used here is a highly characteristic spherical body, 0·5–1 μ in diameter, containing dense granular material and surrounded by a system of compound membranes. In order to detect attachment to a chromatophore a very special plane of section is required, since there is only a narrow neck joining the two organs. However, a fortunate series of sections showing this feature in two separate pyrenoids in one cell is contained in Pls. VI and VII. The pyrenoid is apparently in continuity with the storage part of a chromatophore,[1] though the opacity of the contents is very different. We have no interpretation to offer about the other structural details, detectable especially in Fig. 27, Pl. VII.

Mitochondria

Fig. 27, Pl. VII, also contains a good example of a mitochondrion (labelled *m*). The microanatomy of these organelles is now so familiar that little need be said except to explain that the cylindrical villi which occupy the lumen in place of the cristae of many animal cells differ in no essential way from those of other examples in the lower plants, notably *Synura* (Manton, 1955), the brown algae (Manton & Clarke, 1956; Manton, 1957*a*) and *Vaucheria* (Greenwood, Manton & Clarke, 1957).

[1] See footnote on p. 224.

Golgi apparatus

A slightly less familiar component is illustrated in Pl. X, that has many points in common with the Golgi apparatus of certain animal cells and of some plants, notably *Scytosiphon* (Manton, 1957), wheat roots (Hodge, Martin & Morton, 1957), and *Sphagnum* (Manton, 1957). Attention has already been directed to the position of a compact group of vesicles immediately below the flagellar bases in Fig. 33, Pl. IX. Their characteristically flattened and compact arrangement as well as their position in relation to the nucleus and chromatophores are better displayed in Figs. 35 and 36, Pl. X. The additional details visible in the more highly magnified micrograph of Fig. 37 are in such striking agreement with those of many published micrographs of golgi in animals that the identification as the same organ here seems virtually unassailable unless on biochemical grounds which have not yet been investigated in this material.

Fat bodies

Unsaturated fat bodies, easily identifiable as such by their opacity following osmic fixation, are of common occurrence. They are variable in size and not very numerous; two are contained in Fig. 29, Pl. IX, and also in Fig. 35, Pl. X.

Leucosin

Leucosin in sections is always represented by empty spaces owing to its ready solubility in the alcohols used for dehydration; such spaces are very liable to mechanical distortion, but a demonstrably undistorted cavity bounded by a membrane and almost certainly of this nature is contained in Fig. 29, Pl. IX, beneath the undisturbed covering of scales; parts of very similar cavities are visible in Fig. 34, Pl. IX, and Fig. 36, Pl. X.

Muciferous bodies

The lenticular vesicles of slightly opaque material visible between the leucosin cavity and the covering of scales in Figs. 29 and 34 are the muciferous bodies. As surmised from their behaviour when seen discharging under the light microscope these are not merely amorphous drops or granules of material but organelles with a definite microanatomy. This has not been completely worked out, but from Figs. 29, 31, 32 and 34, Pl. IX, it is clear that each organelle is effectively a small-walled compartment with semi-opaque contents. There are signs of further subdivision of the contents, notably in the faint white internal contours in the central body of Fig. 31, and there is certainly anatomical complexity about the outer wall which cannot yet be defined, though characteristic views of relatively median sections can be studied in Figs. 31, 32 and 34. The apparently translucent thickening of the outer wall causes the contents of an undischarged organelle to appear

somewhat bean-shaped. Further information about the meaning of these structures is greatly to be desired.

The outer membrane

Covering other parts of the cell, beneath the scales, is a delicate outer membrane. This is demonstrable with special clarity in cells which have lost their scales and is visible in most of those illustrated in Pls. VI, VII and X.

Cytoplasmic vesicles

The most conspicuous of the other cell contents are the vast array of vesicles, most of them apparently empty, though doubtless not so in life, which crowd the cytoplasm between the other organs and which are specially conspicuous near the outside of the cell (Pls. VI and VII). They are probably of more than one kind but, though none is as yet chemically identifiable, they resemble at any rate superficially the cytoplasmic vesicles commonly encountered in other cells of the lower plants, cf. *Scytosiphon* (Manton, 1957*a*).

Food vacuoles

Food vacuoles are distinguishable from other cytoplasmic vesicles by their irregular contents, which sometimes include recognizable objects such as ingested scales. One food vacuole with rather indefinite contents is present in Fig. 35, Pl. X.

Cytoplasmic granules

Finally there are cytoplasmic granules. Since extreme metabolic significance has been attributed to certain types of granules (cf. Palade, 1956, etc.), it is important to notice their presence here even though we have no chemical data to add. Granular cytoplasm fills the interstices between vesicles and other cell organs in all parts of the cell. It may be seen specially clearly in Fig. 29, Pl. IX, and Fig. 37, Pl. X.

BEHAVIOUR OF THE LIVING CELL AND REPRODUCTION

Chrysochromulina chiton shows a marked phototactic response, although there is no obvious stigma and so far no cells lacking chromatophores have been observed.

During swimming the haptonema and flagella are directed backwards, the haptonema usually coiled up close to the body (Fig. 1), but sometimes partly or fully extended. Movement can be fairly rapid for short periods, the cells swimming in straight lines rotating slowly with a jerky movement (as if flinging one flagellum round—probably due to excentric insertion on body). The cells are rarely seen moving with flagella and haptonema in front of the body. A characteristic of this species is a gliding movement without rotating,

with flagella held behind body and with haptonema coiled or extended (Fig. 4). As swimming or gliding slows down, the flagella lie further apart from each other.

Quite long periods of anchorage are common. When anchored the haptonema can be extended to any degree from almost completely coiled to fully extended (Figs. 3 and 7). It is, however, rarely entirely straight (Fig. 4); when extended backwards during swimming it is commonly curved at the tip and when attached it is most commonly bent or slightly coiled (Figs. 2, 3 and 5–7).

Phagotrophy is of very common occurrence, the cells ingesting organisms up to about 3 μ in size (Figs. 1–7). The presence of three food vacuoles in one cell at the same time is not uncommon, but one or two is more usual. The method of ingestion is similar to that described for *C. ericina* (1956, p. 397) and the method of discharge of the contents of the muciferous bodies is as described for *C. kappa* (1955, p. 589).

Reproduction of the motile stage of *C. chiton* is similar to that described for *C. kappa* (1955, p. 592), but the lop-sided movement recorded for *C. chiton* is particularly accentuated in incipient fission stages. Double fission stages are more rarely seen in this species than in *C. kappa*. Cultures of *C. chiton* reach a density of $1\frac{1}{2}$–2 million cells/ml. at the peak of growth. Non-motile stages similar to those already described for the other species (1955, 1956) are then produced, but in this species the scale covering of the motile cell appears to remain fairly intact except for a pore through which the contents have come out and masses of these empty 'skins', clearly visible under the light microscope, can be picked up from the bottom of a flask. The large amoeboid and walled cells, both with finely lobed chromatophores, measure from 7×12 to $9 \times 13\,\mu$, while the daughter-cells with smooth thin walls and fairly finely lobed chromatophores measure from $2{\cdot}5 \times 3$ to $3{\cdot}5 \times 5\,\mu$.

DISCUSSION

Most of the more obvious comparisons with other organisms have already been made in passing, such comparisons being necessarily limited at present by the relative scarcity of plant cell types which have been effectively studied by these methods.[1] The most fruitful external comparison at the present stage probably concerns the pyrenoid. This problematical body, or something closely resembling it, has been recognized by light-microscopists in several different groups of non-starch producing organisms (for literature see Fritsch, 1935; Chadefaud, 1936; Chadefaud & Feldmann, 1949; Magne, 1954; etc.),

[1] A paper (Fauré-Frémiet & Rouiller, 1957) which is closely relevant to the present inquiry has reached us too late to be discussed in the text. The authors have investigated a species of *Chromulina* by means of thin sections with the electron microscope. *Chromulina* differs characteristically from *Chrysochromulina* in the external appendages and in the presence of an eyespot but shows many points of resemblance in the other body organs.

always as a body external to the chromatophores though possibly connected to them, and characterized by negative staining reactions when tested by most of the ordinary reagents giving diagnostic colouring. Demonstration of organic attachment to an adjacent chromatophore by a long slender neck has been claimed for *Ochrosphaera neapolitana* (Chrysophyceae) by Schwarz (1932) and Magne (1952). The clarity with which the electron microscope has now revealed, in *Chrysochromulina chiton*, the attachment of the pyrenoids to adjacent chromatophores by a short constricted neck makes this one of the most definite facts which has so far been ascertained about such pyrenoids. The probability is thus greatly increased that they are in fact storage organs concerned with some product of photosynthesis, which the original choice of the name pyrenoid necessarily implies. A resemblance to the 'plastid diverticulum' recently described (Manton, 1957*a*) in the zoospores of the brown alga *Scytosiphon* is also perhaps of importance to note.[1]

Our ignorance of the chemical nature of the contents of pyrenoids only emphasizes what is in fact the most serious gap in our total knowledge of these organisms, and one which requires methods quite other than straight microscopy, namely the general problem of the gross cytochemistry of the cell as a whole. Except for the leucosin, practically nothing is known about the chemical nature of any of the storage products which appear in abundance in various parts of the cell. If this gap could be filled progress in other matters would undoubtedly follow.

An outstanding problem which is both chemical and developmental concerns the structure and mode of origin of the scales, which at present is difficult even to imagine. That there is a delicate cytoplasmic membrane underlying them is a fact which in itself is less explanatory than might at first be thought. The observations on the membrane have necessarily to be made with fixed material, yet we know that in life the cell can engulf solid food through its surface in spite of the covering of scales, etc. The physical nature of the cell surface cannot, therefore, be fully deduced from the anatomical evidence, and we are equally ignorant of the way in which it may perhaps change with time. There are no data, for example, to indicate whether the scales are produced cyclically following a related change in the cell surface prior to their production, or whether they are in fact being produced all the time as a normal process of growth from the surface as we commonly see it. Several details about the shape and arrangement of the scales are difficult to reconcile with the latter view, though anatomy alone is an insufficient guide in such a question.

Purely anatomical information can, however, still add much to our know-

[1] Since this was written further information on pyrenoid structure in the Chrysophyceae is available from two sources. Hovasse & Joyon (1957) describe pyrenoids immersed in the structure of the chromatophore in a species of *Hydrurus*, and we have found a similar condition in some undescribed species of *Chrysochromulina*. The special condition of *C. chiton* is therefore not universal in the group.

ledge of the haptonema, though this must be ascertained comparatively through a range of species before final generalizations can be made. We have shown in *C. chiton* that the anatomical differences from a flagellum are at least as great as had been surmised from their very different behaviour and appearance. We do not, however, yet know the extent of uniformity or diversity within the genus, and in exploring this it is reasonable to hope that our present ignorance about the structure of the extremities, about the mode of attachment to the cell, and, more importantly, about the mechanical principles involved in its movements may also, with further work, be dispelled.

FORMAL TAXONOMIC DIAGNOSIS

Chrysochromulina chiton sp.nov. Parke & Manton

Motile cells showing some metaboly, sphaeroidal to ovoid with a flattened flagellar pole depressed centrally across one axis, 5–8 (exceptionally 4–10) μ in diameter. Two flagella and one haptonema arising close together slightly off-centre from the depression; the flagella subequal, or of equal length, homodynamic, $2\frac{1}{2}$–$3\frac{1}{2}$ times body length, smooth, apex gradually attenuated to a small knob (E. M. observation); the haptonema, thinner than the flagella, 4–5 times body length when fully extended, with a club-shaped tip and an internal structure of three concentric membranes surrounding a ring of seven 'fibres'. The very thin periplast of a pectic nature covered by very thin, transparent, oval, two-layered, sculptured, exceptionally large, dimorphic scales, visible when dry under the light microscope, details visible only under the electron microscope. Large scales oval, bipartite, saucer-shaped, with a wide rim delimited from an oval centre by a ridge, $1{\cdot}9 \times 2{\cdot}4$ to $2{\cdot}2 \times 2{\cdot}9\,\mu$, with the base showing a radiating pattern of ridges on the surface towards the body, the outer surface patternless. The small scales, round to oval, $0{\cdot}7 \times 0{\cdot}9$ to $1{\cdot}1 \times 1{\cdot}4\,\mu$, with the relatively patternless rimmed face away from the body and the face with the pattern of radiating ridges towards the body. Scale distribution a single layer over the body with small scales filling interstices between large.

Cells uninucleate, no stigma. Chromatophores appearing striated, usually two or four, occasionally one or six, golden brown; in motile phase usually ellipsoid or oblong, parietal, with a single globular body (pyrenoid) attached by a constricted neck to inner face near one margin slightly nearer to the non-flagellar pole than the flagellar pole; in non-motile phase deeply lobed or stellate. Lipoids and leucosin produced. Ejectile muciferous bodies generally distributed in peripheral cytoplasm but usually more numerous at flagellar pole. Nutrition phototrophic and/or phagotrophic. Non-toxic to fish.

In motile phase asexual reproduction by fission into two, three or occasionally four daughter-cells of equal or unequal size; in non-motile phase reproduction (asexual?) by successive fission of amoeboid cells to produce four daughter-

cells with thin smooth walls; motile phase probably liberated from walled daughter-cells through a pore.

Isolated by Miss Dorothy Ballantine from sea-water samples collected by her during cruise, May 1955.

