# JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



THE PLYMOUTH LABORATORY

### VOLUME XXX, No. 1

(issued June 1951)

## CAMBRIDGE AT THE UNIVERSITY PRESS 1951

Price Thirty Shillings net

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### A BIOLOGICAL DIFFERENCE BETWEEN NATURAL SEA WATERS

I

#### By Douglas P. Wilson, D.Sc., F.R.P.S.

Zoologist at the Plymouth Laboratory

#### (Plates I and II)

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#### INTRODUCTION

The work of F. S. Russell on plankton animals as indicators of different water masses, particularly the species *Sagitta setosa* J. Müller and *S. elegans* Verrill, and of Atkins, Harvey and Cooper on plant nutrients, have pointed the conclusion that the water in the English Channel, in the neighbourhood of Plymouth, was consistently richer in nutrients and plankton in the 5 or 6 years prior to about 1930 than it has been at any time since. (See Russell, 1935, 1936*a*, 1939; Cooper, 1938; Armstrong & Harvey, 1950; Harvey, 1950.) The comparative infertility of the sea in this area in the 1930's, and until the present time, has been shown not only by the low winter phosphate maximum, and by the sparse plankton characterized by the presence of *S. setosa*, but also by the reduced numbers of young fishes surviving their early larval stages after the summer spawning (Russell, 1939).

It was during the 1930's that I experienced much frustration in numerous attempts to rear the larvae of various species of polychaetes. It may be that the species concerned would in any event have proved difficult, but the meagre results obtained for energy expended, especially when compared with the easier successes of the later years of the 1920's, gradually led to a suspicion that there might be another explanation. As it became clearer, from the work of Russell and Cooper, that local planktonic and hydrographic conditions had changed, it occurred to me that, quite apart from considerations of nutrients and plankton food, the local sea water which I was using for my work might itself be a less favourable medium for developing eggs and larvae than formerly. It seemed at least possible that some species would develop more readily in

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*elegans* than in *setosa* water, and perhaps vice versa. Before 1930 the water used for rearing would very likely have been *elegans* water, while after that date *setosa* water was probably employed. All water used for rearing had been passed through Berkefeld filter candles so that plankton organisms in the water had been removed, any necessary food having been provided from artificial cultures, generally of the diatom *Nitzschia*. Thus if *setosa* water was less favourable than *elegans* water for eggs and larvae, it was not due to the contained plankton. It might be that *setosa* water lacked some vital substance, or alternatively contained in minute amount something harmful to certain species. The effect might be directly on the larvae themselves, or it might be on the maturation of the germ cells so that in some species eggs produced and fertilized under *setosa* conditions are less viable than those matured in an *elegans* environment.

This possibility had become apparent even before 1939, and on discussing the matter with Dr Cooper that year I found that he also, but from a different direction, was approaching similar conclusions. We considered the practicability of obtaining *elegans* water from the westward, but before this could be done war broke out and made it impossible, and it was not until fairly recently that the two kinds of water could actually be tested.

The first tests were made in the summer of 1948. I was at that time engaged in experimenting with larvae of the small polychaete *Ophelia bicornis*. Usually there is little difficulty in rearing this species, but that particular summer several cultures had unaccountably failed. The question of the water was again raised, and on his next cruise to the westward Dr Cooper very kindly obtained some *elegans* water for me. Using this water, exceptionally fine rearings were obtained; at the same time some direct comparative tests of this *elegans* water with our ordinary 'Outside' water were also made. The results were so encouraging (Exps. I and 2, pp. 3–5) that the following spring, and again the next year, more extensive tests were undertaken, using plutei of *Echinus esculentus* which are specially sensitive to cultural conditions. The larvae of another polychaete, *Sabellaria alveolata*, were also tested.

Before detailing the experiments the names adopted for the waters must be explained. It is thought better to refer to them by their location, for although in these experiments the water from the Celtic sea (definition in Cooper & Vaux, 1949, p. 750) always contained typical *elegans* plankton, and the Outside water consistently contained a very sparse zooplankton (a characteristic of *setosa* water) in a further series this might not be so. The plankton was taken, usually at the same time as the water, with a 2 m. stramin ring-trawl. Outside water derives its name from the fact that it is got off Plymouth, *outside* the breakwater and well out to sea, often from beyond the Eddystone.

For much helpful discussion I wish to thank especially Mr F. S. Russell, Dr H. W. Harvey and Dr L. H. N. Cooper. Dr Cooper and Mr F. A. J. Armstrong have given invaluable help technically; they collected, often under

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trying conditions, most of the samples of water tested, and they are responsible for the water analyses and other hydrographical data given in this paper. The plankton was examined by Mr P. G. Corbin who kindly supplied the descriptions.

#### THE EXPERIMENTS

#### General Method

All glassware was cleaned with warm concentrated sulphuric acid and thoroughly washed. Throughout, special attention was given to cleanliness and the avoidance of contamination. Care was taken to treat both sides of an experiment alike. All vessels containing water and larvae undergoing comparative tests were kept side by side under identical lighting and temperature conditions. All waters were filtered through Berkefeld filter candles. In the earlier experiments the test vessels were glass finger bowls of diameter approximately 12.5 cm. and total capacity 500 c.c. They were filled to just over half capacity. In the 1950 experiments, with *Echinus esculentus*, crystallizing dishes 7 cm. in diameter and of approximately 160 c.c. total capacity were used. They were about two-thirds filled, water levels being equal in all dishes in any one experiment.

#### Experiments with Ophelia bicornis Sav.

Fertilizations were made by slitting open mature worms to obtain eggs and sperms. The fertilized eggs were then divided equally into two portions, each of which was thoroughly washed with one of the waters being tested. After 16–20 hr. surface-swimming larvae were poured off into new bowls to separate them from undeveloped eggs, or abnormal larvae which had not swum up. Additional water was added to the new bowls and the levels equalized.

#### Experiment I

*Celtic sea water* collected from the sea surface by means of a wooden bucket. Position:  $50^{\circ}$  00' N.,  $7^{\circ}$  10' W. Sounding: 110 m.

Date: 30. vi. 48. Time: 04.40 hr. G.M.T.

Ship: R.V. Sabella, with Dr L. H. N. Cooper.

Conditions: rough sea and heavy confused swell, in spite of which salinity at 0.5 m. was greater by  $0.09^{\circ}/_{\circ\circ}$  than at 5 m.

Physical and chemical observations:

Depth	Temp	Salinity	Phosphorus (mgatom/m. <sup>3</sup> )				
(m.)	(° C.)	(°/)	Total	Inorganic	Organic		
0.2	13.63	35.21		0.06			
5	13.29	35.12	0.36	0.04	0.35		
50-107	9.83	35.22	0.70	0.44-0.21	0.23		
Integral mean of water column	—	—	0.28	0.33	0.22		

The *Outside water* was from the ordinary laboratory stock, recently collected from the sea surface well offshore from Plymouth. Very little plankton had been present in the area throughout June and early July.

1-2

On the way home from the Celtic sea, hydrographical observations were made at station E I but no water was collected for the present work.

Position: E1, 50° 02' N., 4° 22' W.

Date: 1. vii. 48.

Depth	Temp	Salinity	Phosp	horus (mgatom/m. <sup>3</sup> )		
(m.)	(° C.)	(°/)	Total	Inorganic	Organic	
0.2	13.22	35.34	0.39	0.04	0.32	
5	13.23	35.34	0.39	0.01	0.38	
50-70	11.20	35.37	0.23	0.31	0.22	
Integral mean of water column	. —	—	0.25	0.53	0.29	

The Channel was more saline by about  $0.2^{\circ}/_{\circ\circ}$ , otherwise the two stations differed only in the bottom water which in the Celtic sea was colder and richer in inorganic and consequently in total phosphorus. The phosphorus results do not suggest that *minimum* organic production had been markedly different at the two stations.

The fertilizations of *Ophelia* were made in Celtic water on 8. vii. 48. Three separate fertilizations were made, using three females for each. One male was used to fertilize all the eggs. Each fertilization was immediately divided equally between the two kinds of water and treated as already described.

Results are summarized in Table I. They are positive in favour of Celtic water. After about a week there were many more abnormalities in Outside than in Celtic water. Prototrochal cilia were lost, without proper meta-

#### TABLE I. EXP. I. FERTILIZATIONS OF OPHELIA BICORNIS MADE ON 8 JULY 1948

	Fertilization no. 1 Condition of larvae in		Fertiliza Condition	tion no. 2 of larvae in	Fertilization no. 3 Condition of larvae in		
	Celtic water	Outside water	Celtic water	Outside water	Celtic water	Outside water	
15. vii. 48 19. vii. 48 24. vii. 48 28. vii. 48	Very good Good Fairly good, majority alive	Good Fairly good Poor Poor, majority dead	Very good Good Very good Good, all living	Good Fairly good Fair Poor, many dead	Very good Good Very good Very good, all living	Good Fairly good Fair Poor, many dead	

morphosis, earlier in Outside than in Celtic water. In Outside water very many larvae stuck together in large masses; they did so hardly at all in Celtic water. Larvae in Celtic water outlived those in Outside water.

#### Experiment 2.

Particulars of waters as for Exp. 1.

The fertilizations of *Ophelia* were made in Celtic water on 12. vii. 48. Three separate fertilizations were made, using one female for each. One male was used for all three. Each fertilization was immediately divided into both kinds of water, as already described.

Results are summarized in Table II. They are positive in favour of Celtic water. On 17. vii. 48 large numbers of larvae in all three bowls of Outside

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water were stuck together in very big masses, but larvae in Celtic water were not so massed together and appeared to be much healthier. On this date considerable numbers of larvae were removed from two bowls of Celtic water (Fertilizations 5 and 6) to supply experiments with which this paper is not concerned. After this date these two bowls therefore contained fewer larvae than the corresponding bowls of Outside water.

#### TABLE II. EXP. 2. FERTILIZATIONS OF OPHELIA BICORNIS MADE ON 12 JULY 1948

	Fertilization no. 4 Condition of larvae in		Fertilizat Condition	tion no. 5 of larvae in	Fertilization no. 6 Condition of larvae in		
a	Celtic water	Outside water	Celtic water	Outside water	Celtic water	Outside water	
17. vii. 48 24. vii. 48	Very good Very good	Good Fairly good	Very good	Good	Very good	Good	
26. vii. 48			Very good	Good	Good	Very poor, many dead	
28. vii. 48	Very good	Fairly good	Good	Fair	Good	Almost all	

#### Experiments with Echinus esculentus L.

Recently trawled urchins were used, if possible immediately after trawling, or within a day or two. For each experiment one ripe female and one ripe male were selected by microscopic examination of the gonads after removal of the oral portion of the test. The aboral surfaces were then well rinsed and the inverted urchins spawned into beakers of filtered sea water, either Outside or Celtic. The fertilized eggs were at once divided equally between two beakers and thoroughly washed with six to eight changes, one of Outside water and the other of Celtic water. From each, after thorough stirring to distribute the eggs evenly, equal volumes were removed in a glass dipper to vessels already containing equal volumes of the waters to be tested. Thus very nearly the same number of eggs was added to all vessels. Three or more vessels of each kind of water were tested in a single experiment. After 24 hr. swimming blastulae were poured off into a second set of vessels, to separate them from undeveloped or abnormally developing eggs on the bottom, except in the 1950 experiments when this was not necessary. The water-levels in the second set of vessels were equalized.

#### Experiment 3

*Celtic sea water* collected from the sea surface by means of a wooden bucket. Position:  $50^{\circ}$  36' N.,  $8^{\circ}$  04' W. (over Labadie Bank). Sounding: 70 m.

Date: 10. iii. 49. Time: 01.15 hr.

Ship: R.V. Sabella with Dr L. H. N. Cooper.

Conditions: heavy swell immediately following moderate gale (Beaufort Force 7). Vertical mixing should have been considerable.

Plankton: night haul rich with abundant Sagitta elegans, large Aglantha, Euphausians, etc. No sign of phytoplankton.

Physical and chemical observations were:

Depth	Temp	Salinity	Phosphorus (mgatom/m.*)				
(m.)	(° C.)	(°/)	Total	Inorganic	Organic		
5	9.95	35.24	0.69	0.21	0.18		
25	9.98	35.23	0.76	0.20	0.26		
60	9.98	35.23	0.79	0.49	0.30		
Integral mean of water column		—	0.76	0.20	0.26		

*Outside sea water* collected from the sea surface by means of a wooden bucket by M.F.V. *Sula* during third week of March 1949; exact position unknown. Ordinary laboratory routine collection in glass carboys which had contained sulphuric acid and had previously been well washed out.