Habitat. The sea at position lat. N. 47° 36′, long. W. 04° 18′ (23 May 1955, Plymouth no. 146, type culture) at 50 m; at position lat. N. 47° 44′, long. W. 07° 11′ (17 May 1955, Plymouth nos. 149, 150) at 5 m; at position lat. N. 50° 13′, long. W. 04° 22′ (29 May 1956) at surface; at position lat. N. 50° 09′, long. W. 04° 16′ (19 June 1957, very fine tow-net sample); at position lat. N. 50° 02′, long. W. 04° 22′ (16 July 1957) at surface; at position lat. N. 50° 11′, long. W. 04° 14′ (24 July 1957) at surface; at position lat. N. 50° 02′, long. W. 04° 22′ (17 Sept. 1957) at 5 m, 10 m, 20 m and 50 m, and at position lat. N. 50° 11′, long. W. 04° 14′ (15 Oct. 1957) at surface. Type culture deposited with the Culture Collection of Algae and Protozoa, Cambridge.

Cellula motilis aliquanto formam mutans, sphaeralis vel ovalis; extremitate, qua inserta sunt flagella, centraliter depressa trans alterem axem; lata 5–8 μ (rare 4–10 μ); duobus flagellis sed et uno haptonemate conjunctim exorientibus paululum remotis depressione centrali; flagellis subaequis vel aequis, homodynamicis, longioribus $2\frac{1}{2}$–$3\frac{1}{2}$plo quam cellula, teretibus, apicibus paulatim attenuatis sed terminatis minutis bulbis (ut videtur per microscopiam electronicam); haptonemate teneriore quam flagellis, longiore 4–5 cellula maxime extenso, extremitate claviformi; haptonemate induto 3 membranis concentricis, 7 filamentibus axialibus dispositis in annula ut videtur in sectionibus transversis; periplasto tenuissimo, pectico natura, induto tenuissimis, diaphanis, ovalibus, sculptis squamis, grandibus praeter modum, duobus stratis compositis et sub duabus formas existentibus, visibilibus siccis per microscopiam photicam, minuta structura tamen obscura nisi per microscopiam electronicam; squamis majoribus ovalibus, bipartitis, crateriformibus, longis 2·4–2·9 μ, latis 1·9–2·2 μ, lata margine circumscripta ab ovali areola centrali per angustum jugum, cuius areolae aspecto interno, contra cellulae corpus, sculpto radiantibus rugulis, aspecto externo sine rugulis; squamis minoribus circularibus vel ovalibus, longis 0·9–1·4 μ, latis 0·7–1·1 μ, aspecto interno cum radiantibus rugulis, aspecto externo cum jugo circumscribente, sed rugulis relative defectis; squamis distributis unico strato per superficiem cellulae, minoribus replentibus interstices inter majoribus. Nucleo unico, stigmate defecto; chromatophoris apparente striatis, generaliter 2 vel 4 (rare 1 vel 6), aureobrunneis; in cellulis ad statum motilem chromatophoris generaliter ellipsoidalibus vel oblongis, parietalibus, unico corpore pyrenoidali globulari affixo per isthmum angustum cuique chromatophoro, prope marginem, paululum propiore extremitatem cellulae qua inserta sunt flagella; in cellulis ad statum immotilem chromatophoris profunde lobatis vel stellatis; cellula materias lipoidales et leucosinum producens; ejectilibus corporibus muciferis diffuse distributis in cytoplasmate superficiali, generaliter tamen abundantioribus ad extremitatem cellulae qua inserta sunt flagella. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Cellula ad statum motilem generans per fissionem ad 2, 3 vel rare 4 cellulas filiolas producendas, magnitudine paribus vel disparibus; cellula ad statum immotilem generans (? asexuale) per fissiones sequentes cellularum amoeboidalium ad 4 cellulas filiolas producendas cum parietibus tenuibus et teretibus; ex quibus cellulis filiolis, cellula ad statum motilem per porum liberata (quod maxime potest).

ACKNOWLEDGEMENTS

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SUMMARY

The description of a new species of the genus *Chrysochromulina*, *C. chiton*, includes for the first time some evidence from the electron microscopy of sections in addition to the techniques previously used. Important diagnostic characters are the exceptionally large scales, which are shown to be of two kinds, the larger being saucer-shaped, and the smaller plate-like with a narrow rim flexed towards the upper surface; the structure and arrangement of the two types of scale on the body are described. Anatomical facts are given for the first time for the internal structure of the haptonema; in this species this organ consists of three concentric membranes surrounding a ring of seven fibres or tubes, the centre of the haptonema being hollow; this combination of characters distinguishes the organ fundamentally from a flagellum. Micro-anatomical facts are also given for the following major cell organs: the nucleus, the chromatophores including the pyrenoids, mitochondria, putative golgi material, muciferous bodies, the flagellar bases (preliminary observations only). Other distinguishable cytoplasmic components include the surface membrane, unsaturated fat bodies, vesicles of various kinds and granular protoplasm in the interstices.

REFERENCES

CHADEFAUD, M., 1936. Le Cytoplasme des algues vertes et des algues brunes. Ses éléments figurés et ses inclusions. *Rev. algol.*, T. 8, pp. 5–286.

CHADEFAUD, M. & FELDMANN, J., 1949. Sur une Coccolithophoracée des bacs de l'aquarium de la Station biologique de Roscoff. *Bull. Mus. Hist. nat., Paris*, Sér. 2, T. 21, No. 5, pp. 617–21.

DOFLEIN, F., 1922. Untersuchungen über Chrysomonadinen. I. *Ochromonas granularis* Dofl. *Arch. Protistenk.*, Bd. 44, pp. 147–205.

FAURÉ-FRÉMIET, E. & ROUILLER, C., 1957. Le flagelle interne d'une Chrysomonadale: *Chromulina psammobia*. *C.R. Acad. Sci., Paris*, T. 244, pp. 2655–2657.

FRITSCH, F. E., 1935. *The Structure and Reproduction of the Algae*. Vol. I, 791 pp. Cambridge University Press.

GREENWOOD, A. D., MANTON, I. & CLARKE, B., 1957. Observations on the structure of the zoospores of *Vaucheria*. *J. exp. Bot.*, Vol. 7, pp. 71–86.

HODGE, A. J., MARTIN, E. M. & MORTON, R. K., 1957. The structure of some cytoplasmic components of plant cells in relation to the biochemical properties of isolated particles. *J. biophys. biochem. Cytol.*, Vol. 3, pp. 61–70.

HOVASSE, R. & JOYON, L., 1957. Sur l'ultrastructure de la Chrysomonadine *Hydrurus foetidus* Kirchner. *C.R. Acad. Sci., Paris*, T. 245, pp. 110–13.

LACKEY, J. B., 1939. Notes on plankton flagellates from the Scioto River. *Lloydia*, Vol. 2, pp. 128–43.

LEYON, H. & WETTSTEIN, D. VON, 1954. Der Chromatophoren-Feinbau bei den Phaeophyceen. *Z. Naturf.*, Vol. 9 B, pp. 472–5.

MAGNE, F., 1952. Sur la cytologie d'une Chrysophycée marine vivant en aquarium, *Ochrosphaera neapolitana* Schussnig. *Rev. gén. Bot.*, T. 59, pp. 231–310.

—— 1954. Les Chrysophycées marines de la Station biologique de Roscoff. *Rev. gén. Bot.*, T. 61, pp. 389–415.

MANTON, I., 1955. Observations with the electron microscope on *Symura caroliniana* Whitford. *Proc. Leeds phil. lit. Soc.*, Vol. 6, pt. 5, pp. 306–316.

—— 1957*a*. Observations with the electron microscope on the internal structure of the zoospore of a brown alga. *J. exp. Bot.*, Vol. 8, pp. 294–303.

—— 1957*b*. Observations with the electron microscope on the cell structure of the antheridium and spermatozoid of *Sphagnum*. *J. exp. Bot.* (in the Press).

MANTON, I. & CLARKE, B., 1956. Observations with the electron microscope on the internal structure of the spermatozoid of *Fucus*. *J. exp. Bot.*, Vol. 7, pp. 416–432.

PALADE, G. E., 1956. Electron microscopy of mitochondria and other cytoplasmic structures. *Henry Ford Hospital Symposium on Enzymes*, pp. 185–215. New York: Academic Press.

PALADE, G. E. & PORTER, K. R., 1954. Studies on the endoplasmic reticulum. I. Its identification in cells in situ. *J. exp. Med.*, Vol. 100, pp. 641–56.

PARKE, M., MANTON, I. & CLARKE, B., 1955. Studies on marine flagellates. II. Three new species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 579–609.

—— —— —— 1956. Studies on marine flagellates. III. Three further species of *Chrysochromulina*. *J. mar. biol. Ass. U. K.*, Vol. 35, pp. 387–414.

SCHWARZ, ELISABETH, 1932. Der Formwechsel von *Ochrosphaera neapolitana*. *Arch. Protistenk.*, Bd. 77, pp. 434–62.

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THE BEHAVIOUR OF *LITTORINA LITTOREA* (L.) UNDER NATURAL CONDITIONS AND ITS RELATION TO POSITION ON THE SHORE

By G. E. NEWELL
Queen Mary College, University of London

(Text-figs. 1 and 2)

The four common species of British littorinids are all browsers (mainly on algae), and each characteristically occupies a wide but fairly well-defined zone relative to the tide marks. *Littorina littorea* is a normal and often common member of the gastropod population of the middle shore wherever rock or pebbles are found, and also occurs in numbers on wooden structures such as piers and groynes. More rarely is it found on sandy or muddy shores, particularly if they are sheltered and poorly drained. Only occasionally does it become established above H.W.N., whilst it decreases in numbers below E.L.W.S., even where there is good holding ground. This much is well known, but reasons have been given (Smith & Newell, 1955) for believing that on the shore at Whitstable individual winkles tend to remain faithful to the particular beach level which they adopted during the first year of life after larval settlement.

In contrast to truly sedentary animals, such as barnacles and most lamellibranchs, winkles move about, and were their movements completely at random this constant pattern of zonation would in time be upset. (This must apply not only to littorinids, but to all intertidal animals with broadly similar habits.) An inspection of any shore at low water gives the impression after the tide has receded that the vast majority of animals are settled in situations which best protect them from desiccation, extremes of temperature, insolation and other adversities, but predators and browsers must leave such positions in order to obtain food. Yet, with a few notable exceptions the feeding excursions have not been studied in detail. The present investigation was designed to show how winkles maintain their pattern of zonation, and was done partly on the shore at Whitstable and partly in the laboratory at Queen Mary College.

THE DISTRIBUTION OF WINKLES ON THE SHORE AT WHITSTABLE

The distribution of the winkle population shows no general relation to gravity, light, tidal level or beach slope. Nor does desiccation, itself correlated with time of exposure and tidal level, seem obviously to affect the density of the population. Some winkles are found on top of stones, others at the sides of stones and others again on the vertical surfaces of groynes. It is true that those winkles on vertical surfaces always come to rest with their anterior ends uppermost, but on stones an apparently random orientation prevails.

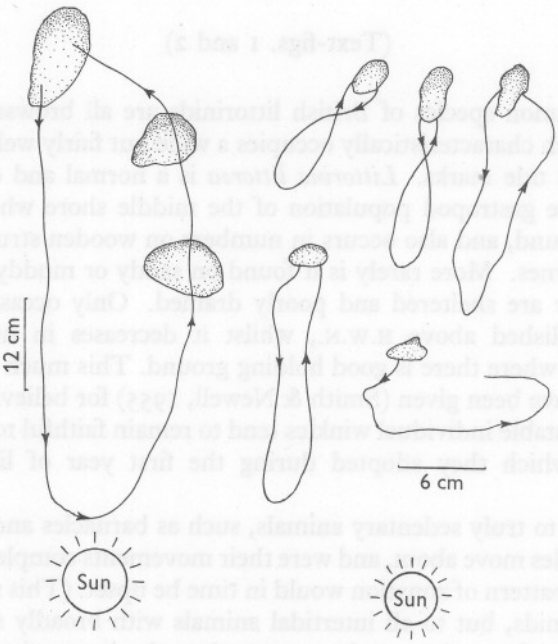

Fig. 1. Selection of tracks made by winkles crawling in the sand near mid-tide level in bright sunlight. September 1956.

Although winkles seem to be distributed in an apparently random way if broadly viewed, they can be noticed to aggregate and form clusters, especially at the junction of two surfaces as, for example, where planking joins a post on a groyne, and also in pools, where winkles are obviously more densely congregated than in drier situations.

On certain parts of the beach, patches of fine sand are interspersed with pebbles and these serve to record the tracks made by winkles when crawling. Such tracks are roughly U-shaped and can often be seen to radiate out from and back to stones on which the animals have settled.

On the vertical surfaces, such as the planking of groynes, winkle tracks are less obvious but, under favourable conditions, they can be seen to take the form of a downward, horizontal and upward limb. In this they resemble the U-shaped tracks in the sand.

During the summer months, when winkles are most active, the tracks in the sand give no clear indication of uniform orientation. Indeed, they appear to be completely random, but, as will be shown later, this appearance is misleading and the apparent randomness of their direction is attributable to the difficulty of following by eye alone a maze of criss-crossing tracks. Yet the very fact that all tracks pursue a fairly straight course for some considerable distance is, in itself, suggestive of orientated responses. The vast majority (roughly 95%) of these tracks consist of an outward and a homeward limb and some that appear to consist of a single limb only may be of the same general form, one limb having been obliterated by wavelets if crawling had begun before the tide had left the zone completely. Examples of some of these tracks are shown in Fig. 1. A few winkles pursue a fairly steady course and show no reversal of direction, but the reason for this is not known.

When crawling on stones winkles can be observed to feed by rasping off organic surface deposits such as diatoms and small algae. They will also browse on *Ulva* and *Enteromorpha*. Indeed, certain areas of groynes are kept clear of *Enteromorpha* by this means, and it must be supposed that any winkle quickly exhausts the food supply in the immediate vicinity of the place on which it has settled.

THE MAINTENANCE OF ZONATIONAL POSITION

Gowanloch & Hayes (1926) presented evidence, from observations on marked specimens, that winkles migrate back to the particular level on which they normally dwelt, after displacement to other situations, whilst Moore (1936), who marked winkles with cellulose paint with a view to following the growth rates of particular specimens, was able to re-collect many of these quite considerable periods after their replacement in roughly the same area. Many of Gowanloch & Hayes's specimens got lost and their results are somewhat equivocal, so that further observations are desirable, particularly since they state that winkles seem to move a few metres at each tide—a view which conflicts with observations recorded in the present paper.

The following observations are typical of several which have been made on the shore at Whitstable, and they show that, if left undisturbed, winkles will return to similar but not necessarily identical positions, and that if removed from the place where they have settled, their chances of getting back home are reduced.

Ten winkles, clustered around a hexagonal nut joining the planking to a wooden groyne post at about the level of H.W.N., were each marked with a dab

of yellow enamel before the tide reached them at 13.15 h on 25 September 1956. Twenty-four hours later, i.e. after two tides, five winkles were in the same position, two were within 1 ft. on the planking whilst three could not be found. On 27 October, 1 month later, five were still within 4 yd. of the nut and of these, one was still on the nut itself. On 28 December 1956, one winkle was still on the nut, three were within 5 yd. on the planking whilst the rest could not be found. In short, these observations show that winkles remain restricted to a particular situation, and it must be stressed that the situation studied was one in which the winkles were in a most unstable state and were subject to severe wave action—far more severe than if they had been on horizontal surfaces.

Twenty winkles were clustered one above the other along the junction of a vertical post with the planking of a groyne. Of these, ten were left undisturbed and were marked with a spot of blue paint, whilst ten were lifted and marked with a spot of yellow as well as blue paint and then placed in the muddy sand 2 ft. away from the base of the groyne. After 24 h, i.e. two tides later, of the undisturbed winkles three were in exactly the same place, two were in a similar situation but on the next post, two were at the base of the groyne whilst three could not be found. Of those which had been removed by 2 ft. from their place of settlement, two were in the same place, two in a corresponding position on the next post and six could not be found. After 4 weeks only five winkles marked with a spot of blue paint could be found, but all were within 5 yd. of their original position. None with yellow and blue spots were found. This shows that displacement from the site of settlement tends to decrease the chances of a winkle getting back to its original position.

It must be realized that under natural conditions winkles are continually liable to displacement by wave-action, but that this is usually limited to the time at which waves are breaking over the zone on which they have settled, and once they have been covered by the advancing tide wave-action is too slight to dislodge them except on very steep parts of the shore. Winkles settled on very dry situations and adhering merely by the adhesive action of a film of dried mucus instead of by the foot are particularly liable to be dislodged, but there is reason to suppose that those animals sticking to stones or groynes by the adhesive action of the foot are not often detached from their place of settlement. This has been confirmed by numerous observations on the shore. It is also clear from considerations of the relation of the efficiency of wave-action relative to beach-face slope (Newell, 1954) that high-level winkles are more liable to displacement by waves than those on the flatter slopes below H.W.N. Yet the previous results (Smith & Newell, 1955) show that the exchange of winkles between beach levels is strictly limited, whilst observations on marked winkles moved to different situations gave no evidence that the animals return to the particular level from which they were collected.

Before the tide has ebbed to its finality, practically all winkles are at rest.

Those on groynes or on dry stones settle down almost as soon as the water leaves them, but those in pools crawl about for periods of up to about 3 h after the tide has receded. But eventually even those left in situations where they are permanently covered by water become quiescent, as is shown by an observation of 14 September (a time of the year when winkles are still very active), when out of forty-five winkles in a shallow pool near mid-tide level, forty-three had settled down $5\frac{1}{2}$ h after full tide. Yet, as soon as the flood tide reaches them, all the winkles begin active crawling once again.

These observations suggest that winkles remain stationary during the greater part of each tide even when they are submerged, whereas Haseman (1911) believed that they crawled continuously 'at random' when covered by water and that their activities ceased only when they were out of water on dry surfaces, a view which is implicit in the writings of others, for example Kanda (1916) and Barkman (1955). It would, of course, be most interesting to follow the movements of individual winkles for some time after the tide had covered them, but this is practically impossible in the turbid water at Whitstable. Nevertheless, results, which are probably comparable to those which would have been obtained under natural conditions, can be arrived at by a study of winkles in a tank with a fluctuating water level.

ADHESION

Winkles on vertical or steeply inclined surfaces almost invariably come to rest with their anterior ends uppermost. After some time of exposure many of them secrete a film of mucus (as noted by Wilson, 1929) around the rim of the shell and this hardens sufficiently to support the animal's weight. Then the foot is withdrawn and the operculum closed so that the animal is in no danger of rapid desiccation. Wilson noted that a mere puff of wind will topple winkles from the rocks to which they are stuck by mucus, and further states that those which come to rest with the head downwards always topple off when the foot retracts. I have been unable to confirm this last observation of Wilson's. Indeed, on the contrary, as can easily be demonstrated, winkles are far more unstable on sloping surfaces when orientated head-uppermost than when the head is downwards. This is at first sight somewhat surprising, but this very instability has survival value in that, should any winkle come to rest above the high water mark, the slightest disturbance will cause it to roll down to a level which will be reached by the next tide and so avoid the possibility of not being wetted, possibly, until a fortnight later.

CRAWLING AND FEEDING

When a winkle is about to begin to crawl, the foot and head are protruded through the shell aperture and the operculum on the metapodial region is turned upwards and backwards above the mesopodial region so that the

creeping sole of the foot can be applied to the substratum. When crawling on either a vertical or a horizontal surface winkles continually 'test' it with their tentacles, which are extended obliquely outwards from the head, but not at any constant angle for they are in almost continual movement, first the right and then the left feeling the substratum. At first sight it would seem that these bending movements of the tentacles, on which it will be remembered the eyes are borne, would preclude the possibility of the animals maintaining any constant orientation to light, but observations made it clear that only that part of the tentacle distal to the eye is involved in the bending and so the eyes maintain a steady position during crawling (Fig. 2). The degree of extension of the head is such that the eyes, placed about one-third of the way up the tentacles, are a short way in front of the pigmented edge of the mantle, which, with the shell, prevents light from the rear from entering them.

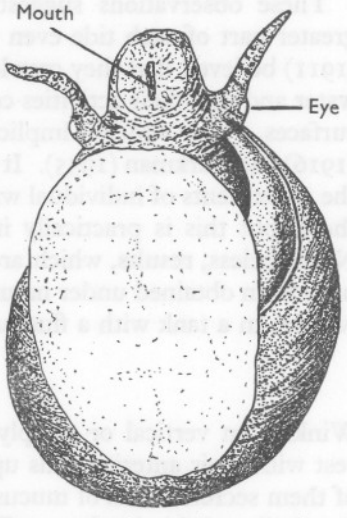

Fig. 2. Winkle crawling on a glass plate—viewed from below.

It can also be noticed that when crawling the animal makes feeding movements, the radula being alternatively protruded and retracted about once every second.

The rate of crawling on wet sand, as determined by an average of twelve specimens on 14 September 1956, was 2 cm/min, which is in very good agreement with the figures of Gowanloch & Hayes (1926) who give an average rate of crawl on rock surfaces as 1·3 m/h which equals 2·1 cm/min. It was found, however, that in the laboratory, either on vertical or horizontal glass plates, winkles can attain a rate of crawl of 10 cm/min. The rate of crawling seems, in part, to depend on the temperature (see p. 236).

In bright light active winkles always respond to a sudden decrease in light intensity by a rapid but momentary withdrawal into the shell. That is, they respond in the same manner as to any other sudden change in the environment, but the withdrawal response to shadows quickly wanes.

FACTORS INFLUENCING THE SEASONAL ACTIVITIES OF WINKLES

So far the account of the behaviour of winkles on the shore at Whitstable has been confined to that seen in the autumn, which is substantially true for the spring and summer months also. But from November onwards, through the winter until March, the winkle population is largely inactive. For example,

on 17 November 1956, a dull, calm day, practically no winkle tracks were discernible. On the following day, with the sun showing as a clear disc through the mist, only one winkle was seen to be moving after the tide had receded. The same inactivity on the shore prevailed throughout December and January, but during the exceptionally warm February of 1957 winkle tracks were again apparent, although in nothing like the profusion noticed in the early autumn of 1956.

Observations of this kind suggest that winkles become inactive when the light intensity and temperature are low, and it is possible that these environmental factors of the winter act either singly or in combination. It is of interest in this connexion that Moore (1936) found that the growth rates of winkle populations in the Plymouth neighbourhood slowed down from October to February.

A few laboratory results will be quoted to show that both low light intensity and decreased temperatures lower the level of activity.

VARIATION IN RATE OF CRAWLING UNDER DIFFERENT LIGHT INTENSITIES

Individual winkles collected from horizontal surfaces and dark-adapted for 6 h were tested for rate of crawling under varying conditions of light intensity. These were obtained with an overhead light and a rheostat. The results are set out below. It will be seen that winkles behave orthokinetically in response to strong variations in light intensity, and it is likely that this has some importance in stimulating the animals to crawl and feed (Table 1).

TABLE 1. DISTANCE (CM) CRAWLED IN 5 MIN AT 19° C

Winkle number	At light intensity of 10 f.c.	3000 f c.
1	22·0	27·0
2	22·5	30·0
3	17·2	24·3
4	16·0	17·0

VARIATIONS IN RATE OF CRAWL AT DIFFERENT TEMPERATURES

Individual winkles collected from horizontal surfaces and dark-adapted for 6 h were tested for rate of crawling at different temperatures (Table 2).

In each instance increase in temperature induced an increased rate of crawl. Further experiments were, therefore, carried out to determine the temperature at which winkles become immobilized.

Twelve winkles from vertical surfaces were placed in a dish of sea water at 0° C and illuminated from above at an intensity at the water surface of 10 f.c. All remained completely inactive for 20 min. Then the water was allowed to warm up gradually (see Table 3).

Twelve winkles from vertical surfaces were placed in a dish of sea water at 11° C illuminated from above at an intensity of 10 f.c. All began to crawl actively straightaway at a rate of about 2·5 cm/min. The water was then cooled gradually (see Table 4).

TABLE 2. DISTANCE (CM) CRAWLED IN 5 MIN

Winkle number	Temperature 12° C	25° C
1	10·5	17·8
2	10·4	15·7
	8° C	19° C
3	12·0	19·5
4	20·5	30·0
5	7·3	22·8
6	9·5	25·5

TABLE 3. EFFECT OF TEMPERATURE ON ACTIVITY

At	3° C	1 became active, i.e. put out its tentacles but did not crawl
	4° C	2 became active, i.e. put out their tentacles but did not crawl
	5° C	7 became active, i.e. put out their tentacles but did not crawl
	6° C	8 became active, and 3 of these began to crawl slowly
	8° C	12 became active, and 8 of these were crawling slowly
	10° C	12 were crawling at a rate of 2·5 cm/min

TABLE 4. EFFECT OF TEMPERATURE ON RATES OF CRAWLING

At	10° C	10 were crawling at a rate of about 1·5–2·0 cm/min
	8° C	8 were crawling at a rate of about 1·0–1·5 cm/min
	7° C	7 were crawling at a rate of about 0·5–1·0 cm/min

(the rest were inactive)

These results show that at temperatures of between 6° and 8° C winkles become inactive even when illuminated at light intensities sufficient to promote crawling at higher temperatures. They serve to explain why they remain largely inactive in the winter months from November to March when air and sea temperatures at Whitstable are usually below 8° C, although bright sunlight may occur at the time of low water. Indeed, observation on the shore makes it clear that low temperatures, rather than low light intensity, inhibit feeding migrations.

An interesting corollary of the relation between activity and temperature is that winkles which have settled on horizontal surfaces and which make their feeding excursions in the shallow layer of water covering them as the tide recedes may, in the warmer months of the year, experience temperatures well above those of the sea itself and so their feeding excursions are correspondingly longer, as can be noticed by a comparison of the tracks in the sand and on groynes.

DISCUSSION

In short, these observations made on the shore give the impression that winkles remain settled for a considerable part (probably the greater part) of each tidal period but that on occasions they undertake feeding excursions.

These excursions, as shown by tracks in the sand or on groynes, are reminiscent of the 'outward and homeward' journeys performed by many invertebrate animals, notably arthropods, but which are also known to be made very precisely by limpets (e.g. various species of *Patella*), less precisely by other shore gastropods, such as *Onchidium* and by chitons, as well as by some land-dwelling gastropods. Few of the homing reactions of intertidal gastropods have received detailed analysis and Thorpe (1956), commenting on the homing of limpets, remarks that: 'Apparent place memory has been observed in a number of other marine and intertidal gastropods, but the basis of it has not been properly investigated.'