Plankton (routine daylight haul 2 miles east of Eddystone) on 21. iii. 49, very, very sparse, hardly any zooplankton, one *Sagitta setosa* present. No sign of phytoplankton. Physical and chemical observations at station E1 were:

						Ph	osphorus (1	ngatom/m.	3)	
Depth	Temp. (° C.)		Salini	ty (°/₀₀)	T	otal	Ino	rganic	Organic	
(m.)	1. iii. 49	13. iv. 49	1. iii. 49	13. iv. 49	I. iii. 49	13. iv. 49	I. iii. 49	13. iv. 49	I. iii. 49	13. iv. 49
Surface	10.1		35.38	—	0.2	_	0.43	_	0.08	_
0.2	-	9.90		35.37		0.23	_	0.28		0.25
5	10.02	9.88	35.35	35.38	0.41	0.47	0.38	0.31	0.03	0.16
50-70	10.05	9.86	35.35	35.30	0.44	0.45	0.41	0.32	0.04	0.13
Integral mean of water column	1 10.03	9.87	35.35	35.29	0.44	0.42	0.39	0.31	0.02	0.19

The Celtic water had evidently been much richer in total phosphorus and by 10. iii there had been more conversion to organic phosphorus than had taken place a month later in the Channel. Again the Channel water was more saline.

An *Echinus esculentus* fertilization was made in Celtic water on 24.iii.49. Three bowls of each kind of water were tested.

Results are summarized in Table III. They are positive in favour of Celtic water, although the larvae in this water were the first to show shrinkage of flesh on the arms, the skeletal rods protruding uncovered (29. iii. 49). Regeneration of the arms of many larvae took place after 31. iii. 49. There was no regeneration in Outside water, although the bowls were kept until 20. iv. 49.

#### Experiment 4.

Particulars of waters, etc., as for Exp. 3, but another pair of E. esculentus used for the fertilization, which was made in Outside water. Three finger bowls of each kind of water were tested.

Results are summarized in Table IV. They are positive in favour of Celtic water, but this fertilization did not do as well as that of Exp. 3. On 28. iii. 49 the larvae in all three bowls of Celtic water were clearly healthier than those in Outside water.

#### Experiment 5.

Particulars of waters, etc., as for Exp. 3.

E. esculentus fertilization made in Celtic water on 30. iii. 49. Four bowls of

each kind of water were tested and, in addition, there were four bowls of a mixture of approximately equal volumes of both waters. Two bowls of each series were given food (*Nitzschia* and an autotrophic flagellate).

Results are summarized in Table V. They are positive in favour of Celtic water. The Mixed water was almost as good as the Celtic. Feeding made no

## TABLE III. EXP. 3. FERTILIZATION OF ECHINUS ESCULENTUS MADE ON 24 MARCH 1949

#### Condition of larvae in Celtic water (bowls 1-3)

26. iii. 49 Many at surface, few on bottom

- 28. iii. 49 Most at surface, some in mid-water, few on bottom
- 29. iii. 49 Bowl 1: about one-third at surface, a few in mid-water, most on bottom. Bowls 2 and 3: most at surface, few in mid-water, about one-third on bottom. Few dead in all three bowls. In most of the living the flesh of the arms shrinking away

30. iii. 49 Few at surface, most on bottom

- 31. iii. 49 All on or near bottom, unhealthy with shortened arms, some dead
- 4. iv. 49 Many at surface or in mid-water, most at bottom, generally short-armed but some long-armed. Some dead
- 6. iv. 49 Numbers at surface or in mid-water, many on bottom, some dead. Swimming larvae with long slender arms, on bottom many with short arms, or with none

Condition of larvae in Outside water (bowls 4-6)

Few at surface, many on bottom Some at surface and in mid-water but most on bottom, some dying

Bowl 4: about one-half mid-water to surface, remainder on bottom. Bowls 5 and 6: fair number between bottom and surface, most on bottom. Many dead in all three bowls. In the living the arms are generally normal. A little shrinkage of flesh

Few at surface, most on bottom

- All on or near bottom, unhealthy with shortened arms, many dead
- All on bottom, majority dead. Living abnormal, with very short arms or none at all
- All on bottom, a few with short arms, most with none, bodies generally shrunken and abnormal. Majority dead

## TABLE IV. EXP. 4. FERTILIZATION OF ECHINUS ESCULENTUS MADE ON 24 MARCH 1949

#### Condition of larvae in Celtic water (bowls 1-3)

- 26. iii. 49 Most at surface, few on bottom
- 28. iii. 49 Most at surface, some in mid-water, few or none on bottom. A few larvae abnormal
- 29. iii. 49 Many at surface, few in mid-water, most on bottom. Flesh of arms shrinking from skeletal rods

30. iii. 49 All in poor condition

Condition of larvae in Outside water (bowls 4-6)

Some at surface, most on bottom

Many at surface and in mid-water, a considerable number on bottom. Many abnormal

Some at surface, some in mid-water, most on bottom. Flesh of arms shrinking from skeletal rods All in poor condition

appreciable difference and did not help the larvae in Outside water. On 6. iv. 49 the pH was the same for all bowls; it did not differ from normal sea water. There was some regeneration of arms of plutei in Outside water after 9. iv. 49, but in only one bowl did this progress to any extent. In this one bowl there were fair numbers of good plutei on 20. iv. 49 when the experiment was ended. Conditions at this time varied from bowl to bowl, rather irrespective of the kind of water.

Larvae from Celtic and Outside water were photographed on 7. iv. 50 (Pl. I, figs. 1, 2).

## TABLE V. EXP. 5. FERTILIZATION OF ECHINUS ESCULENTUSMADE ON 30 MARCH 1949

#### Condition of larvae in Celtic water (bowls 1-4)

- 2. iv. 49 Many near surface, few near bottom
- 3. iv. 49 Many swimming, marked concentration near surface. Many on bottom
- 5. iv. 49 Many swimming, some concentration near surface. On bottom some abnormal
- 6. iv. 49 Many normal plutei concentrating near surface. On bottom most becoming abnormal. None dead

9. iv. 49 Many normal plutei swimming. Most on bottom abnormal

11. iv. 49 Many normal plutei swimming. Most on bottom abnormal Condition of larvae in Mixed water (bowls 5-8)

Many near surface, few near bottom Many swimming, some

- concentration near surface. Many on bottom
- Many swimming, some concentration near surface. On bottom some abnormal
- Many normal plutei concentrating near surface. On bottom most becoming abnormal. None dead Many normal plutei swimming. Most on
- Many normal plutei swimming. Most on bottom abnormal

bottom abnormal

Condition of larvae in Outside water (bowls 9–12)

Many near surface, few near bottom

- Many swimming, some concentration near surface. Many on bottom
- Few swimming, most on bottom becoming abnormal
- Very few swimming and all abnormal, those on bottom often very shrunken; some dead
- Very few swimming and all abnormal. Most on bottom very abnormal or dead
- Fair number swimming, regenerating arms. Most on bottom very abnormal

Condition of larvae in Outside water

swimming, abnormal. Most on bottom, very abnormal, half of them dead

## TABLE VI. EXP. 6. FERTILIZATION OF ECHINUS ESCULENTUSMADE ON 21 APRIL 1949

Condition of larvae in Celtic water

	Bowls 1–3, many larvae per bowl	Bowls 4–6, few larvae per bowl	Bowls 7–9, many larvae per bowl	Bowls 10–12, few larvae per bowl
25. iv. 49	Many on bottom		Few on bottom	
27. iv. 49	Very few swim- ming, most on bottom. All ab- normal, many dead	Some normal plutei swimming. On bottom some abnormal, many dead	Few swimming, most on bottom. All abnormal, many dead	Some normal plutei swimming, more than in Celtic water. On bottom some dead, some abnor- mal, fewer than in Celtic water
3. v. 49	All dead	Some normal plutei swimming. Some abnormal and some dead on bottom	Bowl 7, all dead. Bowl 8, most dead, a few very abnormal living. Bowl 9, a few	A few normal and more abnormal plutei swimming. A few dead

#### Experiment 6.

Particulars of Celtic water as for Exp. 3.

Outside water collected (from sea surface with wooden bucket) by Mr F. A. J. Armstrong in M.F.V. Sula, 13. iv. 49.

Position: E1.

Plankton: daylight haul; very, very sparse, hardly any zooplankton except pilchard eggs in some numbers, one Sagitta setosa present. No sign of phytoplankton.

For physical and chemical properties see under Exp. 3.

After the previous experiments the Berkefeld filter candles had been cleaned and re-sterilized. That previously used for filtering Celtic water was here used for Outside water, and vice versa.

*Echinus esculentus* fertilization made in Celtic water on 21. iv. 49. Four bowls of each kind of water were tested; in two bowls larvae were numerous, in two few in number.

Results are summarized in Table VI. They are negative. The fresh Outside water was slightly better than the 6-week-old Celtic water, but neither was good. Larvae did better when there were relatively few per bowl.

#### Experiment 7

Celtic sea water collected from 20 m. by means of the pump.

Position: 49° 44' N., 8° 50' W. Sounding: 89 m.

Date: 3. v. 49. Time: 20.50 hr. G.M.T.

Ship: R.V. Sabella with Dr L. H. N. Cooper.

Conditions: moderate sea, wind force 5.

Plankton: night haul rich with numerous small Aglantha, Tomopteris and young fish; very abundant plutei, one Sagitta serratodentata; Phaeocystis abundant. No Sagitta elegans.

Physical and chemical observations:

	m	0.11.1	Phosphorus (mgatom/m.*)				
(m.)	(° C.)	(°/)	Total	Inorganic	Organic		
0	11.48	35.37	0.32	0.10	0.27		
5	11.20	35.35	0.33	0.12	0.16		
20	11.20	35.36	0.34	0.15	0.22		
40	11.18	35.37	0.45	0.12	0.30		
80	10.82	35.38	0.63	0.40	0.23		
Integral mean of		_	0.46	0.53	0.53		

Outside sea water collected from sea surface with wooden bucket.

Position: L 5, 50° 11' N., 4° 18' W. Sounding: 68 m.

Date: 2. v. 49. Time: 10.20 hr. G.M.T.

Ship: M.F.V. Sula with Mr F. A. J. Armstrong.

Conditions: fresh north-west wind, sea moderate but increasing.

Plankton: daylight haul, very, very sparse except for pilchard eggs which were abundant. Four *Sagitta setosa*. No sign of phytoplankton.

Physical and chemical observations:

				Phosphorus (mgatom/m.°)			
Depth (m.)	Temp. (° C.)	Salinity (°/)	Total	Inorganic	Organic		
5	10.23	35.26	0.37	0.12	0.22		
10	10.12	35.27	0.38	0.12	0.23		
25	10.08	35.28	0.42	0.22	0.50		
50	10.08	35.31	0.21	0.26	0.25		
Integral mean of	_	· _	0.43	0.31	0.22		
water column							

The Channel water was less saline but there was nothing to choose between the waters in terms of phosphorus content.

Berkefeld filter candles again cleaned and changed over.

*Echinus esculentus* fertilization made on 6. v. 49. Six bowls of each kind of water were tested; in three bowls larvae were numerous, in three, few in number.

Results are summarized in Table VII. They are negative; there was no observable difference between comparable bowls of larvae and in neither water was a satisfactory rearing obtained. Bowls with few larvae did a little better than bowls with many. Regeneration of the arms of some plutei took place in both waters after 14. v. 49.

## TABLE VII. Exp. 7. FERTILIZATION OF ECHINUS ESCULENTUS MADE ON 6 MAY 1949

	Condition of larv	ae in Celtic water	Condition of larvae in Outside water			
	Bowls 1-3,	Bowls 4–6,	Bowls 7–9,	Bowls 10–12,		
	many larvae	few larvae	many larvae	few larvae		
9. v. 49	Normal plutei	Normal plutei	Normal plutei	Normal plutei		
	swimming well	swimming well	swimming well	swimming well		
11. v. 49	Very few swim-	Some swimming,	Few swimming,	Some swimming,		
	ming, most on	most on bottom.	most on bottom,	most on bottom.		
	bottom, becoming	A few becoming	becoming ab-	A few becoming		
	abnormal	abnormal	normal	abnormal		
14. v. 49	All on bottom,	Few swimming,	All on bottom,	Few swimming,		
	very abnormal,	most on bottom,	very abnormal,	most on bottom,		
	about half dead	all abnormal	majority dead	all abnormal		
16. v. 49	Mainly dead or dying	Some normal plutei swimming. On bottom some abnormal, some dead	Mainly dead or dying	Some normal plutei swimming. On bottom some abnormal, a few dead		

#### Experiment 8A

Celtic sea water collected from 20 m. by means of the pump.