The 'homing' reactions of winkles are, perhaps, comparable with those described and analysed by Evans (1951) for the small chiton, *Lepidochitona cinerea*, and evidently they tend to confine any particular winkle for a period of weeks or even months to an area of a few square metres, from which, however, it may become displaced by wave-action. Evans showed that at low tide *L. cinerea* is invariably found on the undersides of damp stones; that the animals move away from a strong directional light source but that they also have a strong positive orthokinetic response (so tending to accumulate in dark situations); that they are positively geotactic when out of water but are indifferent to the stimulus of gravity when immersed, and that a combination of these reactions to common stimuli serves to explain their ability to shelter under stones at low tide, whilst at high water allowing them to carry out feeding migrations. The rather limited excursions of these small chitons receive, then, explanation without recourse to 'place memory'. But, bearing in mind the more varied situations in which winkles are to be found at the time of low water, it is apparent that their reactions are likely to be less stereotyped than those of *Lepidochitona* although their feeding migrations may still be orientated by responses to light and to gravity. Thus, the vertical U-shaped tracks made by those which settle either on the east or west faces of groynes in regions of light or shade, exposed or in cracks, cannot, it would seem, be the result of movements orientated solely with respect to the direction or intensity of light. Rather they suggest excursions determined by gravity responses. On the other hand, it is inconceivable that the tracks made on the virtually horizontal surface of sandy patches owe anything to gravity responses. Indeed, the only directional stimulus would seem to be that of light from the sun to which at least one marine mollusc, viz. the opisthobranch *Elysia viridis* is known to orientate by performing a light-compass reaction (Fraenkel, 1927). A more detailed review of the literature on the behaviour of winkles is given in the article that follows in this Journal (p. 241).

SUMMARY

On the flat shore at Whitstable the population of common winkles is distributed appreciably at random over a variety of substrata ranging from wooden groynes, through shingle to muddy sand, between tidal levels corresponding to H.W.N.–E.L.W.S. but with a maximum density near M.T.L., although aggregations tend to occur in wetter situations and where planking joins vertical groyne posts.

Observations on winkles marked with a dab of paint of distinctive colour show that they tend to remain in approximately the same position for many weeks.

For most of each tidal period winkles remain settled in situations such as stones or groynes, which provide good holding ground, but as the tide recedes or the incoming tide reaches them, they become activated to crawl, feeding meanwhile. These feeding migrations are recorded in the sand by roughly U-shaped tracks and can be seen under favourable circumstances as similar tracks in the vertical plane for winkles on groynes. By such excursions the animals are enabled to move, feed and return to approximately the place from which they started and this serves to explain how they maintain their station on the shore.

When settled on vertical surfaces, winkles always orientate with the head uppermost, when the foot may be withdrawn and the animal become attached by a film of dried mucus.

When crawling the eyes are exposed and the tentacles bend to test the substratum, but only that part of the tentacle distal to the eye bends so that the optical axis of the eye maintains a constant angle to the body axis.

Winkles are inactive during the winter and laboratory tests show that crawling ceases at about 8° C. The animals also behave orthokinetically in response to strong variations in light intensity, crawling faster at higher light intensities.

REFERENCES

BARKMAN, J. J., 1955. On the distribution and ecology of *Littorina obtusata* (L.) and its specific units. *Arch. néerl. Zool.*, T. 11, pp. 22–86. den Helder.

EVANS, F. G. C., 1951. An analysis of the behaviour of *Lepidochitona cinereus* in response to certain physical features of the environment. *J. Anim. Ecol.*, Vol. 20, pp. 1–10.

FRAENKEL, G., 1927. Die Photomenotaxis von *Elysia viridis*. *Z. vergl. Physiol.*, Bd. 6, pp. 167–220.

GOWANLOCH, J. N. & HAYES, F. R., 1926. Contributions to the study of marine gastropods. I. The physical factors, behaviour and intertidal life of *Littorina*. *Contr. canad. Biol.*, N.S., Vol. 3, pp. 135–65.

HASEMAN, J. D., 1911. The rhythmical movements of *Littorina littorea* synchronous with ocean tides. *Biol. Bull., Woods Hole*, Vol. 21, pp. 113–121.

KANDA, S., 1916. Studies of the geotropism of the marine snail, *Littorina littorea*. *Biol. Bull., Woods Hole*, Vol. 30, pp. 57–84.

MOORE, H. B., 1936. The biology of *Littorina littorea*. Part I. Growth of the shell and tissues, length of life and mortality. *J. mar. biol. Ass. U.K.*, vol. 21, pp. 721–42.

NEWELL, G. E., 1954. Animal zones on the North Kent Coast. *S. East. Nat.*, Vol. 59, pp. 34–56.

SMITH, J. E. & NEWELL, G. E., 1955. The dynamics of the zonation of the common periwinkle (*Littorina littorea* (L.)) on a stony beach. *J. Anim. Ecol.*, Vol. 24, pp. 35–56.

THORPE, W. H., 1956. *Learning and instinct in animals*. London: Methuen.

WILSON, D. P., 1929. A habit of the common periwinkle (*Littorina littorea* Linn.). *Nature, Lond.*, Vol. 124, p. 443.

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AN EXPERIMENTAL ANALYSIS OF THE BEHAVIOUR OF *LITTORINA LITTOREA* (L.) UNDER NATURAL CONDITIONS AND IN THE LABORATORY

By G. E. NEWELL

Queen Mary College, University of London

(With Text-figs. 1–10)

The previous paper (Newell, 1958) was mainly an account of straightforward observations of the winkle population on the shore at Whitstable. These suggested additional work on the activities of the animals, particularly their responses to light and to gravity, which are important in the maintenance of their position on the beach.

RESPONSES TO SUNLIGHT ON THE BEACH

The fact that individual tracks in the sand seem to follow an apparently orientated course suggests that winkles are reacting to visual clues, since the terrain is flat and featureless. To test this supposition a winkle crawling from east to west with the sun on its left-hand side was shaded from the sun by a piece of cardboard. Then the image of the sun was shone on its right-hand side by means of a plane mirror. Immediately the winkle changed direction and crawled back on a track parallel to its original course. This experiment was repeated with several other specimens, some of which were moving from approximately north to south and in various other directions, but with the invariable result that their direction of crawling was reversed when the apparent direction of the light was altered. A slightly different experiment was one in which winkles were lifted from the sand and placed on a moist plate. Most began crawling in more or less straight lines and when the plate was turned through 180 degrees the direction of crawling was reversed, each winkle countermarching approximately along its original path.

These results seem to show that winkles crawling on wet sand are orientating to the direction of the sun in a manner which seems likely to be a 'light-compass reaction' (Fraenkel & Gunn, 1940), since, whilst individuals pursue an orientated course for some time, yet the direction of crawling is capable of reversal and, moreover, there is a good deal of variation in the direction of movement as between individual winkles. The maze of trails in the sand, despite the statement of Schwarz (1932), gives no immediate impression of

constancy of direction for the population as a whole and the discovery that each trail is, in fact, a record of orientated crawling was made possible only because attention was focused on small instead of large samples of animals. Nevertheless, it does not follow that there is no pattern of orientation for all the winkle trails even if it is not at first sight obvious.

A survey made at 13.30 to 14.30 h on 14 October at the time of low water, roughly 4 h after the tide had uncovered the area, showed the usual maze of tracks in the sand, but only a few winkles were still crawling. The bearing of 125 of these tracks was measured to the nearest 10 degrees (magnetic) by means of a prismatic compass and the results are given in the form of a polar diagram (Fig. 1A). From this it is at once obvious that the majority of tracks are approximately towards and away from the sun but that there is a good deal of variation and 15%, approximately, are at right angles to the sun's rays. It must be remembered, however, that the tracks are not all made at the same time even at one tidal level, and, as the tide recedes, the apparent motion of the sun will cause tracks on lower levels to differ in direction from those higher up the shore with the result that the records made on any survey will be expected to show some 'scatter'. Nevertheless, polar diagrams (Fig. 1B–D) similar to that in Fig. 1A, but made from tracks recorded at different times of the day and for different tides in the lunar cycle from neaps to springs, fully confirm the idea that winkles crawl mainly along the direction of the sun's rays, whilst observations as the tide recedes show that at first the direction of crawling is mainly towards the sun and later, mainly away from it. Fig. 1D, although very similar to the three previous diagrams, was constructed from results obtained on a dull summer day and suggests, therefore, that the animals were orientating to the brightest patch in the sky. It remained possible, however, that the pattern of polarized light in the sky might be providing the visual cue to which the responses were made. To test this animals were completely shaded by a large sheet of 'Polaroid' which was rotated to varying degrees and the effect on the direction of crawling noted. Of fifty winkles tested in this way none showed any clear-cut alteration of the direction of movement, most of them continuing to crawl in the same direction as when they were fully exposed.

Similarly, winkles tested in the laboratory with a sheet of 'Polaroid' interposed between them and a 200 W. lamp, also seemed not to orientate to the plane of polarization. These observations are, admittedly, insufficient to state definitely that winkles do not orientate to the plane of polarization but, even if further experiments show that this can affect their direction of crawling under laboratory conditions, it is unlikely that responses of this kind play any part in the lives of animals in natural conditions, a point of view clearly expressed by Stephens, Fingerman & Brown (1953) in a discussion of the orientation mechanism of *Drosophila*. These authors also point out that, as expressed by Fresnel's Laws, at all angles of incidence except 0 and 90 degrees, 'light

vibrating in the plane defined by the incident and reflected rays will be more efficiently refracted than light vibrating in a plane normal to this'. This applies even to light entering a non-birefringent medium, and so an apparent response to the plane of polarization of the light may be an indirect one, the animal in reality responding to the amount of light entering the eye.

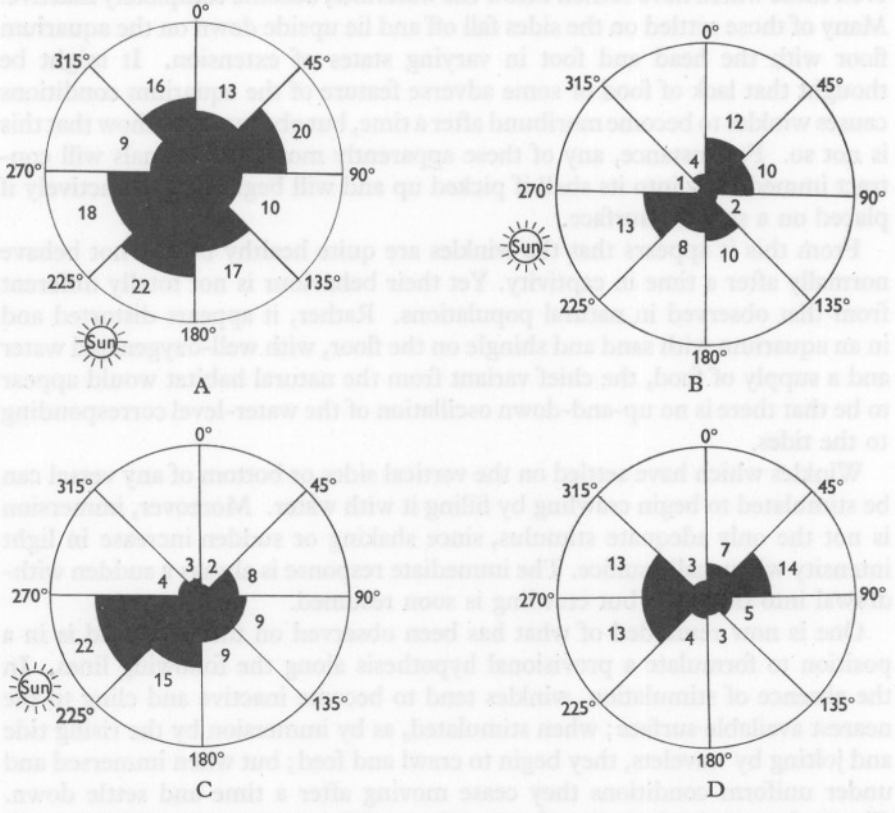

Fig. 1. Polar diagrams to show the directions of tracks and numbers of winkles moving along various tracks in the sand. Bearing measured to the nearest 10° magnetic. (A) 14 October 1956, bright sunlight, low water, neap tides; (B) 25 March 1957, 5 h after low water, sun shining only occasionally between clouds; (C) 27 March 1957, 1 h after tide had receded from M.L.T., sun's disc showing through faint haze; (D) 31 July 1957, low water, overcast sky, position of sun, 290°.

BEHAVIOUR OF WINKLES IN AN AQUARIUM

If winkles are collected at random from a variety of situations on the shore which include vertical, sloping and horizontal surfaces, their behaviour in an aquarium is also very variable. Soon after being tipped in, practically all of them begin to crawl about, but this activity is not continued indefinitely. After some hours of what appears to be random crawling, they settle down,

some on the sides at varying heights relative to the water-level and some on the bottom. In these situations the vast majority remain motionless for hours or days but very occasionally one will become active and crawl about. Here again there is no uniformity in the direction of crawling.

After being kept in captivity for periods of 2 weeks or longer, all the winkles, even those which have settled below the waterline, become completely inactive. Many of those settled on the sides fall off and lie upside down on the aquarium floor with the head and foot in varying states of extension. It might be thought that lack of food or some adverse feature of the aquarium conditions causes winkles to become moribund after a time, but observations show that this is not so. For instance, any of these apparently moribund animals will contract immediately into its shell if picked up and will begin to crawl actively if placed on a suitable surface.

From this it appears that the winkles are quite healthy but do not behave normally after a time in captivity. Yet their behaviour is not totally different from that observed in natural populations. Rather, it appears distorted and in an aquarium with sand and shingle on the floor, with well-oxygenated water and a supply of food, the chief variant from the natural habitat would appear to be that there is no up-and-down oscillation of the water-level corresponding to the tides.

Winkles which have settled on the vertical sides or bottom of any vessel can be stimulated to begin crawling by filling it with water. Moreover, immersion is not the only adequate stimulus, since shaking or sudden increase in light intensity will usually suffice. The immediate response is always a sudden withdrawal into the shell, but crawling is soon resumed.