Position: station 64, 50° 33' N., 8° 51' W., 25 miles west of Labadie Bank. Sounding: 125 m.

Date: 29. iv. 50. Time: 01.30 hr. G.M.T.

Ship: R.V. Sabella with Mr P. G. Corbin.

Conditions: wind force 4, sea slight.

Plankton: night haul, rich, abundant Sagitta elegans, two or three S. serratodentata, a few Limacina, Tomopteris, fairly numerous Euphausians. No sign of phytoplankton. Physical and chemical conditions:

Depth	Temp	Salinity	Phospl	norus (mgat		pigments	
(m.) (° (	(° C.)	(°/)	Total	Inorganic	Organic	Silicate	I m. <sup>2</sup>
20	9.78	35.19	0.61	0.47	0.14	3.3	
Integral mean		_	0.65	0.49	0.16	3.3	0

*Outside sea water* collected from sea surface with wooden bucket. Position: E I, 50° 02' N., 4° 22' W. Sounding: 74 m. Date: 3. v. 50. Time: 12.30 hr. G.M.T.

Ship: M.F.V. Sula with Dr L. H. N. Cooper.

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Plankton: daylight haul, very sparse zooplankton except for abundant pilchard eggs. *Phaeocystis* very abundant.

Physical and chemical conditions:

Depth	Temp	Salinity	Phosphorus (mgatom/m. <sup>3</sup> )		
(m.)	(° C.)	(°/)	Total	Inorganic	Organic
0	10.86	35.14	0.49	0.13	0.33
Integral mean of water column			0.25	0.12	0.32

The resources of total phosphorus were greater at the Celtic sea station but *minimum* production as assessed from organic phosphorus was less.

Two new, previously unused Berkefeld filter candles (both of same batch from maker), specially cleaned and sterilized by Mr F. A. J. Armstrong, were used for filtration.

#### TABLE VIIIA. EXP. 8A. FERTILIZATION OF *ECHINUS ESCULENTUS* MADE ON 5 MAY 1950. THICK CULTURES

	Condition of larvae	Condition of larvae	Condition of larvae
	in Celtic water	in mixed water	in Outside water
	(dishes 1-5)	(dishes 6-10)	(dishes 11–15)
6. v. 50	Majority concentrating	Majority concentrating	Majority concentrating
	near surface	near surface	near surface
8. v. 50	Most at surface, many in mid-water, few on bottom. Larvae normal	Many at surface, many in mid-water, some on bottom	Some at surface, some in mid-water, most on bottom. Larvae mainly abnormal
9. v. 50	All swimming with concentration at sur- face. Normal plutei, abnormalities rare	Many swimming with slight concentration at surface. Fair number on bottom. Larvae mainly normal	Fair number swim- ming in mid-water, most on bottom. Majority abnormal
10. v. 50	About 20% swimming.	About 20% swimming.	Few swimming.
	Larvae becoming ab-	Larvae fairly normal;	Majority abnormal,
	normal, very few dead	fair number dead	many dead
12. v. 50	Single dish. Some swimming. Larvae rather abnormal, many dead	Single dish. A few living, abnormal. Most dead	Single dish. All dead

*Echinus esculentus* fertilization made in Outside water on 5. v. 50. Three sets, each of five crystallizing dishes, used to test each kind of water and a mixture of both waters in equal proportion. Larvae were highly concentrated, there being several hundreds per dish.

Results are summarized in Table VIIIA. They are positive in favour of Celtic water and the mixed water was nearly as good as the Celtic. After 10. v. 50 only one dish of each kind of water was retained.

Two dishes, one of Celtic and the other of Outside water, were photographed side by side on 9. v. 50 (Pl. II, fig. 1). These dishes were typical of all the others. Larvae from both natural waters were photographed on 8. v. 50 (Pl. I, figs. 3, 4).

#### Experiment 8B.

Particulars of waters and fertilization as for Exp. 8A. No mixed water used. Three dishes of each kind of water were on 6. v. 50 supplied with a few surface-swimming blastulae pipetted from the two beakers containing the original fertilization (see p. 5, describing method). On 12. v. 50 to one dish of each kind of water were added, for food, a few drops of a mixture of autotrophic flagellates from culture. This was repeated on 17. v. 50.

Results are summarized in Table VIIIB. They are positive in favour of Celtic water. Larvae did badly in Outside water in spite of the low concentration. Fed larvae did a little better than unfed. Food did little to improve the

#### TABLE VIIIB. EXP. 8B. FERTILIZATION OF *ECHINUS ESCULENTUS* MADE ON 5 MAY 1950. THIN CULTURES

	Condition of larv (dishes	ae in Celtic water 16–18)	Condition of larvae (dishes	e in Outside water 19–21)	
6. v. 50	Most swimming near surface		Most swimming near surface		
8. v. 50	All in mid-water or normal	at surface. Larvae	Few at surface, Larvae mainly ab	most on bottom. normal	
9. v. 50	All swimming w plutei	ell up. Normal	Few swimming, Majority abnorma	most on bottom. d	
10. v. 50	All swimming wel well developed	l up. Most plutei	Some swimming, Majority abnorma	most on bottom. al	
	Dishes 16-17	Dish 18—fed	Dishes 19-20	Dish 21—fed	
12. v. 50	Most swimming well, normal. A few slightly ab- normal	All normal swim- ming strongly	All on bottom, all abnormal	All on bottom, majority ab- normal	
17. v. 50	Thin-armed plutei swimming well	Normal plutei swimming strongly	All on bottom, all abnormal	One or two swim- ming. All ab- normal	
20. v. 50	Thin-armed plutei swimming well	Normal plutei, half of them swimming. A few dead	All on bottom, abnormal de- generating. A few dead	One or two swim- ming, one fairly normal pluteus. Majority abnor- mal degenerat- ing. A few more dead	
25. v. 50	No change	Majority swim- ming, fairly normal plutei	All on bottom, all abnormal	One or two swim- ming. All ab- normal, often degenerating	

condition of the larvae in Outside water. The food was eaten, but it may not have been entirely suitable.

#### Experiment 9.

Particulars of waters as for Exp. 8A, but another portion in another pair of carboys used. The Berkefeld filter candles were cleaned and changed over, that used to filter Celtic water in Exps. 8A and B now being used to filter Outside water, and vice versa.

*E. esculentus* fertilization made in Outside water on 10. v. 50 and, as usual, immediately divided into halves and each washed off with one of the waters.

After  $\frac{3}{4}$  hr. it was noticed that most of the still undivided eggs were irregular, not normally rounded, inside the fertilization membrane, and this condition was more marked in Outside than in Celtic water. Two dishes of each kind of water were tested.

Results are summarized in Table IX. They are positive in favour of Celtic water. The difference was shown unusually early; this may have been due to over-ripeness of the eggs, almost all *Echinus* having spawned by this date, those few which had not usually showing histolysis of the ova. The female used for the experiment had only four ovaries. Another factor to be considered is the air temperature which was considerably higher than at any other time during the course of these *Echinus* experiments (both years). The temperature was high from 10. v to 14. v (16–19° C. outside in the shade at 10 a.m., room temperatures were two or three degrees higher) and then dropped several degrees centigrade, remaining relatively cool until the end of the month.

## TABLE IX. EXP. 9. FERTILIZATION OF ECHINUS ESCULENTUSMADE ON 10 MAY 1950

Condition of larvae in Celtic water (dishes 1-2)

- 11. v. 50 Blastulae swimming strongly, many at surface
- 12. v. 50 Many at surface, few on bottom. Few at surface, many on bottom. abnormal, few dead Abnormals and dead fairly numerous
- 14. v. 50 Few swimming; most on bottom abnormal; a few dead
- 16. v. 50 Large number swimming but majority on bottom. Arms being regenerated
- v. 50 Large number swimming strongly, majority on bottom. Further regenerated, some almost normal. A few degenerate, a few dead

Condition of larvae in Outside water (dishes 3-4)

Most on bottom, few swimming

Few at surface, many on bottom. Abnormals and dead fairly numerous Few swimming, most on bottom, abnormal, about 50% dead

Almost all dead. A very few living, abnormal

All dead

One dish of each kind of water was photographed side by side on 16. v. 50 (Pl. II, fig. 2). Each dish was typical of all others in its series.

#### Experiment 10.

Particulars of waters as for Exp. 9. This experiment ran concurrently with the latter, a different pair of adults being used for the fertilization, which was made in Celtic water. The female appeared to be partially spawned. The fertilized eggs produced normal fertilization membranes, and cleavage was also normal. Five dishes of each kind of water were tested.

Results are summarized in Table X. They are positive in favour of Celtic water. At first, larvae were weaker than usual in both waters, due possibly to the high air temperatures 10. v-14. v, or to over-ripe ova, or to both. Improvement in the Celtic water coincided with lower air temperatures from 15. v onwards.

## TABLE X. EXP. 10. FERTILIZATION OF ECHINUS ESCULENTUS MADE ON 10 MAY 1950

#### Condition of larvae in Celtic water (dishes 5-9)

# 11. v. 50 Blastulae swimming up to surface12. v. 50 A few swimming up: majority on bottom, some abnormal

- 14. v. 50 Most on bottom, a few dead
- 15. v. 50 Some fairly normal plutei swimming well up. Most on bottom, shortarmed or armless
- 17. v. 50 Many fairly normal plutei swimming, with some concentration at surface. Most on bottom, slightly abnormal

Condition of larvae in Outside water (dishes 10-14)

Blastulae swimming up to surface A few swimming up: majority on bottom, some abnormal

Most on bottom, many dead

None swimming, a large proportion dead. Living larvae very abnormal

A very few swimming; majority on bottom. All still living very abnormal

#### Experiment with Sabellaria alveolata (L.)

The success of the early experiments with *Ophelia* and with *Echinus* made it desirable that a trial should be made of other species. So far only one other has been tried; this was *Sabellaria alveolata* which on 12. iv. 49 was collected at Duckpool, near Bude, on the north coast of Cornwall, where it is abundant.

#### Experiment 11.

Particulars of waters as for Exps. 3-5.

A fertilization was made in Celtic water on 13. iv. 49. This was equally divided and washed off in both kinds of water in the usual way. Surface-swimming larvae were decanted into clean bowls on 14. iv. 49. One finger bowl of each kind of water was tested. For food, a few drops of a culture of an autotrophic flagellate was added to both bowls on 14. iv. 49 and again on 20. iv. 49.

## TABLE XI. EXP. 11. FERTILIZATION OF SABELLARIA ALVEOLATA MADE ON 13 APRIL 1949

	Condition of larvae in Celtic water	Condition of larvae in Outside water
20. iv. 49	Very few abnormal larvae	Some larvae abnormal, losing bristles
25. iv. 49	Majority normal, fast swimming. Very few abnormal	Many abnormal. All larvae swimming slowly
27. iv. 49	Majority normal, fast swimming. Very few abnormal	Many abnormal. All larvae swimming slowly
3. v. 49	Many abnormal, many dead	Many abnormal, many dead
10. v. 49	Many abnormal, many dead, but some normal, though lethargic, larvae	Similar to Celtic water

Results are summarized in Table XI. They are positive in favour of Celtic water. At first larvae in both bowls were similar, but after a week some of these in Outside water were losing the long provisional bristles characteristic of this species (Wilson, 1929). On 25. iv. 49 a big proportion of these larvae were abnormal. They had few or no bristles, shrunken bodies, and were slow swimming. In Celtic water, on this date, there were very few abnormal larvae to be seen; almost all of them were quite normal and fast-swimming. After another week, however, both lots of larvae were in poor condition. They were

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too crowded for there to have been any possibility of normal growth through their long pelagic development.

#### Other Experiments

A few other experiments were undertaken; they may be mentioned briefly. An experiment with *Ophelia bicornis* similar to those already described was begun 5 days later than Exp. 2, using waters from the same collections. This fertilization of *Ophelia* made on 17. vii. 48 gave only a relatively small number of larvae. Those in Celtic water did better than those in Outside water, but the difference was not as great as in the earlier experiment, and in neither water did the larvae do well. The fertilization may have been poor initially, or the Celtic water with longer storage was losing its health-imparting property.

Using the Outside water obtained at L 5 on 2. v. 49 (details as for Exp. 7), some tests were made to see if it could be improved by the addition of various substances. A fertilization of *Echinus esculentus* was made in this water on 20. v. 49 and distributed in equal quantity among a number of finger bowls. Addition of soil extract in the proportion used by Gross (1937) to two of the bowls did not improve matters, and the larvae after 5 days were in poorer condition than in three bowls of untreated water. At the suggestion of Dr H. W. Harvey other bowls were treated with vitamin B<sub>1</sub> (aneurin hydrochloride), and another set with biotin in various minute proportions. These appeared to have no effect. In this experiment the larvae did rather better than in the same Outside water in Exp. 7, but there was no test of Celtic water with this particular fertilization.

The observation that larvae sometimes regenerated the larval arms led to an experiment to establish the fact more firmly. On 6. iv. 49 a small number of larvae with very short stumpy arms were picked out from one bowl of Celtic water and one of Outside water (bowls forming part of Exp. 5) and placed in new bowls of Celtic water and given autotrophic flagellates for food. On II. iv. 49 it was observed that a good proportion of these larvae had fairly long arms, and by 20. iv. 49 most of them had normal arms. At first the arms were thin and needle-like, but later were of normal thickness. The larvae transferred from Celtic water did rather better than those from Outside water where a higher proportion failed to regenerate any of the arms.

#### DISCUSSION

In experiments of the character just described, one of the most fruitful sources of error would be concealed contamination. In spite of every effort to achieve complete cleanliness, and to treat both sides of an experiment alike, a small unobserved detail of procedure might bias one side more favourably than the other. The technique adopted has been carefully considered with this in mind.

If we take the 1950 experiments alone, it could be pointed out that whereas the Celtic water was collected from 20 m. with a pump, the Outside water was

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obtained by another ship from the surface with a bucket. The original intention had been to collect both samples with the pump, but weather and other uncontrollable factors had made this impossible. Incidentally, when collecting with the pump the water was sucked through a hose and through the carboy to the pump so that water in the carboy had no contact with the pump itself. A thorough wash through was given before any water was actually taken. Similarly with the wooden bucket method of collection, great care was taken to wash out well the bucket and carboy before the latter was filled. The carboys were thoroughly cleaned in the chemical laboratory ashore, before being taken to sea. (For full details of the method of collecting water samples see Cooper, 1951.) Even so, the objection may be raised that the methods of collection for the 1950 experiments were not the same for both waters. Criticism along these lines, however, can probably be discounted when it is remembered that for the 1949 experiments both waters were collected with wooden buckets.

Another criticism, raised during the experiments, was the possibility of some form of contamination by the Berkefeld filter candles. It might be that one candle affected the water differently from the other. This criticism was met by changing over the filter candles so that the one previously used for Outside water was now used for Celtic water, and vice versa. This was done both in 1949 (see notes to Exps. 6 and 7) and in 1950 (see note to Exp. 9) and in both years, particularly in 1950, it is very evident that the results were unaffected by this procedure (Pl. II, figs. 1, 2). Moreover, the pair of candles used in 1950 was not the same as the pair used in 1949. There is no reason to continue to suspect the filter candles, although it will still be wise to change them over with each new experiment.

The technique used would result in a slight variation in the volume of water and number of larvae per test vessel in each experiment. The variation would, however, be very small and would not be biased in favour of either kind of water. Larvae behaved alike in all test vessels of water of one origin. The small variation in volume and number has therefore no significance. Moreover, consideration of Exp. 7, and comparison of Exp. 8A with 8B, shows that acceleration of the onset of abnormality and death is not unduly accelerated by crowding.

Apart from the experimental details just discussed and discounted no other possible sources of error have been discovered. Thus we conclude that the difference between the waters is real and natural. It should now be considered whether the difference is in any way related to seasonal changes in the plankton, for it is possible that past floras and faunas will have extracted or excreted substances, the absence or presence of which will affect developing larvae. Lucas (1949) has discussed the ecological significance of metabolites, some of them stimulating, others the reverse, in marine and other environments.

The sample of Celtic water which in Exp. 7 proved to be no better than Outside water was observed, before filtration, to contain abundant *Phaeocystis*.

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This colonial flagellate was also abundant, before filtration, in the Outside water used in Exps. 8A, 8B and 9. It was not, however, seen in other Outside water samples. It is just possible that it was responsible for the poor condition of the Celtic water used in Exp. 7, but in view of the fact that Outside water, whether it contained *Phaeocystis* or not, was always poor, such an interpretation must be regarded with suspicion. However, the possibility cannot be ruled out entirely.

Except for Phaeocystis there is no other correlation of poor water conditions with the observed plankton content, with the possible exception of the occasional abundance of pilchard eggs. It seems therefore more likely that the observed difference is due to something more fundamental, the presence or absence of traces of one or more elements or perhaps traces of organic materials. The results obtained by mixing equal quantities of the two waters (Exps. 5 and 8A) suggest that Celtic water contains something essential for normal development of some species which is lacking in Outside water. Other explanations of the effect of mixing may be possible, but this is perhaps the most reasonable assumption to take as a starting-point for investigation. The importance of various micro-constituents of sea water, especially organic substances and manganese, in the growth of phytoplankton has been emphasized in the work of Harvey (1947, 1949, 1950); it is very probable that the health of zooplankton is likewise dependent on the presence of trace substances, though not necessarily the same substances, and that different species have different requirements.

The bodily forms of the unhealthy plutei (Pl. I, fig. 2) which developed in Outside water are certainly suggestive of a deficiency disease. They show analogous resemblances to plutei experimentally produced by workers concerned with the mechanics of sea-urchin development. With increasing age these abnormal larvae became more and more shrunken, unless they died first.

In the experiments there is evidence (see Exp. 6 and p. 15) that Celtic water deteriorates with storage in ordinary glass carboys at room temperature. Adsorption of the health-promoting factor by the glass is one of various possibilities.

Altogether, during two breeding seasons, eight distinct fertilizations of *Echinus* have been used to test comparatively Celtic water collected on three cruises, and Outside water collected on four separate occasions. In addition, there were separate collections of water for the tests with *Ophelia*; the *Sabellaria* tests used water collected for *Echinus*. Of the eight *Echinus* tests, six were positive in favour of the Celtic water and two were neutral. Possible reasons for the two latter have already been discussed above. The *Ophelia* and *Sabellaria* tests were all positive in favour of Celtic water. In all these experiments the only good rearings have been in Celtic water and all Outside water rearings have been bad. It does not follow, however, that Outside water is always bad. At present, the most that can be inferred is that

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good rearings are more likely to be obtained by using Celtic water than by using water from the Channel in the region of the Eddystone. So far, no comparative tests have been made with inshore water.

This is the first observation that has been made to show that the difference in bottom faunas from one region to another may be related to the ability, or otherwise, of larval stages to develop in the overlying water mass. For instance, Luidia ciliaris (Philippi) is at the present time infrequent off Plymouth where about eighteen years ago it was common, but it is known that its larvae are indicators of *elegans* water and were present in the area when it was covered with *elegans* water (Russell, 1936*b*, p. 597). Other species dependent to a greater or lesser extent on *elegans* water are likely to have been adversely affected when that water mass moved away from the area. Thus Munida bamffica (Pennant), formerly abundant on the Plymouth trawling grounds, is now at best exceedingly rare, while there has been a reduction in the abundance of Echinus esculentus itself which when needed in quantity has to be trawled more to the westward than formerly. Sabellaria alveolata, although still present at the mouth of the Erme estuary, also seems less common than it used to be. It must not be thought, however, that the fauna of the Plymouth area shows a general allround reduction; on the contrary, it is probably true to say that most species are as plentiful as ever, and some may even be more plentiful. It is evident that many animals find no difficulty in living and reproducing under water conditions that seem to affect some other species adversely. Nevertheless, it is possible that at least some of the former are invigorated by an elegans environment, and that under laboratory conditions their larvae may succeed in elegans water when they will fail in that obtained from another water mass. Ophelia bicornis is an instance of a species thriving in a non-elegans environment, though there is no doubt that its larvae did better in the laboratory when kept in Celtic water (which in these experiments seemed always to have been elegans water) than in Outside water from the Eddystone region. It should be noted that Ophelia larvae have not been tested with the inshore estuarine water of their natural environment, which may well be more suitable for them than Outside water.

#### SUMMARY

Eggs and larvae of *Echinus esculentus*, *Ophelia bicornis* and *Sabellaria alveolata* developed abnormally, or were in poor health, in sea water collected from the English Channel in the region of the Eddystone, although in water collected from the Celtic sea development was generally normal and healthy. Experiments indicated that the Channel water lacked some unknown constituent, essential for healthy development of these species, present in the Celtic sea. The results accord with some of the hydrographical and biological changes in the area which have taken place within recent years.

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2-2

#### EXPLANATION OF PLATES

#### (All photographs on both plates are by electronic flash at about $\frac{1}{3000}$ sec.)

#### PLATE I

- Fig. 1. Normal swimming plutei from Celtic water, Exp. 5. Photographed alive on 7. iv. 50 when 8 days old,  $\times 43$ .
- Fig. 2. Abnormal swimming plutei from Outside water, Exp. 5. Photographed alive on 7. iv. 50 when 8 days old,  $\times$  43. At this time no normal plutei were present in any of the Outside water dishes. Many lying on the bottom were in worse condition than these.
- Fig. 3. Normal plutei from Celtic water, Exp. 8A. Larvae in Celtic water in Exp. 8B were indistinguishable from these. Photographed alive on 8. v. 50 when 3 days old, ×25.
- Fig. 4. Abnormal plutei from Outside water, Exp. 8A. Larvae in Outside water in Exp. 8B were indistinguishable from these. Photographed alive on 8. v. 50 when 3 days old, × 25.

#### PLATE II

- Fig. 1. Typical dishes from Exp. 8 A photographed on 9. v. 50 when the larvae were 4 days old. On the left is an Outside water dish with most of the larvae concentrated in a patch on the bottom, although a fair number are still swimming in mid-water. The other four Outside water dishes were similar. On the right is a Celtic water dish with all the larvae swimming well up. The other four Celtic water dishes were similar.
- Fig. 2. Typical dishes from Exp. 9 photographed on 16. v. 50 when the larvae were 6 days old. The Berkefeld filter candles had been changed over since Exps. 8A and B (see p. 12). On the left is a Celtic water dish with many larvae swimming well up although some are resting on, or are close to, the bottom; few are dead. The other Celtic water dish was similar. On the right is an Outside water dish with almost all the larvae dead in a large patch on the bottom. The other Outside water dish was similar.

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# WILSON. PLATE I



# PLATE II WILSON.

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### CHEMICAL PROPERTIES OF THE SEA WATER IN THE NEIGHBOURHOOD OF THE LABADIE BANK

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#### (Text-figs. 1-3)

A number of excursions to the Celtic Sea have been made, first, to obtain for Dr D. P. Wilson water as contrasted as possible with that in the English Channel near Plymouth, and secondly to obtain answers to hydrographical queries. This paper presents some of the chemical findings which may prove to be relevant to Wilson's investigations on the development of the young planktonic stages of invertebrates. Since not all sea water in the Celtic Sea was likely to be equally suitable for his work, some means was needed for quickly assessing waters on the spot. There are a number of objections to the use of phosphate or other chemical analyses on board so that examination of plankton hauls was chosen. Suitable water was selected on the basis of the bulk and variety of zooplankton and the presence of species known to indicate '*elegans*' water. The 2 m. stramin ring-trawl, fished for the standard half-hour oblique haul, was used.

On the earlier cruises the water was sampled at the surface with a wooden bucket and filled into carboys through a wooden funnel fitted with a 200-meshesto-the-inch silk strainer. The occasional high values for phosphorus compounds and iron found in surface water suggest that various kinds of dross may accumulate there so that, in the later work, collection was made from 20–30 m. depth through a rubber hose. This water did not pass through the pump used.

All carboys were very well cleaned by Mr F. A. J. Armstrong personally, using water-white technical grade sulphuric acid, followed by Plymouth tap water and plenty of distilled water. Chromic cleaning mixture was never used. The bark bung was covered with a clean piece of waterproof paper. The wooden bucket and funnel are well cared for and were vigorously swilled with sea water on the spot. Soap was never used. The rubber hose was washed out by running water through it to waste for several minutes.

'Celtic' water, shown by Wilson's experiments to promote more healthy growth and development than 'Outside' or 'Channel' water, was drawn from the following positions which are shown by circles in Fig. 1.

Date	Lat.	Long.	General position
30. vi. 48	50° 00' N.	7°10' W.	35 miles west of Scilly Isles
10. iii. 49	50° 36' N.	8° 04' W.	Over Labadie Bank
29. iv. 50	50° 33' N.	8° 51' W.	20 miles west of Labadie Bank (Station 64)

L. H. N. COOPER



Fig. 1. Areas of mud and ooze (stippled) in the Celtic Sea (after Laurie Norie and Wilson's chart (1919) of the Outer Grounds of the English Channel). Stations, marked by crosses, worked in April 1950 (prefixed by letter A) and in June 1950 (prefixed by letter J). Stations, marked by circles and dated, where waters for Dr Wilson's experiments (1951) were collected.