One is now reminded of what has been observed on the shore and is in a position to formulate a provisional hypothesis along the following lines. In the absence of stimulation, winkles tend to become inactive and cling to the nearest available surface; when stimulated, as by immersion by the rising tide and jolting by wavelets, they begin to crawl and feed; but when immersed and under uniform conditions they cease moving after a time and settle down. Clearly those on horizontal surfaces, and especially on wetter parts of the shore, will experience different conditions from those settled on groynes. The available evidence suggests that the stimulus to crawl on flat surfaces is an increase in light intensity and jogging by the breaking wavelets as the tide falls. Winkles on vertical surfaces are similarly activated but they are also induced to settle by desiccation, which in its extremer conditions causes withdrawal of the foot and adhesion solely by means of hardened mucus (Wilson, 1929).

REACTIONS TO GRAVITY

So far, attention has been paid only to winkles crawling on the virtually horizontal surface of wet sand, but an appreciable proportion of the population lives, often densely aggregated, on the wooden groynes running at right angles

to the shoreline from H.W.S. to a varying distance below H.W.N. Tracks made by the periodic feeding excursions are not easy to follow in these situations, but, as has been mentioned, on some groynes tracks can be seen to be made up of a downward, horizontal and upward limb. They thus resemble in general form those made on horizontal surfaces, but since winkles occur on either the west or east face of groynes, in light or in shade, it would seem unlikely that the movements are orientated solely with respect to light but rather that gravity provides the necessary directional stimulus.

A chance observation is of interest in this connexion and serves also to reinforce the idea that the clue to an understanding of the behaviour is to pay regard to individual winkles, taking note of the exact situation from which they were collected. A batch of winkles was collected from the top surfaces of flat stones and a similar batch from the vertical face of a groyne. Each batch was placed in a separate tin and after 2 h those from flat surfaces, without exception, were still at the bottom of the tin, whereas those from the groynes were settled on the sides of their tin, clear of the bottom. This shows that those from horizontal surfaces are indifferent to gravity, whereas those from vertical surfaces behave, at least for a time whilst in the dark, in a negatively geotactic fashion. This observation has been confirmed on six subsequent occasions. On one of these, namely on 26 September 1956, the winkles were examined at intervals of 2, 6, 12, 16 and 72 h. Those from vertical surfaces remained settled on the sides of the tin, whereas those from horizontal surfaces were all massed at the bottom. It remains to be seen if winkles from vertical surfaces are indifferent to the stimulus of directional light, but this can be tested only under laboratory conditions (p. 255).

As has been mentioned winkles react to the stimulus of gravity in a variable manner according to the slope of the position on which they have settled. It is this which accounts for variations in behaviour in an aquarium when animals are collected at random from the shore. To eliminate this variable, a batch of twelve winkles was taken from the strictly horizontal surfaces of flat stones left high and dry by the tide. Each animal was marked with a dab of yellow paint. A second batch was collected from the tops of flat stones in a pool of water and marked with blue paint whilst a third batch from vertical surfaces was marked with a spot of red paint. Each of these batches of twelve winkles was placed in an aquarium with sand and shingle in the bottom, the larger stones projecting above water-level. After crawling and feeding for some hours, all the animals settled down.

A summary of their subsequent movements is set out below, from which it will be seen that those collected from flat surfaces tended to settle in somewhat similar situations, irrespective of whether they were dry or not, whereas those from vertical surfaces tended to settle on the sides of the aquarium, i.e. on vertical surfaces. This confirms previous findings, viz. that the past history of a particular winkle influences its geotactic response.

Twelve winkles from flat, dry stones, marked with yellow paint. None climbed up the tank in the first hour. After 2½ h four were on the sides at the surface film and the rest on stones lying on the bottom of the tank. After 3 days seven were on stones and five on the sides of the tank at the surface film.

Twelve winkles from flat, submerged stones, marked with blue paint. None climbed up the sides of the tank in the first hour. After 2½ h two were on the sides of the tank but below the waterline. After 3 days six were on stones, four were on the sides of the tank and two on the bottom.

Twelve winkles from vertical surfaces marked with red paint. Twelve climbed above the waterline in 6, 8, 9, 11, 14, 15, 17, 22, 24, 25, 25 and 31 min respectively. After 2½ h eight animals had moved down and settled at the waterline, head uppermost. After 3 days, nine were settled on the sides and three were crawling on the bottom of the aquarium.

Results confirming those set out above were obtained by collecting winkles from the vertical faces of groynes and from the tops of flat stones and testing their geotactic responses in separate vessels. For example, twelve winkles from groynes were placed in a large bottle full of sea water. Six immediately crawled up and reached the top in 2 min. They stayed there until accidentally dislodged. Three crawled up and settled on the sides but below the waterline within 2 min, whilst after 2 min the remaining three were still crawling on the bottom. This experiment was made in diffuse light but essentially the same result was obtained in bright sunlight. Varying the direction of the light rays entering the bottle appeared to have no effect on the behaviour of the animals. The bottle was then put in complete darkness and after 2 h seven were at the top, two were on the sides but just below the waterline and three were on the bottom.

This seems to show that winkles from the sides of groynes are predominantly negatively geotactic; but subsequent observations show that this view, which accords with most previous workers, requires modification, for, as will be seen, under suitable conditions winkles reverse the sign of their response, and it is this reversal which accounts for the lack of a 100% uniformity in the previous experiment.

Twelve winkles collected from horizontal surfaces were placed in a small glass tank in diffuse light. All began to crawl but repeatedly turned as if unable to orientate. After 4 h, although apparently random movements brought them into contact with the sides of the tank, none had climbed the walls. Thus, winkles from flat surfaces are not negatively geotactic, at least for some time. Yet, after 24 h in the tank all the winkles of this same batch had settled on the waterline.

It can, therefore, be assumed that all winkles have a tendency to climb and settle on vertical or steeply sloping surfaces, but that if this opportunity is denied them, the response becomes abolished for some time, which other experiments show may be as long as 10 days. This seems to be a process of habituation as defined by Thorpe (1956).

GRAVITY REACTIONS OF WINKLES AFTER DRYING

Bearing in mind that all winkles settled intertidally on vertical surfaces experience a semi-diurnal drying after the tide has receded, it seemed important to test if drying has any effect on the responses to gravity. Therefore, instead of beginning observations, as in previous experiments, as soon as possible after collecting the animals, time was allowed for them to dry.

Twelve winkles collected from groynes at the time of low water were left in a tin for 5 days. Then, three at a time, they were allowed to settle on a horizontal moist glass plate. This was then placed vertically in a large beaker of sea water. Immediately all the winkles began to crawl *downwards*. That is, contrary to previous occasions, they behaved *positively* geotactically. At levels of from 3 to 5 cm below the surface they moved about more or less horizontally and then crawled up to settle at or above the waterline. This was the invariable result and shows that after a period of desiccation winkles behave positively geotactically, are then neutral to the stimulus of gravity for a time and finally reverse the sign of their response. Light can be ruled out as an essential orientating stimulus since the experiments were carried out in diffuse light whilst exactly comparable results were obtained with light from directly above the beaker or shining through the sides.

Additional results are set out in tabular form (Table 1).

TABLE 1

Winkle number	Time in minutes under water
1	7
2	9
3	10
4	15 (but then returned for 4 min)
5	15
6	12
7	72 (fed on *Ulva* until it was all gone)
8	6
9	12
10	9
11	9
12	7
13	11
14	6
15	3
16	3
17	settled at waterline
18	6

The results of the previous experiment have an obvious bearing on the interpretation of the behaviour of winkles under natural conditions on the beach groynes, since they clearly point to the conclusion that the animals settle down in the head-up position but become positively geotactic after a period of desiccation. This enables them to carry out feeding migrations

when reached by the incoming tide. A reversal of the gravity response, so that its sign becomes negative, causes them to return approximately to the position they had left, when they settle down and later on experience yet another period of drying...and so on. One is reminded of Mitsukuri's results on *Littorina exigua* (p. 259) which showed that this winkle also moves downshore after a period of desiccation, although Mitsukuri believed that this was because they became positively phototactic.

It was obviously of interest to repeat the previous experiment on winkles which had not been desiccated for a period of as long as 5 days. A summary of the results of a further series of tests is set out below.

Eight winkles were collected from the vertical face of a groyne at 09.00 h at approximately low water and allowed to settle on a moist glass plate at 10.30 h. The plate was then placed vertically in a beaker of sea water in diffuse light. Each specimen moved down, then moved horizontally and finally returned to the waterline, the times for these migrations being as follows (Table 2).

TABLE 2

Winkle number	Time in minutes of excursions	
1	10	
2	6	
3	15	
4	12	
5	10	
6	15	
7	55	(fed on a piece of *Ulva* until it had all gone)
8	9	

This experiment confirms previous results and also shows that a period of desiccation of about $1\frac{1}{2}$ h is sufficient to initiate what must now be regarded as the normal tidal feeding excursion. Here, as in the previous series, one winkle stayed below water for a much longer time than any of the others, owing, it would seem, to the chance finding of food. Thus the behaviour of winkles collected from vertical surfaces becomes predictable in the laboratory under conditions which simulate those on the beach. They become active after immersion and crawl, first downwards, then horizontally and then upwards, attempting to feed meanwhile.

That feeding does, indeed, modify the duration of the feeding excursion is shown by the following experiment.

A batch of ten winkles was divided into two equal groups and each was placed in a tank with only 1 cm water in the bottom. One batch was allowed to feed for 2 h on *Ulva* whilst the other batch was left unfed. Each batch was then immersed by running in sea water. The results are set out in Table 3. This clearly shows that fed winkles are less active than unfed ones and that the presence of food prolongs the period of immersion.

TABLE 3

Fed	Unfed
None moved at once. Four stayed immersed for 1½ h. After 3 h, four had climbed above the waterline but one remained below it still feeding.	All became active at once and all five crawled down, then horizontally and then up above the waterline in 45 min.

Another experiment which gave a more clear-cut result was carried out on winkles which had been collected 10 days previously from a groyne. From these, two batches of five each were taken at random. One batch was allowed to feed on lettuce for 2 h. The other batch had been starved during the 10 days' captivity. Each batch was then allowed to settle on the sides of a jar with a little water in the bottom. All settled above the waterline. The experiment began when water was run into the jars so as to submerge all the winkles. The results are summarized below.

Fed winkles. All five animals were near the top of the jar. When submerged none became active for some minutes and none crawled about under the water. After 35 min all had crawled up out of the water.

Starved winkles. One winkle was near the top of the jar and four on the sides lower down. All became active immediately after immersion, crawled down, then horizontally and finally up above the waterline in times which varied from 7 to 38 min.

This not only confirms the view that fed winkles are less active than starved ones, but shows also that feeding abolishes the positively geotactic behaviour which is always evoked by the immersion of starved winkles. The longer times of the feeding migrations of these winkles which had been starved for 10 days, when compared with those carried out on animals kept in captivity for shorter periods suggest that the time spent in an attempt to feed is related to the degree of starvation.

The experiments described so far have indicated that the behaviour of winkles collected from the shore at Whitstable varies a good deal according to the situation on which the animals have come to rest, but observations on gravity responses have been chiefly on winkles collected from vertical surfaces. Comparable experiments on those from horizontal surfaces give different results, as is shown, for example, by those summarized below.

Twelve winkles were collected from the tops of flat stones at 09.00 h at low tide and were tested for gravity responses at 11.30 h. Each winkle was placed on a moist glass plate. After settling, the plate was placed vertically in a beaker of sea water illuminated by diffuse light from a north window. Each winkle was, in the first instance, placed so that it was orientated transversely across the plate. Most stayed in this position but four re-orientated to assume the head-up position directly the plate was tipped up so that it looked like a mechanically caused slip, and three out of these four animals later orientated across the slope. Three slipped off the plate but none crawled up or down.

These observations show that winkles from horizontal surfaces are, indeed, as has been suggested by previous experiments, indifferent to the stimulus of gravity.

So far the gravity reactions of winkles have been tested only in response to strictly defined surfaces, but on rocky shores winkles will experience slopes intermediate between the horizontal and the vertical. It is, therefore, of interest to find the minimum slopes which will evoke geotactic responses. This was done by placing winkles on horizontal surfaces and then tilting up the plate, 10 degrees at a time. As has already been seen, winkles from horizontal surfaces pay no regard to the stimulus of gravity and so only animals from groynes were tested in this manner. Several experiments of this nature were carried out. Practical difficulties arise. For example, not all the winkles used adhere to the plate and so some fall off when the plate is tipped at angles of 40 degrees to the horizontal or greater; some settle down and adhere but fail to crawl; others crawl horizontally and then off the plate and up the sides of the containing vessel.

Twelve winkles collected from groynes at time of low water were placed on a wooden plate with their heads downwards in diffuse light. The plate was then tipped up 10 degrees at a time and left on each occasion for 2 min. The minimum angle at which any winkles orientated to the head-up position was 10 degrees.

Twelve winkles collected from groynes at low water were placed with the longitudinal axis across the plate which was then tipped up, 10 degrees at a time. The plate was kept moist but was not immersed in water. From Table 4 it will be seen that at the lower angles winkles tended to retain the orientation at which they were placed on the plate, but that at 90 degrees all twelve assumed the head-up position, either statically or by crawling up.