For each of these waters a description of the chemical properties has already been given in Wilson (1951, pp. 3, 5, 10).

No attempt has been made to contrast one Celtic Sea water with another. We cannot say whether waters which yielded sparse catches with the ring trawl would have caused poor development of *Echinus* and other invertebrates. The positive conclusions were: (a) water collected in spring over or a few miles to the west of the Labadie Bank was favourable for the development of young *Echinus esculentus*, a sea-urchin, and *Sabellaria alveolata*, a polychaete worm; (b) water collected at midsummer 35 miles west of Scilly was favourable for development of the polychaete *Ophelia bicornis*.

It is not yet possible to say how far these conclusions may be generalized for other seasons and other areas in the Celtic Sea. None the less, the March 1949 search for a suitable water was conducted much at random and several poor plankton catches were taken before the rich one over the Labadie Bank. In April 1950, also, suitable water was found not far away. Since other lines of evidence are pointing to the Labadie area as important in the economy of the Celtic Sea, it is proposed to report them here.

Around the Labadie Bank weak tides should facilitate the accumulation of detritus in the deeper water and the deposition of fine sediment. Much mud and ooze on the sea-bed surrounding the bank is shown on the chart of the outer grounds of the English Channel, published in 1919 by Laurie Norie and Wilson and based on Admiralty soundings with the primed lead (Fig. 1). The areas of mud and ooze are stippled. Richness of this mud in organic matter still needs to be demonstrated.

In April 1950 hydrographical stations were worked on a course laid over the bank. Due to overcast weather there is a possible error of 5 miles or so in the dead reckoning of some of the positions so that stations 68 and 81 shown as lying outside of the muddy area may actually have lain within it. It was anticipated that regeneration and breakdown of suspended detritus and of mud would be revealed by a notably higher content of total and phosphate phosphorus in the bottom water. The results confirmed the hypothesis although they did not prove it (Fig. 2).

At stations 62, 61, 53 and 52 on Section I (Fig. 2) and at station 67 on Section II (Fig. 3) which all overlay mud or ooze, the content of phosphatephosphorus in the bottom water was relatively high. Total phosphorus was high by the same amount, providing strong confirmation of this enrichment of the bottom water. At station 81 a single analysis on the bottom water gave a high content of total phosphorus but no parallel analysis of phosphatephosphorus was made.

At station 85 over another area of mud in the 'submerged estuary' of the 'Irish Channel River', high content of total- and phosphate-phosphorus was also observed in the deeper water. The neighbouring station 86 over mud did not show the phenomenon, whilst station 65 over mud to the west of Labadie Bank showed it only weakly.

There was no evidence of bottom regeneration of any form of phosphorus at the remaining forty-three stations worked in April 1950 over non-muddy



Fig. 2. Vertical Section I of stations worked over mud or ooze for (A) total phosphorus, (B) phosphate-phosphorus and (C) organic phosphorus in the Celtic Sea, 25–29 April 1950. Areas of mud and ooze are shown by thickening of the bottom lines of the sections.

bottoms. On the June cruise also there was no evidence of bottom regeneration from the non-muddy areas; the course from stations 12 to 17 (Fig. 1) lay over small muddy patches, although only one actual station was so placed. Inter-

pretation of the small differences observed, complicated by thermal stratification, is difficult.

Enrichment of the bottom water by breakdown of suspended detritus or of ooze and mud is unlikely to be confined to phosphate. Other nutrients and



Section II

Fig. 3. Vertical Section II of stations worked over mud or ooze, as in Fig. 2.

growth-affecting substances are likely to accompany it. Rich catches of the larger zooplankton may be had from many parts of the Celtic Sea where a number of very different methods of enrichment are possible. None the less, it is a suggestive coincidence that two of the water samples which proved successful in Wilson's experiments were taken in the Labadie area, where regeneration of phosphate and presumably other growth-promoting substances was occurring in the deeper water. The neighbourhood of the Labadie Bank should repay intensive study.

Thanks are tendered to Mr F. S. Russell, Dr H. W. Harvey, Mr G. A. Steven, Dr D. P. Wilson and Mr P. G. Corbin for valuable discussions, to Mr F. A. J. Armstrong who carried out all the chemical analyses and with great care prepared all the gear for sea, and to Lieut.-Cmdr. C. A. Hoodless, D.S.C., R.N.R., and the crew of R.V. *Sabella*.

#### SUMMARY

The manner of collecting water samples for Dr D. P. Wilson's experiments on a biological difference between natural sea waters has been described. Two of these samples were from the neighbourhood of the Labadie Bank.

Chemical results are presented which suggest that extensive regeneration of phosphate is occurring from the mud and ooze deposits which surround the Labadie Bank or from detritus accumulating in the deeper water due to weak tides.

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# THE DEVELOPMENT AND EARLY STAGES OF THE ASCIDIAN PYURA SQUAMULOSA (ALDER)

#### By R. H. Millar

The Marine Station, Millport

#### (Text-fig. 1)

In the family Pyuridae the larval and post-larval stages are known only in *Halocynthia pyriformis* (Rathke) and *Boltenia echinata* (L.). Berrill (1948) has pointed out that this is the least specialized family of the Pleurogona and that its larvae are therefore of particular interest.

On I August 1950 specimens of *Pyura squamulosa* (Alder), up to 36 by 30 mm., were collected from boulders at low-water level in the Sound of Kerrera, Argyll. They were taken on the following day to the Marine Station, Millport, and there kept in running sea water until 4 August, when artificial fertilizations were carried out.

Egg (Fig. 1A). The ripe ovum is spherical, orange-pink, and 160  $\mu$  in diameter. It is surrounded by loosely arranged inner follicle cells, a chorion, and a complete layer of rounded outer follicle cells. When the egg is freshly drawn from the ovary there is a narrow perivitelline space, but after some minutes in sea water the space is enlarged by the osmotic uptake of water and the diameter of the chorion increases from 175 to 190  $\mu$ . The final external diameter of the layer of outer follicle cells is about 220  $\mu$ .

Sperm (Fig. 1D). The form of the sperm is typical of that of many ascidians. The rod-like head is  $9\mu$  long and has a protoplasmic bulge near the point of attachment of the tail. The tail is a flagellum about  $50\mu$  long.

*Fertilization and development.* The eggs and sperm from two individuals were mixed in sea water, and in spite of the alleged difficulty in obtaining fertilized eggs of pyurid ascidians (Berrill, 1950), development proceeded, and apparently normal larvae were obtained in large numbers. Cleavage follows the pattern which is now well established in ascidians. A deep orange crescent (Fig. 1B) appears in the fertilized egg like the yellow crescent of *Styela partita* (Conklin, 1905) and the bright orange crescent of *Boltenia echinata* (Berrill, 1948); pigmentation of this kind is unknown in other ascidians. The pigment is later localized in the tail of the larva (Fig. 1C). Most of the eggs fertilized about 11.30 a.m. on 4 August, and kept in Petri dishes at 18–19° C. had hatched as active larvae by 9.30 a.m. on the following day.

Larva (Fig. 1 E). The trunk of the larva is about  $200 \mu$  long and the tail about  $730 \mu$  long, inclusive of the posterior projection of the fin. There are two dorsal and one ventral anterior papillae of conical shape. The fin runs round the



Fig. I. Development of Pyura squamulosa (Alder). A, fertilized egg showing concentration of pigment forming orange crescent. B, first cleavage, showing orange crescent. C, embryo with trunk and tail differentiated and orange pigment localized in tail. D, sperm. E, larva. F, sagittal section through larval sensory vesicle. G, metamorphosing larva. H, post-larval stage with ampullae. I, young ascidian. amp., ampulla; at. op., atrial opening; br.s., branchial sac; ep., epidermis; es., endostyle; fn., fin; le.c., lens cell; lo., anterior lobes; nch., notochord; oc., ocellus; ol.op., oral opening; or.c., orange crescent; or.pg., orange pigment; ot., otolith; pp., papillae; sen.pg., remains of larval sensory pigment (otolith and ocellus); sen.ve.w., wall of sensory vesicle; st., stomach; ta., tail being resorbed.

trunk in the sagittal plane and bifurcates on the dorsal surface of the trunk to send a short ridge forward to each dorsal papilla. On the ventral surface of the trunk the fin remains simple and runs forward to the single ventral papilla. The rudiment of the endostyle occupies the ventral part of the trunk, and the sensory vesicle lies near the dorsal surface. Both otolith and ocellus are present and well developed (Fig. 1F). The otolith is contained in a single cell projecting up from the floor of the sensory vesicle, and consists of a spherical or ovoid mass of black pigment. The mean value of the greatest diameter of this pigment mass is  $132 \mu$ . On the postero-dorsal wall of the sensory vesicle lies the ocellus. This has a cup-shaped mass of black pigment of which the mean diameter across the opening is  $14.3 \mu$ . In addition to a number of retinal cells there are, opposite to the opening of the pigment cup, three cells each containing a large clear body acting as a lens. The distal ends of the lens cells are closely applied to the lower surface of the dorsal epidermis. No trace was found of the rudiments of siphons, or peribranchial sacs. The tail has the usual single row of notochordal cells surrounded by muscle cells, and also contains the pigment which formed the orange crescent of the fertilized egg. Before the larva hatches the tail is coiled in a horizontal plane.

#### METAMORPHOSIS AND POST-LARVAL DEVELOPMENT

Within 12 hr. of hatching resorption of the tail is in progress both in larvae attached to the glass of the vessel and in those unattached (Fig. IG). At the same time the trunk becomes shorter and wider. Eight lobes now appear round the anterior margin of the trunk, and the tail begins to shorten; the test round the tail, however, is not resorbed. By the time the tail has disappeared the eight anterior lobes have developed into eight ampullae growing out laterally over the substratum. At this stage two black pigment spots represent the remains of the larval otolith and ocellus, and the orange pigment from the larval tail forms a band across the body of the young ascidian (Fig. 1H). Later the ampullae become irregular in outline and constricted at the base, and soon some of them disappear. At about this time the endostyle becomes clearly visible. At the next stage the functional ascidian is recognizable, having oral and atrial openings, active body musculature, branchial sac with protostigmata, and stomach containing food masses (Fig. 11). The ampullae are represented by one or two small irregular projections from the basal part of the body. The remains of the larval otolith and ocellus show as two black spots on the right side of the body, and not, as found by Berrill (1929) in Halocynthia pyriformis, near the adult ganglion.

Subsequent development was not followed, as all the major changes in organization from larva to adult were now complete.

The development time-table is given below.

4 August: 11.30 a.m., fertilization; 1.00 p.m., 1st cleavage; 2.30 p.m., 4th cleavage; 3.30 p.m., 5th cleavage; 5.00 p.m., gastrulation; 6.00 p.m., deep gastrula; 9.00 p.m., differentiation of trunk and tail. 5 August: 9.30 a.m., hatching of larvae almost complete; 7.30 p.m., tail resorption in progress. 7 August: tail completely resorbed; ampullae well developed. 8 August: diameter of post-larva over ampullae 0.33 mm. 10 August: diameter of post-larva over ampullae 0.48 mm. 14 August: oral and atrial openings, branchial sac and gut differentiated. 17 August: feeding first observed.

The temperature from the time of fertilization until the end of tail resorption was  $18-19^{\circ}$  C. and thereafter  $23^{\circ}$  C.

In Table I some of the important features in the development of *Halo*cynthia pyriformis, Boltenia echinata and Pyura squamulosa are compared.

	I ABLE I		
	Halocynthia	Boltenia	Pyura
Pigmented crescent in develop- ing ovum	0	+	+
Diameter of ovum (mm.)	0.26	0.18	0.16
Larval sense organs	Otolith and ocellus	Otolith and ocellus	Otolith and ocellus
Number of lens cells in ocellus	3	3	3
Rudiments of peribranchial sacs in larva	÷	÷	ō
Number of ampullae in post- larva	4	4	8

The Pleurogona (= Stolidobranchiata Lahille, 1886) comprises the families Styelidae, Pyuridae, and Molgulidae. In these families the sensory equipment of the larva is variously modified or reduced. Thus the ocellus has disappeared in the larvae of all the Molgulidae, the most highly developed of pleurogonid ascidians. In the Styelidae the ocellus may be combined with the otolith (subfamily Botryllinae), or absent (Dendrodoa, Polycarpa), or merely reduced (Styela). The larvae of the Pyuridae have hitherto been known only from *Halocynthia pyriformis* and *Boltenia echinata*. In both of these the ocellus is present and of normal structure (Berrill, 1950), though not so well developed as in the larvae of the enterogonid ascidians.

In Pyura squamulosa the larger size of the ocellus represents a more primitive condition. The larva is also less specialized in that it shows no trace of precociously developed peribranchial sacs or siphon rudiments such as those of *Boltenia*. It is indeed nearer to the enterogonid type of larva than that of any other known pleurogonid ascidian. The small size of the egg also indicates a condition at least as primitive as *Boltenia* and considerably more primitive than *Halocynthia*. The presence of a pigmented crescent in the developing egg of *Pyura squamulosa* and *Boltenia* suggests that these forms may be closer than *Halocynthia* to the styelid stock, at least one member of which shows a similar pigmented crescent.

#### DEVELOPMENT OF PYURA

Evidence gained from a study of the development of *Pyura* therefore indicates it to be more primitive than either *Boltenia* or *Halocynthia*, the other two pyurid ascidians whose development is known.

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### CORELLA HALLI N.SP., A NEW ASCIDIAN FROM THE ENGLISH CHANNEL

#### By Patricia Kott, M.Sc.<sup>1</sup>

From the Plymouth Laboratory

#### (Text-fig. 1)

In September 1950 Dr H. W. Parker of the British Museum (Natural History) kindly sent me a specimen of an ascidian collected by the yacht, *Manihine*, for identification. This has proved to belong to an unnamed species of the genus *Corella*, here described as *C. halli*. The unique type specimen was obtained from dredgings on a shingle bottom at 35 fathoms, 10 miles WSW. of the Casquets in the English Channel, 30 August 1950 (*Manihine*, Station no. 81).

*External features.* The animal (Fig. 1A) is enclosed in a transparent test, laterally flattened, circular, of 4 mm. diameter and fixed basally to pieces of sand; there is no stalk. The branchial aperture is terminal anteriorly; the atrial aperture is also anterior and dorsal to the branchial aperture. Both apertures are sessile.

*Mantle.* The animal inside the test is 2 mm. in diameter. The mantle musculature is weakly developed (Fig. 1B): there is one circular muscle band around the base of each siphon, five narrow muscle bands run transversely across the anterior aspect of the body ventral to the branchial siphon, another five bands radiate from each side of the branchial siphon and two similar bands radiate from the atrial siphon. In the postero-dorsal corner of the left side of the body there are seven narrow bands of muscle set obliquely.

The atrial siphon is bordered by six small pointed lobes and the branchial siphon has eight similar lobes.

*Tentacles*. The branchial tentacles are simple; there are fifteen long slender tentacles alternating with the same number of very short tentacles.

The dorsal tubercle (Fig. 1 C) is anterior and to the left of the neural ganglion and slightly on the left side of the peritubercular prebranchial space. It is of the usual semicircular form typical of this genus, with the concavity directed anteriorly and to the right.

*Dorsal lamina* (Fig. I C). Languets representing the dorsal lamina are short, broad laterally, and flattened antero-posteriorly. Terminally they come to a sudden small point and are not long and tapering as are the dorsal languets of *C. parallelogramma* (Müll.). In this specimen there are eleven languets.

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Branchial sac. The form and arrangement of the stigmata (Fig. 1D) are similar to those found in immature specimens of C. parallelogramma: the stigmata are arranged in pairs in fourteen transverse rows. There are twenty longitudinal vessels on each side of the body supported by papillae between each of the stigmata; intermediate transverse vessels are present and these occasionally carry a small papilla between two successive longitudinal vessels. The anterior and posterior corners of the stigmata of each pair are turned inwards



Fig. 1. Corella halli n.sp. Type specimen. A, zooid from the right side showing alimentary canal,  $\times 24$ ; B, zooid from the left side showing mantle musculature,  $\times 24$ ; C, region of the neural ganglion showing the anterior part of the dorsal limina, the dorsal tubercle and neural ganglion,  $\times 72$ ; D, part of the posterior end of the branchial sac showing one pair of the stigmata joined in the horizontal plane,  $\times 72$ .

and in the posterior part of the sac there are still a few stigmata joined in the horizontal plane (Selys-Longchamps, 1901, pl. XXIV, fig. 7).

Alimentary canal (Fig. 1A). The oesophagus is short and narrow and opens into a spindle-shaped stomach with twelve longitudinal furrows and a short pyloric caecum. The anal opening is fringed by eight rounded lobes. The alimentary canal is completely on the right side of the body. Gonads. Gonad cells are present and spread diffusely over and between the loop of the alimentary canal as in other species of this genus.

Relations. In the form of its branchial sac this specimen might be identified as a young specimen of C. parallelogramma (Müll.); it is here described as a new species, however, since there are certain distinctive features which do not occur in C. parallelogramma and since the presence of gonad cells indicate a mature form. The species is distinguished from C. parallelogramma by the following characters: the tentacles are of two orders, long alternating with very short; the alimentary canal is on the right-hand side of the body, whereas in the young forms of C. parallelogramma which have been described the alimentary canal is posterior to the branchial sac; the stomach is longer and more spindle-shaped and there are lobes present around the anus while the anal borders of several specimens of C. parallelogramma examined are smooth; the languets of the dorsal lamina are fewer. Unless further collection and study of the development of C. parallelogramma indicates that this specimen is merely an individual variant of a young form, the species is particularly interesting and shows a primitive condition of the branchial sac in the evolution from straight to coiled stigmata. It is natural to assume that there may be some species in the transition stage which the form described above would represent; a stage in the evolution of Corella which is recapitulated in the life history of the more highly specialized C. parallelogramma.

*Corella halli* n.sp. is named after Major H. W. Hall, M.C., who has made his yacht *Manihine* available to the British Museum for collecting; the specimen comes from the *Manihine* collections of the British Museum (Natural History) where it is deposited under the type number B.M. 1950.9.21.1. My thanks are due to Dr H. W. Parker for giving me the opportunity of describing it.

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# TURBONILLA ELEGANTISSIMA (MONTAGU), A PARASITIC OPISTHOBRANCH

# By Vera Fretter

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# (Text-figs. 1-2)

Turbonilla elegantissima (Montagu), though one of the larger and more familiar members of the family Pyramidellidae, is known only by the shell and the appearance of the animal when crawling. Forbes & Hanley (1853) and Jeffreys (1867) state that it is common around the British Isles, and that its foreign distribution is from Tromsö to the Mediterranean. In the fauna list of the Marine Biological Association (1931) it is recorded as T. lactea (L.) 'occasionally under stones, particularly where there is a certain amount of silt' from low water to a depth of 10 fathoms, and, in the Salcombe estuary, 'verv common on the Zostera south of Pilworthy Pt., (Allen & Todd, 1900)'. Actually this opisthobranch has its own extremely specialized habitat, as do, apparently, all other members of its family (Fretter & Graham, 1949): like them it is an ectoparasite, restricted to the vicinity of its host. Specimens have been collected from the laminarian zone along the south coast of Devon and Cornwall, the largest number from Church Reef, Wembury, where silt and sand around boulders and between shale ledges provide suitable niches for such sedentary worms as Audouinia tentaculata and Amphitrite gracilis. When covered by the tide the tentacles of these polychaetes extend through the silt and sand and spread over the surface of the rocks for a considerable distance, and it is in the vicinity of these tentacles that Turbonilla lurks to suck liquids from them by means of a long proboscis (Fig. 1). Audouinia and Amphitrite were also recorded at Pilworthy Pt., but their association with Turbonilla was then unknown. When the laminarian zone is uncovered the mollusc may wander some distance from its host, in a more superficial position on the shore, clinging to the half-buried stones or just hidden by the silt. It has never been collected from exposed sandy shores, which might offer a suitable habitat for the host, but no safe crannies for the protection of the parasite. Thus Turbonilla burrows to seek its food, and in this respect differs from the Odostomia spp. which have been described (Fretter & Graham, 1949), for they rest on or near the entrances of the tubes of tubicolous worms, and when they feed the extending proboscis is directed upwards through the water, free of contact with the mentum beneath it, arching over in a U-shaped bend to reach the tissues of the host. Turbonilla, by contrast, is subterranean, ploughing its

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way forwards in search of food and, on sensing it, thrusting out a stout proboscis which is supported by the mentum (M) and pushed through the silt to reach the host. When the feeding process of *Turbonilla* has been observed in the laboratory the proboscis has been seen to suck blood from the tentacles of *Audouinia*, even a solitary writhing tentacle fragmented from the worm being pursued and sucked, whilst, if *Amphitrite* be the prey, either tentacles or gills may be attacked. On no occasion have I seen the proboscis fix itself to the body wall to pierce the worm: presumably this is too stout for penetration to occur.





The differences between the structure of *Turbonilla* and *Odostomia*, some of which may be correlated with the differences in habit, will be emphasized in the following description.

The slender shell, so easily pulled through the silt, is too long to be held erect when the mollusc comes to the surface and creeps along the shore, and it is then dragged awkwardly behind the short foot. The exposed parts of the body are white and, as in closely related opisthobranchs, are withdrawn into the calcareous shell and shielded by the operculum when the animal is disturbed. The operculum is orange and lacks the teeth which in *Odostomia* project into the flesh of the foot. The foot is truncated in front, the antero-lateral corners slightly emphasized, and posteriorly it tapers to a blunt point. The sole is

## TURBONILLA ELEGANTISSIMA

covered with columnar epithelium, strongly ciliated, and the same epithelium spreads over the upper surface of the propodium to surround the opening of the anterior pedal mucous gland, and covers the dorsal surface of the mentum. Elsewhere over the foot cilia are more infrequent. The mentum (Fig. 1, M) is a rectangular outgrowth from the foot, narrow in the transverse plane, and it lies between and beneath the tentacles. It differs from the mentum of *Odostomia* in that the introvert, instead of opening above it, extends through it and opens subterminally on the dorsal surface by an inconspicuous aperture. The introvert opens in the same position in *Turbonilla jeffreysii*. A median longitudinal furrow runs along the dorsal surface of the mentum above the introvert, and extends to the tip, that is, beyond the mouth. The furrow is lined by cuticularized cells. When the proboscis is to be used the mentum is extended in the direction of the food and held taut, and while the proboscis is gradually everting and pushing through the sand the antero-lateral corners of the tip of the mentum may be pressed against the substratum to give support.

Within the tissues of the foot, and spreading into the haemocoel of the head anterior to the nerve ring, are large numbers of mucous cells. Their contents are discharged by ducts passing between the epithelial cells of the foot. Such cells comprise the anterior pedal gland which opens on the dorsal surface of the propodium. A second large grouping discharges to the entrance of the penial sheath situated beneath the mentum, and a third, more diffuse, lubricates the surface of the sole especially the posterior half. Another type of gland cell is found within the foot forming on each side a lateral glandular streak as in *Odostomia* and in *Patella* (Yonge, 1947). Each cell is spherical, filled with globules responding to basic stains and surrounded by a lattice of muscle fibres which may help to discharge the secretion. The epithelium along the sides of the foot, on to which the ducts of these glands discharge, has scattered cells bearing bundles of long cilia; these may be sensory in nature. Similar cells are also found on the ventral surface of the mentum which is otherwise free from cilia.

The head bears a pair of black eyes closely set between a pair of tentacles (Fig. 1, T). The latter are of the typical pyramidellid type, shaped like the ears of a rabbit with a concave outer surface, though freely movable so that the concavity may face in a variety of directions. Invariably when the mollusc is burrowing and the tentacle is pressed back the concavity is lateral; the anteriorly directed surface which is pushed through the silt and sand is thickly ciliated, the cilia directing particles towards the tip. These cilia, together with those on the propodium and the dorsal surface of the mentum, form a cleansing current which sweeps sand and silt posteriorly over the head and out to each side. The posterior surface of the tentacle is not ciliated except for the rim bordering the concavity, and the epithelium of the concavity has only scattered tufts of cilia; these may be sensory. The tentacles of *Odostomia* are held erect when the mollusc is active, perhaps to sample the water, or to sense currents in its

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vicinity: this function would appear to be less significant in *Turbonilla*, which also lacks the ganglionic mass in the core of each tentacle.