TABLE 4

Angle (degrees)	Orientation		
	Up	Down	Horizontal
10	3	2	7
20	3	4	5
30	2	3	7
40	3	3	6
50	2	2	8
60	2	0	10
65	7	0	5
70	7	1	4
80	11	0	1
90	12	0	0

All that can be inferred from the results is that the minimum slope to which winkles can orientate is probably about 10–20 degrees, that at slopes of 60–65 degrees most of them assume the head-up position and that at 90 degrees all winkles orientate head-uppermost. This is rather different from saying

that at steep angles winkles tend to behave in a negatively geotactic fashion, since many animals remained in the same position although re-orientating with respect to gravity. Indeed, in other experiments some of the more active specimens crawled right to the top of the vessel and then over the top and *downwards* on the outside surface.

Attempts by previous workers, as for example Kanda (1916), to assess the strength and nature of the response of winkles to gravitational stimuli gave conflicting results, and from what has been said it will be realized that this can be attributed to two main causes. In part it could have been due to a failure to appreciate that winkles collected from steeply sloping surfaces normally alter the sign of their response to gravity after crawling for some time, so that intermittent observations might catch a winkle during its downward, horizontal or upward phase of movement. It is also true that previous workers paid insufficient attention to the exact situation from which winkles were collected, so that those from sloping surfaces were mixed with those from horizontal ones, when, of course, a variety of responses was detected. Gowanloch & Hayes (1926) showed that, statistically, winkles from higher levels on the shore tended to crawl upwards more strongly than those from lower levels, the implication being that in some way this difference in behaviour was related to the position on the shore, but they paid no regard to the winkles as individuals. It seems likely, however, that the differences they noted are due, not to a difference in shore levels or zones as such, nor to time of exposure to air, etc., but, instead, to a difference in beach slope at various positions relative to tide marks. I am indebted to Mr Derek Mills for the information that at New Brunswick (where Gowanloch & Hayes made their collections), the shore has a steep upper beach and a more or less horizontal rock platform near low water-level, so that the differences in behaviour with respect to the stimulus of gravity may be attributed to habituation to a horizontal or steeply sloping surface, such as has been noticed for the winkle population at Whitstable.

Summary of results of observations on the reactions of winkles to gravity

(1) Winkles have an inherent tendency to settle on vertical or steeply sloping surfaces, when they assume the head-up position.

(2) Provided that the surface is moist, when stimulated to crawl the animals move first downwards, then horizontally and then up again, to regain approximately their original position. Immersion in water, mechanical agitation or a sudden increase in light intensity are stimuli which are adequate to promote crawling.

(3) The excursions from the position of settlement can be regarded as feeding migrations.

(4) Winkles collected from horizontal surfaces are indifferent to gravitational

stimuli for periods which vary from some hours to 10 days. They have become habituated to flat surfaces but this habituation gradually diminishes, so that when presented with an opportunity to crawl upwards they revert to their inherent pattern of response.

(5) Fully fed winkles do not respond by crawling downwards when stimulated to move, but starved winkles make longer feeding excursions below water if they happen to find food.

(6) The minimum slope to which winkles orientate is about 10–20 degrees.

REACTIONS TO LIGHT

Winkles collected from horizontal surfaces

The following observations are a selection from very many which were made and serve to show that winkles from horizontal surfaces react to the stimulus of light in a way which is consistent with the form of the tracks seen on the sand at low tide.

Twelve winkles were taken from the tops of flat stones shortly before the incoming tide reached them but no note was taken of their exact orientation on the stones. The animals were placed in a shallow glass dish of sea water in bright sunlight, the sun being nearly due south.

The animals began to crawl almost at once. Seven crawled away from the sun and five towards it. Not all the paths were directly towards or away from the sun but any obliquity of the paths brought the winkles to the sides of the dish, when, of course, the paths of necessity became straight. After 3 min seven were at the end away from the sun and five at the end nearest the sun. After about 5 min three animals which had formerly been photopositive became photonegative and crawled to the opposite end of the dish, whilst the two which were photopositive became photonegative. This reversal of response was confirmed by turning the dish through 180 degrees, when the five winkles under consideration also turned round and maintained the same orientation to the sun's rays. This chance observation also confirms what had been seen only rarely on the beach but which had been inferred from the form of the tracks in the sand, viz. that winkles on flat surfaces reverse the sign of their response to light. From this it is also obvious that the immediate past history of an animal will modify the results obtained when testing its light reactions, and winkles collected at random from horizontal surfaces would not be expected to display any uniformity of orientation to light. Indeed, the result set out below is typical of many which were obtained even when winkles were collected from exactly similar situations.

Twelve winkles were collected from horizontal surfaces, all with their heads facing the sun, and placed in a shallow dish of sea water. Three behaved photopositively, three behaved photonegatively, and six moved across the sun's rays.

The experiments, an account of which follows, were designed to test the reactions to light of winkles which had received uniform treatment for some time before the observations.

Eight winkles from horizontal surfaces were kept in a tin for 48 h and then placed in a shallow dish with the bottom and three sides covered by black paper. A beam of parallel light was shone through the uncovered end of the dish from a microscope lamp. Two winkles crawled towards the light; after 2 min two winkles crawled away from the light, four winkles crawled transversely; and after 6 min eight winkles crawled towards the light. This shows that unfed, dark-adapted winkles are positively phototactic, but that there is some variation in the initial direction of their crawling.

In order to record the paths followed by winkles crawling when illuminated by a parallel beam of light, animals which had been dark-adapted for 4 days were placed in a large developing dish whose walls were covered with black paper and which was floored with cm graph paper. Light beams from microscope lamps gave an illumination of 250 ft. candles at the floor of the dish and could be shone either at right angles or from opposite ends of the dish. The movements of the winkles were recorded at half-minute intervals on another piece of graph paper.

The selection of tracks which follow (Figs. 2–5) shows that all the winkles behaved positively phototactically but that when two lights were shone at right angles a crawling animal usually disregarded the second light and continued to crawl towards the first one. This suggests that they are behaving telotactically (Fraenkel & Gunn, 1940), a type of behaviour which has much in common with light-compass reactions.

In a repeat of the above experiment twelve dark-adapted winkles collected from horizontal surfaces were placed in a dish of sea water lined by black paper. A beam of parallel light was shone in at one end. All were strongly photopositive for 12 min but at times varying from 12 to 20 min they became photonegative. This, it will be realized, confirms observations made previously (*a*) on the shore, and (*b*) in a dish using the sun as a light source. But such clear-cut results were not always obtained. On some occasions winkles reached the sides of the dish before finally orientating towards or away from a light source and then the direction of crawling in part became determined by the sides of the dish, for the winkles move along the line of junction of sides and floor.

Winkles collected from vertical surfaces

It has already been shown that winkles from vertical surfaces make feeding excursions during which they orientate with respect to gravity, but this does not preclude the possibility of reactions to light entering into their responses. Their place of settlement, however, introduces a factor quite different from that affecting winkles on horizontal surfaces, for, since the groynes are opaque,

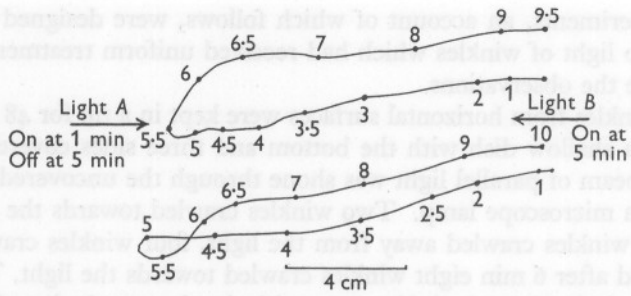

Fig. 2. Tracks of two winkles collected from horizontal surfaces and dark-adapted for 4 days. Numbers along tracks indicate time intervals in minutes. Lights *A* and *B* are microscope lamps.

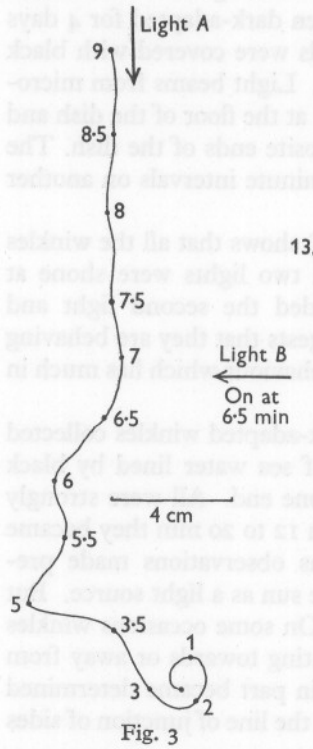

Fig. 3

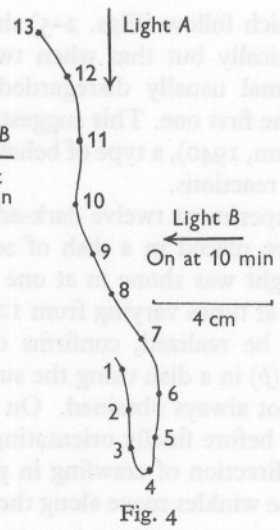

Fig. 4

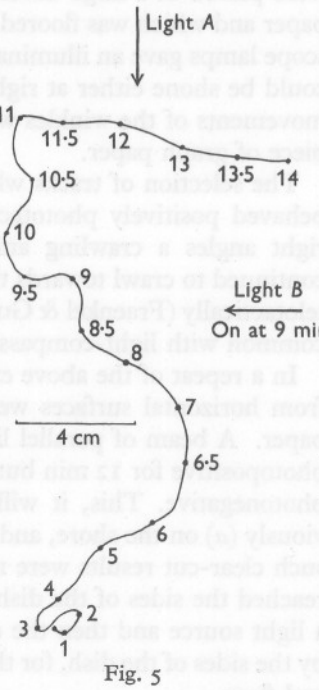

Fig. 5

Figs. 3–5. Tracks of winkles collected from a horizontal surface and dark-adapted for 4 days. Numbers along tracks indicate time intervals in minutes. Lights *A* and *B* are microscope lamps.

the incident light must always be mainly from above. It will, however, vary in intensity as the tide rises and falls. When covered to the depth of a metre or more by the turbid water at Whitstable, winkles will be almost in darkness, since even a depth of 14 cm of water cuts out 95% of the light reaching the

surface (Aleem)[1] and they will become dark-adapted during a period of some hours. As the tide falls the light intensity increases and their behaviour might be expected to vary in a way comparable to that noticed for winkles from horizontal surfaces, viz. they might reverse the sign of their response to light after some time of exposure to higher intensities of illumination. In order to rule out gravitational responses the reactions to light of winkles collected from vertical surfaces were tested by observing their crawling reactions on the floor of a dish.

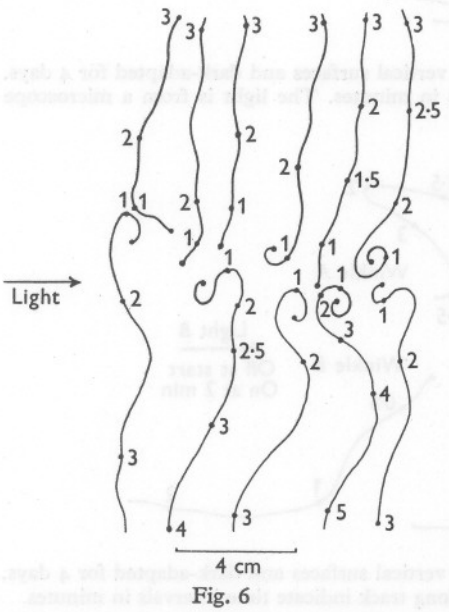

Fig. 6

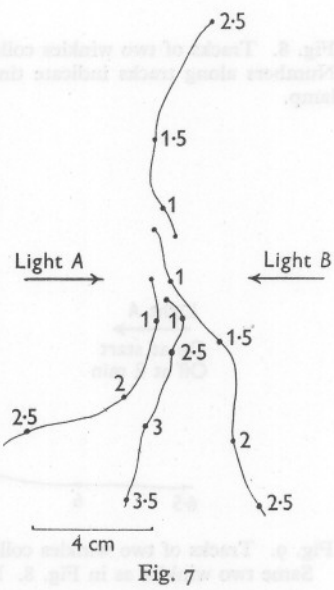

Fig. 7

Fig. 6. Tracks of winkles collected from vertical surfaces and kept in diffuse light for 4 days. Numbers along tracks indicate time intervals in minutes. The light is from a microscope lamp.

Fig. 7. Tracks of winkles collected from vertical surfaces and dark-adapted for 4 days and then exposed to diffuse light for 2 h. Numbers along tracks indicate time intervals in minutes. Lights *A* and *B* are microscope lamps.

Numerous observations on the reactions of winkles from vertical surfaces kept in diffuse light for periods of up to several days showed that the animals, when placed in a dish of sea water and illuminated by a beam of parallel light from a lamp, crawled mainly across the light beam. A selection of tracks made under these conditions in given in Figs. 6 and 7.

Fully dark-adapted animals behave somewhat differently. At first most animals crawl away from the light source, but after some time of exposure to illumination they orientate across the light beam, and later still some become photopositive. Some of these responses are shown in Figs. 8–10.

[1] University of London, Ph.D. Thesis, 1948.

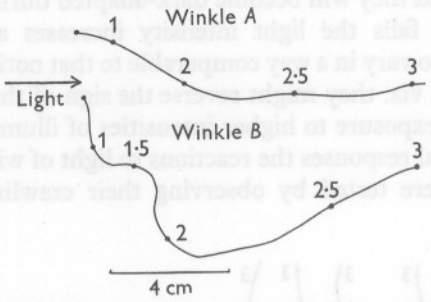

Fig. 8. Tracks of two winkles collected from vertical surfaces and dark-adapted for 4 days. Numbers along tracks indicate time intervals in minutes. The light is from a microscope lamp.