The mantle cavity is deep and narrow. From it have disappeared the osphradium and ctenidium, and the opening of the hermaphrodite duct has been removed to a more anterior position. The rectum and kidney discharge into the posterior half. The anus is on the floor at the posterior end on the extreme right side, and the renal aperture is on the roof at the apex of a papilla which is at the anterior end of the kidney, about halfway along the mantle skirt, slightly to the left of the median line. The kidney lies wholly within the mantle skirt, occupying the posterior half of the left side, and its blood drains to the large efferent pallial vessel which courses along the left wall of the kidney to the auricle. A low, cubical, or even squamous, epithelium lines the greater part of the mantle cavity and permits ready exchange of respiratory gases between the water in the cavity and the blood vessels in the mantle skirt. Beneath this epithelium along the right wall is a strip of gland cells arising near the anus, which broadens as it passes anteriorly. Here the gland cells become larger and fewer. There appear to be two types of cell comprising this strip: one, the larger, elaborates secretory masses of irregular shape which stain deeply at first with iron haematoxylin, but near the time of liberation stain less intensely; whilst the second is filled with granules shaped like rice grains and always staining deeply with basic dyes. The secretion from these glands presumably helps to lubricate the passage of the faeces, supplementing the meagre secretion from the short intestine. On entering the anterior half of the mantle cavity the glandular strip leaves the mantle skirt and passes ventrally to the dorsal wall of the body along which it runs, first to the right of the pallial hermaphrodite duct and then above it.

The anterior half of the mantle cavity contains two important structures: the hypobranchial gland, which spreads along the roof anterior to the kidney and approaches the pallial edge, and, along the right wall, an exhalant gutter which is extended anteriorly beyond the mouth of the shell into a spoonshaped siphon. The gutter arises anterior to the point at which the strip of subepithelial gland cells, running forwards from the anus, passes medially on to the body wall. It is bordered on either side by a narrow strip of tall columnar epithelium, about a dozen cells broad, which is covered with long, closely set cilia. Between these bands the epithelium contains brown pigment granules and is not ciliated. One band runs along the mantle skirt on its extreme right side, and the other lies on the body wall immediately beneath it keeping to the right of the genital duct. Posteriorly the two converge and unite. The effective stroke of the cilia directs particles mixed with hypobranchial secretion forwards into the exhalant channel. The same general arrangement of this cleansing path from the mantle cavity is found in *Odostomia* (Fretter & Graham, 1949) and also in Omalogyra and Rissoella (Fretter, 1948); it appears more important in Turbonilla, and, while the animal burrows, a rope of detritus adhering to

secretion is poured from the mantle cavity by its activity. In all of these gastropods gill filaments have been lost, and their function of maintaining a stream of water through the mantle cavity is taken over by the ciliated strips of columnar epithelium along the exhalant path; the vascularized mantle skirt takes over the respiratory function of the gill.

At the anterior edge of the mantle skirt the epithelium is columnar, and in the pallial thickness is a complicated musculature and many mucous glands which discharge on to the surface.

In Odostomia the opening of the hermaphrodite duct is just within the mantle cavity, and from it a ciliated tract directs spermatozoa around the base of the right tentacle to the penial sheath. In *Turbonilla* the pallial duct is extended forward in the direction of the ciliated tract and opens on the propodium in front of the right tentacle and to the right of the mentum: in fact the common genital aperture is anterior to the opening of the penial sheath. Yet another opening lies on the side of the head, just behind the genital one and slightly more median. It is the duct of a muscular pouch which lies in the haemocoel and may be associated with the genital system.

Differences between the alimentary canal of Turbonilla and Odostomia are for the most part insignificant. The acrembolic proboscis grips the tentacle of the host with a sucker, formed from modified lips, and the piercing action of the stylet is synchronized with the attachment. The stylet is short, occupying only one-tenth of the length of the fully extended proboscis as compared with one-third in Odostomia; such a short stylet is not found in Turbonilla jeffreysii, an ectoparasite of hydroids and the only other member of the genus which has been studied. As in this species, however, and in contrast to Odostomia, the oral tube is not divided into a dorsal channel for the stylet and a ventral food channel: the sucker surrounds a single opening through which the stylet protrudes and into which the food is drawn. The cuticle comprising the stylet is secreted from the dorsal wall of the oral tube, like the jaws of more unspecialized gastropods. It extends forwards to embrace, and to envelop anteriorly, a projection arising from a more posterior part of the dorsal wall of the oral tube and passing through it to the sucker. This projection, forming the core of the stylet, contains the fused salivary ducts which open at the tip of the spike, and must pour secretion into the wound inflicted by it. Posteriorly the median salivary channel enlarges to a club-shaped origin, a region that invariably contains some saliva. Its walls are thickened by a complexity of muscles in which are embedded the ends of the salivary ducts and which are responsible for the movements of the stylet.

The oral tube has a low cubical epithelium which turns outwards over the sucker to meet the epithelium of the introvert; only at the periphery of the sucker is the epithelium ciliated. The ciliated cells, forming a ring not more than a dozen cells broad, flick particles away from the mouth as the proboscis is pushed through the silt. The intrinsic musculature of the sucker is arranged

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as in Odostomia, though the longitudinal fibres, attached at their outer ends to the underside of the epithelial cells, are longer, giving the sucker a greater depth. This muscular region is followed by a short section of the tube in which there are large subepithelial glands discharging their secretion into the lumen; in their detailed structure these resemble glands which occur in the long oral tube in Turbonilla jeffrevsii (Fretter & Graham, 1949, fig. 5). The buccal pump arises from the ventral wall of the tube at about two-thirds of its length from the anterior end. Its low, cuticularized epithelium overlies a thick wall composed chiefly of radial muscle fibres with antagonistic transverse fibres. This musculature sets up a rhythmical pumping action when the parasite is feeding. The pump is small as compared with that of *Odostomia*, lies hidden beneath the oral tube and has no caecum; its size may be correlated with the shortness of the oral tube. All its contractile cells contain groups of fibrillae which are striated. There are some mucous cells in and under the epithelium at the posterior end of the pump, especially around its opening to the oesophagus which is on a small papilla projecting into the narrow oesophageal tube.

The epithelium of the introvert is not complex as in other pyramidellids. It is columnar and cuticularized, showing no regular papillation. In the living tissue two types of subepithelial gland cell can be distinguished: in one the cytoplasm contains colourless spherules and in the other it appears as a granular fluid of homogeneous consistency. Occasionally these gland cells occur in the epithelium. Circular muscle fibres constituting a coat of considerable thickness lie directly beneath the epithelium, and then come the longitudinal muscles which are responsible for the retraction of the extended proboscis. They take the same course as the retractors of the proboscis of Odostomia. As the proboscis is retracted small particles of detritus and diatoms are frequently drawn in with it (Fig. 1, PD): on its protrusion they fall off the smooth surface or are scraped off as it pushes through the substratum. The eversion of the proboscis is mainly brought about by the hydraulic action of the blood in the cephalic haemocoel. To provide for the disturbances in the vascular system which such profound changes in shape of the anterior part of the body must entail the arrangement of this system is modified to what must be regarded as the typical pyramidellid plan. A single aorta arises from the ventricle and passes up the visceral mass to open into haemocoelic spaces. The anterior aorta has no direct connexion with the heart, but runs between a vast haemocoelic sinus which bathes the pericardium and adjacent reproductive organs, and the haemocoelic spaces of the head and foot. Blood will flow anteriorly through the aorta when the proboscis is thrust out, and when it is withdrawn may be forced back In this way the reverse direction to fill the posterior haemocoelic reservoir. rapidly in injury to the heart by the sudden pressure of returning blood is prevented.

In *Odostomia* the large buccal ganglia are situated at the anterior end of the buccal pump which is regarded as part of the modified buccal cavity, the tube

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of capillary dimensions which leaves the pump being the oesophagus. In Turbonilla the ganglia are more posterior and lie near the point at which the salivary ducts leave their glands. This point may appear half-way along the muscular tube which connects the pump with the stomach, though when the proboscis is in use the part of the tube posterior to the ganglia may be extended to twice or even thrice the length of the anterior section. With the extreme specialization of the anterior part of the alimentary canal in the pyramidellids the buccal ganglia may shift from their original position. In Cerithiopsis tubercularis (Fretter, 1951), which shows modifications associated with a much less specialized type of proboscis, the ganglia lie at the posterior end of the anterior oesophagus where the salivary ducts leave the haemocoel and penetrate the musculature of the oesophagus within which they make their way to the buccal cavity. A similar course is followed by these ducts in Turbonilla. The region of the alimentary canal between buccal pump and buccal ganglia may, therefore, be regarded as the anterior oesophagus. It is a long muscular tube of uniform diameter which lies in tangled coils in the haemocoel when the proboscis is retracted, and is carried out into the proboscis when it is everted. The section of the oesophagus behind the buccal ganglia is of uniform structure throughout, and is only distinguishable from the anterior part by its musculature. The oesophageal epithelium is similar from one end to the other, being composed of low columnar cells.

The intrinsic muscles of the anterior oesophagus constitute a coat of circular fibres beneath the basement membrane of the epithelium, and an outer coat of longitudinal fibres; the salivary ducts run just within the latter. These muscles set up a peristaltic movement which can be seen when the living animal is dissected. In the wall of the posterior part of the oesophagus there are no circular muscles: its whole movement is controlled by a complex arrangement of longitudinal fibres grouped into compressed sheets which lie radially with respect to the lumen; the nuclei of the muscle cells border the haemocoel. Only the anterior part of this section of the oesophagus is drawn out into the proboscis.

Each salivary gland consists of a narrow tube, ciliated and surrounded by subepithelial gland cells except at the innermost end where the lumen swells into a thin-walled bladder lined by cubical cells without cilia. Strands of muscle attach this bladder to the wall of the posterior part of the oesophagus. The gland is connected to the stylet by a long duct which leaves its anterior end and, after a very short course in the haemocoel, passes into the intrinsic longitudinal muscles of the anterior oesophagus. The duct appears minute in transverse section, with three low epithelial cells surrounding the lumen and beneath these muscle cells containing circularly running fibrillae. Anteriorly the two ducts leave the oesophagus and pass dorsally to enter the median salivary channel which lies within the stylet. Most of the secreting cells of the gland have a similar appearance—very finely vacuolated cytoplasm which

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stains lightly with basic dyes and also with stains specific for mucus. The nuclei are relatively enormous and have many granulations and three or four nucleoli. There may be, as in *Odostomia*, a holocrine secretion occurring without a restitution phase: there is evidence from the appearance of the gland that new cells are formed only at the innermost end, near the terminal bladder, and spent cells, destroyed after secreting, are restricted to the region at the beginning of the duct.

When the proboscis is withdrawn the muscles of the posterior half of the oesophagus contract, shortening and broadening that tube, which comes to rest beneath the coiled anterior oesophagus and just above the columellar muscle. Posteriorly the oesophagus opens to the stomach, which is ill-defined except for its anterior wall, and which cannot elsewhere be distinguished from the digestive gland. The anterior wall is made of a small area of columnar epithelium which surrounds the oesophageal opening and the opening of the intestine lying above this. Thus the food, which is liquid and so needs no mechanical treatment before it can be absorbed, is passed from the oesophagus directly to the cells of the digestive gland; in these cells it appears, at first, in the form of brown spherules. The only waste matter which is seen in the intestine is derived from the digestive cells of the digestive gland, and is in the form of small yellow and green concretions. The intestine passes from the stomach directly to the upper end of the mantle cavity. The epithelium is ciliated and has a few scattered gland cells in which secretion spherules stain readily with basic dyes.

The important dissimilarities between the hermaphrodite reproductive systems of *Odostomia* spp. and *Turbonilla elegantissima* concern the length of the pallial duct and the organs of copulation. Perhaps of less significance are other divergent characters which are related to the accessory glands, both albuminous and mucous, and the histology of the pallial duct.

The gonadial channel which links the hermaphrodite gland with the pallial duct receives, in *Turbonilla*, a single duct which drains both the albumen and mucous glands. These glands, lying in the upper visceral coils, have, in *Odostomia*, separate ducts which converge on approaching the gonadial duct so that their openings to it are set very close together. The ducts have apparently fused in *Turbonilla*. A second mucous gland occurs in *Odostomia* and surrounds the upper end of the pallial duct, where the body broadens to its last coil, and when the female system is mature this gland spreads half-way along the duct. In *Turbonilla elegantissima* the distal mucous gland is confluent with the proximal one: a single gland, lobed and presumably not entering the last coil, has one outlet to the genital duct and a second, by a broad secretory channel, to the innermost end of the pallial duct.

The pallial duct extends on to the propodium and has, for more than half its length, an epithelium of tall columnar cells bearing long cilia. This distal region of the duct is very muscular. Elsewhere the ciliated cells lie between gland

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cells swollen with secretion. The gland cells are of three principal types. The most important, occurring in the centre of the secreting area and—a few—at the proximal end of the duct, contains large spherules of mucus; distally, between these and the ciliated part, and sometimes scattered amongst the mucous cells, are glands with smaller secretion globules which stain deeply with basic dyes; whilst at the proximal end of the duct the secretion within the cells, again in the form of spherules, is not mucus and stains with basic dyes only lightly. Surrounding the opening of the gonadial duct is yet another type of gland cell: the enormous nucleus is probably indicative of intense metabolic activity; the spherules of secretion stain deeply (iron haematoxylin) during their period of elaboration, but lightly near the time of liberation. This varied histological structure of the duct contrasts with the uniformity in *Odostomia*.