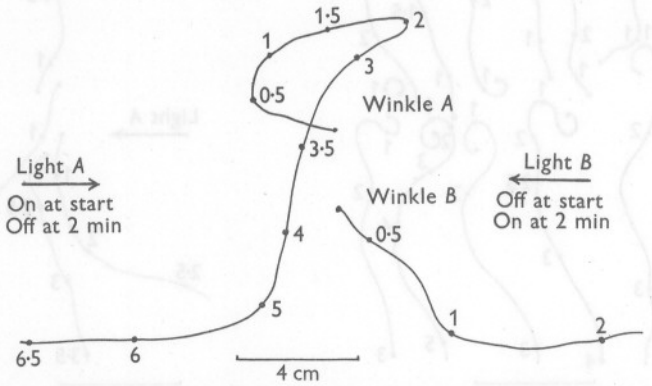

Fig. 9. Tracks of two winkles collected from vertical surfaces and dark-adapted for 4 days. Same two winkles as in Fig. 8. Numbers along track indicate time intervals in minutes.

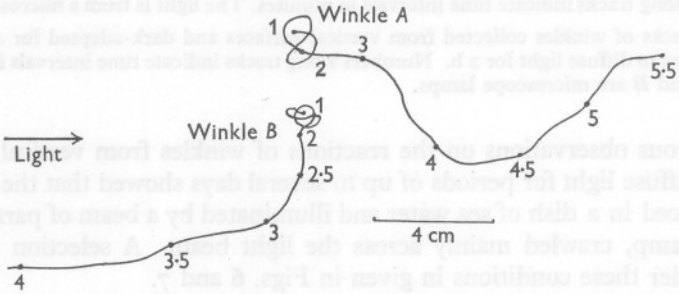

Fig. 10. Same two winkles as in Figs. 8 and 9. Winkle *A* remained photo-negative but winkle *B* became photopositive after 2 min and remained so when repeatedly retested.

In addition, it should be mentioned that of fifteen winkles collected from vertical surfaces and dark-adapted for 8 h, thirteen were at first photonegative, one was photopositive and one crawled across the light beam. Six which were at first photonegative reversed their direction of crawling after 5 min.

Thus, the feeding migrations of winkles on vertical surfaces are probably, in part, determined by their responses to light. When dark-adapted the animals behave photonegatively, but after a time they orientate across the light beam and they then crawl upwards, having reversed the sign of their response to light. In contrast to winkles from horizontal surfaces, those from vertical surfaces are at first photonegative and their feeding migrations are determined in part by gravity and to a lesser extent by responses to light. Further light is thrown on this point by the observations which follow and which were made to see how winkles behaved when (*a*) the light stimulus is removed, and (*b*) when it is acting against gravity.

(i) Dark-adapted winkles from horizontal surfaces were allowed to settle on horizontal glass plates in darkness, when they orientated at random. Viewed in ruby light they crawled, when stimulated by immersion, in a very sluggish fashion and within 5 min had settled again.

(ii) When twelve dark-adapted winkles from vertical surfaces were allowed to settle in darkness on a vertical glass plate in an empty beaker, all crawled upwards and settled. In ruby light all at first crawled down and then up above the waterline when the beaker was nearly filled with sea water. This shows that in darkness all the winkles when stimulated crawled downwards but that all behaved in a negatively geotactic manner before settling down, although the downward limb of the track was short and the crawling was sluggish.

(iii) The experiment was repeated but with the difference that, when the winkles were submerged, illumination from directly below was provided by a 75 W. lamp. Then eight winkles crawled horizontally, five crawled upwards, two crawled downwards and then up. All settled just above the waterline after 12 min.

This shows that the behaviour pattern which is usual for 'natural conditions' is upset by illumination from below. It also suggests that the winkles which settle on vertical surfaces begin their feeding excursions partially as a negative phototactic response, as probably do also those which have settled on sloping rock surfaces. Other observations on the behaviour of winkles in diffuse light confirm the view that under these conditions they crawl across the light rays. For example, it is the almost invariable rule that winkles placed in a glass aquarium some distance from a north window settle on east and west walls. That is, after a preliminary period of crawling, they ascend and settle on the walls which are not directly facing or away from the light. This behaviour is quite consistent with that observed for the distribution on the beach groynes, on which it is noticed that the vast majority of the animals are clustered against the junction of the vertical posts with the planking.

Summary of observations on the reactions of winkles to light

(1) Dark-adapted winkles from horizontal surfaces almost invariably be have positively phototactically but after some time of exposure to a light source they crawl horizontally across the beam and then, later still, behave negatively phototactically.

(2) Winkles from horizontal surfaces kept for some hours in diffuse light usually crawl transversely to a light beam.

(3) Winkles usually, but not always, disregard a second light source when this is switched on after they have orientated to the first source but sometimes they re-orientate towards the second light. They are thus able to behave telotactically to light.

(4) Winkles from vertical surfaces behave to light in a way which in several respects resembles the behaviour of those from horizontal surfaces. Thus, after being kept in diffuse light, they orientate and crawl across the light beam, and reverse the sign of their response when exposed to light after being dark-adapted. But there is an important difference, namely that most winkles from vertical surfaces are, when first tested, photonegative.

(5) The behaviour in the laboratory seems to be similar to that noticed on the shore, but suggests that winkles both from horizontal and vertical surfaces carry out feeding migrations which are influenced by light. Those from horizontal surfaces orientate *solely* by reactions to the direction of light, whilst those from vertical surfaces orientate to the direction of the light (which is always mainly from above) and also to gravity, the two sets of responses normally reinforcing each other.

DISCUSSION

The results of previous work on the behaviour of littorinids can now be considered, but, as will be apparent, the accounts of shore observations and the results of laboratory experiments on the reactions of winkles to light and to gravity are so conflicting that they give little help in the solution of the problem of the relation of these reactions to observed distribution of *Littorina littorea*. Indeed, the interpretation of these results is itself an interesting study, since it cannot be reasonably doubted that they are based on accurate, even if incomplete, observations. But the explanations offered in the present paper are at variance with previous accounts. To what, it may be asked, can these discrepancies be attributed? In part the answer is that previous workers failed to take due note of the immediate past history of the animals they studied, the exact situation from which the winkles were collected, the degree of dark-adaptation and so on. In part they are due to failure to make continuous observations on individual winkles, with the result that few authors noticed the regularly recurring reversal of the responses to light and gravity and none appreciated their significance in the mode of life of the animals. On the

other hand, many, if not most, of the observations previously recorded can be confirmed, or partially confirmed, although receiving quite different explanations.

Bohn (1904*a*) stated that *Littorina rudis* has a natural rhythm of active and passive life, the whole cycle lasting 15 days, which is due to the varying heights reached by the tides over the lunar cycle. At spring tides the animals are active whilst at neaps the operculum closes and the animals are inactive. This is certainly not true for *L. littorea* and has not been confirmed for *L. rudis*. Bohn also stated that this rhythm is retained by *L. rudis* for several months in a laboratory, and he notes that at the time of spring tides the animals become more strongly positively geotactic and phototactic. Bohn (1904*b*, 1905*a*) states that on a horizontal surface an animal will crawl towards a light and then later reverse its direction, the time of reversal corresponding with the time at which in nature it would have experienced the maximum degree of dehydration on the shore. Again, referring to 'les littorines', Bohn (1904*c*) believed that on sloping surfaces winkles orientate along a resultant of the 'forces' due to negative phototaxis and negative geotaxis. Yet, when an animal is upside down on a vertical surface, negative phototaxis is said to be replaced by positive phototaxis. It can be said that these observations have not been confirmed for *L. littorea*, although, as has been seen, reversal of the sign of the response to light and gravity play a most important part in the feeding excursions. Later, Bohn (1905*b*) seemed to show that *L. littorea* is always negatively phototactic, but, as in previous papers, no details are given of his experimental procedure.

Mitsukuri (1901), working with the Japanese snail, *L. exigua*, stated that as a rule they are negatively phototactic and that, since the light is always more intense offshore(???), the animals are driven upshore away from the sea. The animals show a 'disinclination' to become submerged, as is shown in an aquarium by raising the water level. This he terms 'negative hydrotaxis'. But, as has been mentioned (p. 248), an exactly comparable behaviour has been seen for *L. littorea*, for which it is suggested that this is a result of the animals becoming quiescent after the termination of their feeding excursions. Mitsukuri believed that some winkles get left above the waterline but when re-wetted they become positively phototactic and move down the shore. Mitsukuri, it should be noted, paid no attention to gravity responses. Morse (1910) believed that his observations showed that *L. littorea* is negatively phototactic during the daytime in June but becomes positively phototactic as night approached. But, after 18 June, the numbers of winkles behaving in a positively phototactic manner predominated even during daytime, whilst the period of transition of the light responses corresponded with the time of change from spring to neap tides during which time winkles in a natural state on the rocks were exhibiting a corresponding change in phototaxis because the tide did not reach them. Morse therefore believed that he had confirmed the

observations of Mitsukuri who stated that winkles, when desiccated, become positively phototactic and when submerged negatively phototactic. It is to be noted that Morse overlooked any possible reactions to gravity and that he gave no description of his experimental procedure. Haseman (1911), working on *L. littorea* at Woods Hole, was unable to confirm Bohn's results on tidal rhythmicity. He observed that winkles placed on horizontal surfaces do not show rhythmical movements corresponding with the rise and fall of the tides, but that snails located on more or less vertical surfaces follow the surface film of the water as it is raised or lowered. I have not been able to confirm this, but as has been stated (p. 248) winkles after a quiescent period crawl down and then up again to settle at or near the surface of the water. One curious observation which may be mentioned is that the winkles with which Haseman worked stopped opposite olive-green sectors in an aquarium in preference to sectors coloured yellow or brown. Haseman explained the zonation of winkles at Woods Hole along the following lines. Submerged animals crawl at random but do not crawl on dry surfaces and are not found above tidemarks. As the tide rises some, by mere chance, crawl above the waterline but the dryness and unevenness of the surface slows them down so that the tide overtakes them and they become submerged. When the tide falls, a few may get left behind and cling to the rocks.

Kanda (1916) also made studies on *L. littorea* and pointed out that earlier workers had omitted to take into account reactions to gravity. When winkles were allowed to crawl on a glass plate submerged in water he found that the steeper the angle of the plate, the greater was the number of negatively geotactic animals, but even on slopes as gentle as 11 degrees, 55% of the animals were negatively geotactic whereas, at angles of 90 degrees, 100% were negatively geotactic. Similar results were got with winkles crawling on a plate in air, but when a ground-glass plate was used 15% of the specimens behaved positively geotactically even at slopes of 90 degrees. Kanda interpreted this to mean that winkles tend to become positive when dry and points out that this 'would evidently serve as a protective reaction when they are left by the retreating tide'. When Kanda's results are examined in detail the striking thing is the irregularity of the gravity responses. For example, when a wooden plate was substituted for a glass one, in air, at an angle of 90 degrees, 52% of the winkles were positively geotactic, 16% were negative whilst 15% crawled horizontally. The rest fell off the plate.

The result of this experiment, which was made in darkness, was interpreted to mean that *positive* geotaxis predominated, but since Kanda failed to notice the reversal of the sign of the response to gravity the difference in the result from that of previous experiments may well have been due to the circumstance that he was observing downward, horizontal and upward parts of U-shaped tracks now known to be typical of winkles on vertical or steeply sloping surfaces at Whitstable. In nature Kanda thought that 'the limitation of upward

movement is due chiefly to the exhaustion of moisture carried by the foot from the sea. They seldom, if ever, crawl on dry rocks much higher than high tide mark....They seem to stop crawling when the moisture which they carry themselves is exhausted.' When tested under similar conditions, except that the apparatus was half-filled with water with the plate at 90 degrees, no winkles were positively geotactic but twelve out of 150 crawled horizontally on reaching the surface film. In direct sunlight with the light parallel to the plate the winkles which were 'negatively heliotropic' showed a variety of reactions: 10% crawled diagonally, 16% went horizontally whilst 74% crawled downwards, so that both light and gravity seem to affect the direction of travel. Discussing these results and those of previous workers, Kanda states that *L. littorea* is, as believed by Bohn (1905*a*, *b*), 'negatively phototropic'. Indeed, Kanda places great emphasis on this point which, he says, is 'the unmistakable reaction of the animal to light'. Winkles (he says) do not, as supposed by Morse, crawl upwards because of 'positive heliotropism' but on account of 'negative geotropism', and in spite of 'negative heliotropism', and he further believes that Morse's view that as night approaches winkles become increasingly positively phototactic is incorrect. Haseman's results also come in for criticism by Kanda who (quite correctly) says that they are not clearly set out and that no account was taken of possible reactions to the sun's rays. Here again, it may be remarked, Kanda failed to notice the regular reversal of responses to light.

Gowanloch & Hayes (1926) were primarily concerned with the behavioural features affecting the zonation of *L. littorea* on a rocky shore. They state that winkles are never found on areas of fine sand or mud but are typically inhabitants of exposed rock surfaces, from which it is obvious that their remarks do not apply to the many situations other than rocks where winkles flourish. They believed that, although not expressible in a quantitative way, their observations on marked winkles showed that the animals returned to a particular tidal level when displaced. No evidence for this has been found in the present investigation. Their results also seemed to show that winkles varied in the degree of their response to light and to gravity. Those collected from high up on the shore are more strongly positively phototactic than those from lower down and desiccation steadily increases the degree of 'negative geotropism'. Later (1929) Hayes presented evidence for the view that increasing the time during which winkles are desiccated decreases the degree of 'negative geotropism' and that, conversely, prolonging the time of immersion increases 'negative geotropism'. Colman (1933), in a valuable discussion of the factors underlying zonation, pointed out that 'the combined effect of these reactions is to make it difficult for each species to get away from its normal habitat'.

This may well be true, but, as has been mentioned (p. 251), these differences in behaviour were probably caused not by differences in shore level as such

but by variations in the steepness of beach slope, the lower levels being less steep than the upper ones.

On the flat type of shore, such as the 'wattens' and 'waddens' of the north German and Danish coasts, Schwarz (1932) stated that gravity responses play little or no part in the orientation of the winkles found there. When the sun is low and the light is directional, winkles behave positively phototactically when uncovered by the tide. Thus, they move from east to south in the morning and from south to west in the evening. On dull days and when the sun is high in the sky they move in a random manner. Ankel (1936) was unable to confirm what he terms 'this remarkable finding', yet had Schwarz noticed the reversal of the direction of crawling, his observations would have been substantially correct.

Reference may be made to papers dealing with the behaviour of species of *Littorina* other than *littorea*. Fraenkel (1927) carried out a thorough investigation of the reactions of *L. neritoides* to light and to gravity and convincingly showed that when submerged the animals are photonegative except when upside down (as when crawling on the ceiling of a rock crevice) when they are photopositive. Moreover, they are usually geonegative and so tend to crawl up rock faces. These reactions serve to explain why *L. neritoides* moves to the upper shore and splash zone. If when moving under water an animal encounters a crevice its negatively phototactic behaviour takes it inwards along the floor; its negative geotaxis causes it to climb the end wall and brings it to an upside-down position when it behaves positively phototactically and crawls along the ceiling out of the crevice. Yet when out of water, as when its movements have brought it above the tidal level, upward crawling is continued and if it enters a crevice as a result of negative phototaxis, it stays there, for the sign of this response does not reverse until the animal becomes submerged. This analysis goes a long way to explain the observed distribution of *L. neritoides*, which commonly inhabits crevices near and even above H.W.S., but that it is incomplete is shown by the observations (Lebour, 1935; Lysaght, 1941) that the animals are sometimes found in numbers in situations where they are permanently submerged. Moreover, at whatever tidal level it is found, *L. neritoides* must, presumably, leave its crevice on occasions in order to feed. It must be supposed, therefore, that the behaviour of this winkle is less stereotyped than Fraenkel's account would indicate.

A recent paper by Barkman (1955) deals with the reactions of *L. obtusata*. He states that this species is negatively geotactic and that this agrees with the results of all previous observers of littorinids except Mitsukuri (1901) and Morse (1910). *L. obtusata* is also invariably negatively phototactic. Barkman could detect no tidal rhythms and found that in a tank with a sloping floor and with an arrangement for simulating a rising and falling tide with an oscillation of 33 cm, the animals came to rest at about 6 cm above the waterline. This he explained by assuming that winkles have a tendency to crawl

upwards until halted by desiccation. Similar movements occur on the shore, it is said.

The view of the locomotory pattern of behaviour of the common winkle, *L. littorea*, presented in the present paper, reduces the responses to a rather simple but reversible reaction to the direction of the incident light, which can be reinforced by gravity responses. All the winkles seem to spend most of the time in which they are submerged by the tide or exposed to the air, settled on places which offer good holding ground. But when stimulated, as by increased wave action during a rising or falling tide, they are activated and make feeding excursions of limited duration out from and back to their approximate place of settlement, thus tracing out roughly U-shaped tracks on the substratum, whether it be the sand or the vertical face of a groyne. The physiological explanation for the reversal of the sign of responses is not known, but, since it occurs even when the external environment is maintained constant, as under laboratory conditions, it must depend on some internal mechanism, and, following Pittendrich (1956), this may be termed a biological clock but one which does not fall readily into any one of the categories he specifies.

On very flat shores, such as occur in the Whitstable area, only two types of situation are (apart from very occasional small boulders) available for winkles to settle on, viz. the practically flat beach and the vertical surfaces of groynes, but on rocky shores winkles are presented with surfaces which have a great variety of slopes. It is highly likely, therefore (and preliminary observations at Plymouth reinforce this view), that the behaviour of winkles on rocky shores is more complex than that on flat shores in the sense that their feeding excursions are always determined in part by the direction of the light and in part by gravity, the two stimuli varying in importance according to the position of settlement, whereas, as has been seen, dwellers on flat surfaces orientate solely in response to light and those on vertical surfaces mainly in response to gravity.

The ecological importance of this type of behaviour would seem to be that a mainly sedentary animal is able to maintain its station of the shore in a position of security, but also, by reacting to environmental clues, to move out and return to a place of settlement, feeding meanwhile. The kind of behaviour exemplified by winkles finds a more precise expression in the movements of other intertidal animals, of which the common limpet, *Patella vulgata*, may be singled out for comment. Here the feeding excursions, which have repeatedly been described, end with a return to an exact spot or scar on the rock from which the animal began its crawl. Limpets can be truly said to return to a home. But it cannot for a moment be supposed that this type of behaviour, although outstanding in its precision, is unique. Rather it would seem proper to regard it as the extreme of a series at one end of which are intertidal animals that wander 'at random', and at the other those which regularly leave and return to a strictly localized spot. Winkles seem to occupy

an intermediate position in this series and are, perhaps, representative of the vast majority of shore animals, although this is not to say that the environmental clues selected and the reactions to them are identical in all instances. Some, indeed, are unexpectedly complicated. For example, Papi & Pardi (1953) and Pardi & Papi (1953) showed that *Talitrus saltator* makes excursions after emerging from the sand and orientates by means of a light-compass reaction, moving up and down the beach at right angles to the shoreline, no matter what the time of day, and so returning to the zone of moist sand. This implies that in some way the animals make allowance for time in fixing their angle of orientation to the sun.

There can be little doubt that, as stressed by Ewer (1956), the distribution of animals on the shore is a dynamic process initiated by larval behaviour and in some instances maintained by the behaviour of the adults.

Winkles are structurally adapted in several obvious ways to life in the intertidal zone, to which, indeed, they are virtually restricted, few if any occurring below E.L.W.S. even when the nature of the sea floor is suitable and food is abundant. Bearing in mind that *L. littorea* can withstand prolonged immersion—for more than 50 days (Hayes, 1929)—the reasons for a fairly sharply defined lower limit to the zonation are obscure, whereas, other considerations apart, inability to withstand continued exposure and lack of suitable food suffice to account for the upper limit at approximately H.W.N. The results of the present investigation suggest that the lower limit of zonation may be that at which there is too little mechanical disturbance and too low a level of light intensity to stimulate winkles to embark on the feeding excursions, so that any which by chance settle below tidemarks are by their behaviour inhibited from feeding and so fail to become established.

SUMMARY

Winkles dwelling on horizontal surfaces orientate by means of a light-compass reaction. As the tide recedes, most of the tracks in the sand are towards the sun but after a time each animal reverses its direction of crawling, so tracing out a roughly U-shaped path which leads it back, approximately, to its starting-point.

When kept in an aquarium winkles tend to settle on the sides above the waterline and to become inactive. When stimulated, as by immersion, they crawl downwards, then horizontally and then upwards before settling above the waterline.

Winkles collected from horizontal surfaces at first show no tendency to climb vertical surfaces in an aquarium. After a period which varies from a few hours up to 10 days they do climb the sides of the tank. Animals collected from vertical surfaces climb the sides of the aquarium at their first opportunity and then settle. It can be concluded that winkles have an inherent tendency to

climb and settle in the head-up position on vertical or steeply sloping surfaces but that if this opportunity is denied them they become habituated to horizontal surfaces.

The minimum angle to which winkles can react by gravitational responses is of the order of 10–20 degrees.

In an aquarium starved winkles prolong their excursions below water when presented with suitable food but fully fed animals respond less strongly to the stimulus of immersion.

Dark-adapted winkles from horizontal surfaces are at first positively phototactic, but after a time varying from 15 to 20 min, they become negatively phototactic. Tested with the 'two-light' experiment most animals disregard the second light (telotaxis).

Dark-adapted winkles from vertical surfaces are at first negatively phototactic and then from periods of 5 min and upwards photopositive. This behaviour agrees with that seen on the shore, where their feeding migrations are orientated responses to gravity but are reinforced by reactions to light.

The relations of the responses of winkles to light and to gravity to the observed pattern of zonation are discussed.

REFERENCES

ANKEL, W. E., 1936. Prosobranchia. *Tierwelt N.-u. Ostsee*, Teil 9, b, 240 pp. (Lief. 29).

BARKMAN, J. J., 1955. On the distribution and ecology of *Littorina obtusata* (L.) and its specific units. *Arch. néerl. Zool.*, T. 11, pp. 22–86.

BOHN, G., 1904*a*. Periodicité vitale des animaux soumis aux oscillations du niveau des hautes mers. *C.R. Acad. Sci., Paris*, T. 139, pp. 610–11.

—— 1904*b*. Oscillations des animaux littoraux synchrones de la marée. *C.R. Acad. Sci., Paris*, T. 139, pp. 646–8.

—— 1904*c*. Influence de la position dans l'éspace sur les tropismes. *C.R. Soc. Biol., Paris*, T. 57, pp. 351–3.

—— 1905*a*. Attractions et oscillations des animaux marins sous l'influence de la lumière. *Mem. Inst. gén. psychol.*, T. 1, pp. 1–111.

—— 1905*b*. L'éclairement des yeux et les mouvements rotatoires. Essais et erreurs dans les tropismes. *C.R. Soc. Biol., Paris*, T. 59, pp. 564–7.

COLMAN, J., 1933. The nature of the intertidal zonation of plants and animals. *J. mar. biol. Ass. U.K.*, vol. 18, pp. 435–76.

EWER, D. W., 1956. Animal ecology and behaviour. *S. Afr. J. Sci.*, Vol. 52, pp. 211–15.

FRAENKEL, G., 1927. Beiträge zur Geotaxis und Phototaxis von *Littorina*. *Z. vergl. Physiol.*, Bd. 5, pp. 585–97.

FRAENKEL, G. & GUNN, D. L., 1940. *The Orientation of Animals, Kineses, Taxes and Compass Reactions*. Oxford.

GOWANLOCH, J. N. & HAYES, F. R., 1926. Contributions to the study of marine gastropods. 1. The physical factors, behaviour and intertidal life of *Littorina*. *Contrib. Canad. Biol.*, N.S., Vol. 3, pp. 135–65.

HASEMAN, J. D., 1911. The rhythmical movements of *Littorina littorea* synchronous with ocean tides. *Biol. Bull., Woods Hole*, Vol. 21, pp. 113–21.

HAYES, F. R., 1929. Contributions to the study of marine gastropods. 3. Development, growth and behaviour of *Littorina*. *Contrib. Canad. Biol.*, N.S., Vol. 4, pp. 413–30.

KANDA, S., 1916. Studies on the geotropism of the marine snail, *Littorina littorea*. *Biol Bull., Woods Hole*, Vol. 30, pp. 57–84.

LEBOUR, M., 1935. The breeding of *Littorina neritoides*. *J. mar. biol. Ass. U.K.*, Vol. 20, pp. 373–8.

LYSAGHT, A. M., 1941. The biology and trematode parasites of the gastropod, *Littorina neritoides* (L.) on the Plymouth breakwater. *J. mar. biol. Ass. U.K.*, Vol. 25, pp. 41–63.

MITSUKURI, K., 1901. Negative phototaxis and other properties of *Littorina* as factors in determining its habitat. *Annot. zool. Jap.*, Vol. 4, pp. 1–19.

MORSE, M. W., 1910. Alleged rhythm in phototaxis synchronous with ocean tides. *Proc. Soc. exp. Biol., N.Y.*, Vol. 7, pp. 145–6.

NEWELL, G. E., 1954. Animal zones on the North Kent Coast. *S. East. Nat.*, Vol. 59, pp. 34–56.

—— 1958. The behaviour of *Littorina littorea* (L.) under natural conditions and its relation to its position on the shore. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 229–239.

PAPI, F. & PARDI, L., 1953. Ricerche sull' orientamento di *Talitrus saltator* (Montagu) (Crustacea Amphipoda). 2. Sui fattori che regolano la variazione dell' angolo di orientamento nel corso del giorno. L'orientamento di notte. L' orientamento di un altro populazione. *Z. vergl. Physiol.*, Bd. 35, pp. 490–518.

PARDI, L. & PAPI, F., 1953. Ricerche sull' orientamento di *Talitrus saltator* (Montagu) (Crustacea Amphipoda). 1. L'orientamento durante il giorne in una populazione del littorale tirrenico. *Z. vergl. Physiol.*, Bd. 35, pp. 459–89.

PITTENDRICH, C. S., 1956. Perspectives in the study of biological clocks. *Perspectives in Marine Biology*. University of California Press.

SCHWARZ, S., 1932. Der Lichteinfluss auf die Fortbewegung, die Einregelung und das Wachstum bei einigen niederen Tieren, (*Littorina, Cardium, Mytilus, Balanus, Teredo, Sabellaria*) *Senckenbergiana*, Bd. 14, pp. 429–54.

STEVENS, G. C., FINGERMAN, M. & BROWN, F. A., 1953. The orientation of *Drosophila* to plane of polarized light. *Ann. ent. Soc. Amer.*, Vol. 46, pp. 75–83.

THORPE, W. H., 1956. *Learning and Instinct in Animals*. London: Methuen.

WILSON, D. P., 1929. A habit of the common periwinkle (*Littorina littorea* Linn.). *Nature, Lond.*, Vol. 124, p. 443.

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