Fig. 2. Turbonilla elegantissima. A, Copulatory organs. The right wall of the sperm sac, the penial sheath, the vas deferens and of the posterior part of the penis has been removed. × 122. B, Transverse section through penis and its sheath. × 271. D, dilator muscles of penial sheath; L, longitudinal muscles of penis; M, muscles running between epithelia of penis; os, opening of sperm sac into penial sheath; PE, penis; PO, opening of penial sheath beneath mentum; SS, sperm sac; VD, vas deferens; W, wall of penial sheath.

The penis is invaginable (Fig. 2 A, PE), as in other pyramidellids, and when at rest lies in the haemocoel, retracted into a tubular sheath (w) with which its walls are confluent. The sheath is broad posteriorly where the retracted penis lies, and its narrow anterior end passes through the nerve ring to open beneath the mentum (PO). It is ventral to the introvert. A large muscular pouch, the sperm sac (ss), lined by a low columnar epithelium on which fine cilia are closely set, opens into the dorsal wall of the sheath, in front of the tip of the retracted penis. The opening is surrounded by a sphincter. The structure of the penis is unusual in that it contains neither blood spaces nor duct. When retracted into the sheath it has the appearance of a long muscular scoop

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attached posteriorly to the wall of the sheath. On to its concave surface, at the posterior end, opens the vas deferens, which is a deep longitudinal groove running along the dorsal wall of the sheath between the mouth of the sperm sac and the base of the penis. The lips of this groove (at least in fixed and dissected specimens) are firmly pressed together so that it may function as a duct. The vas deferens, the rest of the sheath and the penis are covered by cuticularized epithelium. The entire space between the upper and lower epithelia of the penis is occupied by three sets of intrinsic muscles: one series of fibres runs between the two epithelia (Fig. 2B, M); antagonizing these are bundles of longitudinal fibres under the lower epithelium (L); and, beneath the upper epithelium, on to which the vas deferens discharges, fibres run transversely from edge to edge with a few running longitudinally. It is assumed that, as in Odostomia spp., the sperm sac is filled with spermatozoa prior to copulation, for it is difficult to see how they could reach it when the penis is protruded. There is another sac, similar in histology and slightly smaller, which lies in the haemocoel above the anterior end of the retracted introvert and opens on to the propodium not far from the genital aperture. The opening is median to that of the pallial duct and posterior to it. There is no evidence as to the use of this pouch, since it has always appeared empty. Perhaps it is concerned with copulation and functions as a bursa, receiving the sperm from the penis and storing them temporarily, and expelling them later to the groove by which it is connected to the genital duct.

The penis in most gastropods is distended with blood at the time of copulation and at other times is relatively small. In *Omalogyra atomus* (Fretter, 1948) and *Turbonilla elegantissima*, both highly specialized species, the organ is unusual in that it contains neither blood spaces nor glands and the musculature is pronounced. The protrusion of the penis in *Turbonilla* is presumably brought about by pressure on the blood in the haemocoel aided by the muscles of the penial sheath.

The eggs of *T. elegantissima* are not known. Lebour (1936) describes and figures the young which even when very small (0.25 mm. across the shell) may be found with the adults in muddy and stony ground. She suggests that there is a very short free-swimming stage in the life history, and this would agree with what happens in species of *Odostomia*.

# SUMMARY

Turbonilla elegantissima is a pyramidellid, ectoparasitic on Audouinia tentaculata and Amphitrite gracilis, attaching to the tentacles, and sometimes to the gills of Amphitrite, by means of an oral sucker, piercing by a stylet—a modified jaw—and sucking its food by a buccal pump. It burrows in silt.

The alimentary canal varies from the structure in Odostomia and Chrysallida in details which are relatively unimportant and confined to its highly modified anterior part. Thus the stylet, and consequently the oral tube, is very short, and in the tube there is only one channel for both stylet and food; the opening of the introvert is on the dorsal surface of the mentum, subterminally, and the surface of the introvert is not papillated; the buccal pump is short, arising behind the sucker, and has no caecum; the oesophagus is divisible into anterior and posterior sections, distinguished by their musculature and by the fact that the salivary ducts run within the longitudinal muscles of the anterior part.

In the reproductive system there is greater specialization than in Odostomia and Chrysallida: for the opening of the pallial hermaphrodite duct is on the propodium. The penis, scoop-shaped and concealed within a sheath, has neither blood spaces nor duct, and its musculature is pronounced. There is a muscular pouch in the haemocoel of the head, opening on the propodium close to the hermaphrodite genital aperture, which may be a bursa copulatrix.

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# THE BREEDING OF CREPIDULA FORNICATA (L.) IN THE RIVER BLACKWATER, ESSEX

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# (Text-figs. 1-4)

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#### INTRODUCTION

The chain-forming prosobranchiate gastropod Crepidula fornicata is not indigenous to British waters, but has been introduced during the last 70 or 80 years, probably from North America, upon imported oysters, and, during this period, has spread to most of the oyster beds on the south-east and south coasts of this country (see Orton, 1950b, for a recent discussion of its present distribution). In spite of the interest shown in this species because of its detrimental effect upon oyster culture, and because of its well-known change of sex from male to female, little is known in the literature of its breeding habits here, or in North America. Conklin (1897) states that the spawning period in New England lasts from early summer to about mid-August, no larvae being found in late August. Murie (1911), in discussing the introduction of this species, elaborates upon Conklin's observations, and gives evidence that females, in samples of Crepidula taken from the River Colne in May and September 1898, contained spawn beneath their shells. Orton (1912a, p. 438) states that he is 'informed by Professor Conklin that American Crepidulae begin to spawn in May and possibly in April, whilst English Crepidulae begin

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to spawn in early March'. In a footnote, he adds 'Crepidula spawned in tanks at Plymouth in early February'.

Later workers upon *Crepidula* have added little to our knowledge of the breeding period, although Coe (1936, 1938) has investigated the sex change in several species, in greater detail than Gould (1919). Orton (1909) has described the occurrence of protandric hermaphroditism in *C. fornicata* and (1912*b*) the method of feeding. Thorson (1946), whilst conducting extensive plankton investigations in Danish waters, recorded the early free-swimming stages of *C. fornicata* off north-west Jutland, in July 1940, but observed no later stages.

The author is indebted to Messrs Wombwell and French, of the Tollesbury and Mersea Native Oyster Co., for arranging the collection of regular samples of *Crepidula*. The author is also indebted to Emeritus Professor J. H. Orton, of Liverpool University, for generous advice and criticism, to his present colleague Dr D. J. Crisp for much useful advice, particularly upon statistical problems, and to the Directors of Imperial Chemical Industries Paints Division, under whose auspices the investigation was conducted.

# SUMMARY OF THE LIFE HISTORY OF CREPIDULA FORNICATA

The anatomy and histology of the reproductive system of *C. fornicata* are well known (Orton, 1909; Gould, 1917; Coe, 1936).

Briefly, the gonad consists of an irregular lobulated organ lying along the intestine and between the lobes of the digestive gland. In the functional males it is reddish brown in colour, whilst the female gonad is bright yellow. The diverticulae of the gonad give rise to the gonoduct anteriorly. The gonoduct in the functional male consists of a very convoluted seminal vesicle, which is continued as a narrow vas deferens to open upon a ciliated groove, which runs forward, on the right side of the animal, to a large grooved muscular penis, situated behind the base of the right tentacle.

On the completion of the functional male phase, the penis degenerates, cells being sloughed off from the brown tip (Gould, 1917). As the penis becomes smaller, the width of the gonoduct increases and the inner walls become folded longitudinally. The distal part of the gonoduct develops into a prominent uterus with folded walls, into which a number of seminal receptacles open.

Whilst these changes are taking place, the penis becomes greatly reduced, and is finally represented by a minute brown scar. The gonad, which even in the male phase contains small ova, develops rapidly as oogenesis takes place, becoming a bright yellow when fully functional. Coe (1938) found that the period of sexual transformation from male to female in isolated individuals was 61 days, but was considerably longer for males forming part of a 'chain'.

The eggs are laid in a bunch of stalked balloon-shaped capsules, united basally to a common stem, fastened to the substratum and, as in *Vermetus*,

protected by the parent whilst development is in progress. The capsule has thin, cream-coloured walls, and contains *c*. 250 eggs, each 150–170  $\mu$  in diameter when initially spawned, floating in the intra-capsular fluid.

The embryonic development has been described in great detail by Conklin (1897). At the end of embryonic development, the larvae escape as advanced veligers through an apical split in the egg sac, to lead a free-swimming life until settlement occurs. The free-swimming veliger larvae possess shells  $260 \mu$  long,  $190 \mu$  broad and  $170 \mu$  high (mean of 100 measurements), with a two-third to three-quarter whorl.

In the adults, the functional male phase can be readily distinguished externally by the development of a coiled seminal vesicle, a long black penis, and a red brown gonad, whilst the functional female possesses a prominent uterus and a yellow gonad. Not infrequently a female is found with a small brown rudimentary penis. Orton (1909) gives diagrams of the functional male and female phases, with several intermediate stages.

# MATERIALS AND METHODS

The investigation of the breeding of Crepidula fornicata was commenced in May 1946, and was continued until December 1947. In 1946, samples of about 50 'chains' (i.e. c. 150 females) of Crepidula were obtained fortnightly from the oyster beds in Thirslet Creek, near Tollesbury (River Blackwater), Essex, although larger samples from Mersea Quarters (West Mersea) were examined when personal visits were made to the locality. No samples could be obtained from the River Blackwater during February and early March 1947, owing to the presence of extensive ice floes. From mid-March, however, until November, regular weekly samples of the same size (i.e. c. 50 chains) as employed in the previous year (1946), were obtained from Mersea Ouarters (see Admiralty Charts 3740-3741). Except when personal collections were made, all samples were wrapped in damp sacking, packed in a wicker-work basket, and were dispatched by rail on the afternoon of the day of collection. They were usually received at Liverpool late the following morning, in good condition, even during the period of high temperatures during the summer of 1947. A few observations were made as opportunity arose at Burnham-on-Crouch in 1948, 1949 and 1950, but no systematic study was attempted.

The samples were examined for sex, gonad development, and presence of, and stage of development of the embryos. Shell lengths were usually recorded. Complete chains (i.e. those unbroken with the basal member present) only were employed.

The embryos were referred to seven categories, each corresponding to a readily distinguished stage in development as follows:

*Stage* o. Comprising those females with well-developed ovaries, containing ripe ova, and which are ready to spawn.

4-2

Stage I. I- to 8-celled stage, usually an orange or deep yellow in colour (Fig. 1*a*).

Stage 2. 8-celled stage, to the end of gastrulation (Fig. 1 b). The colour of the embryos in this stage is predominantly yellow but is sometimes cream. Owing to the amount of albuminous fluid contained within the egg cases, the embryos are normally difficult to separate for examination.



Fig. 1. Crepidula fornicata. Stages of embryonic development: a, Stage 1; b, Stage 2; c, Stage 3; d, Stage 5.

Stage 3. The 'pre-veliger' stage, in which gastrulation is complete, and the archenteron and stomodeum are well formed, the latter usually being prominent. The shell gland can usually be observed. The embryo is well ciliated (Fig. 1c). The colour is usually yellow to light yellow and the embryos are normally more readily separated than in the previous stage.

*Stage* 4. Early veliger stage, with well-developed velum, and shell, but with a less advanced internal organization than the next stage. The colour is usually a light yellow to yellow-brown. The embryos are readily separable in sea water.

Stage 5. Later veliger stage, ready to hatch (Fig. 1d). The shell is well formed, of a broad tumid appearance. The velum is slightly 4-lobed and very strongly ciliated. The margin of the velum is usually darkly pigmented. The foot is well developed and ciliated, with a dark pigment spot anteriorly. The

gut is filled with granules, and is darkly pigmented. The embryos at this stage appear brown or dark grey to the naked eye.

Stage 6. Hatched stage, represented by empty egg capsules.

The stage of development of the embryos was determined microscopically, the criterion of colour alone not being sufficiently definite to ensure accurate classification into the appropriate category.

During both seasons, the percentage of females bearing embryos in Stage 1, except at the beginning of the period of spawning in 1947, was low, and followed the general course of spawning closely. It is possible that these embryos were the result of forced spawning, extrusion of the ova occurring during the process of dredging, and hauling the trawl. As 24 hr. normally elapsed before examination could be commenced, there was ample opportunity for early segmentation to occur. It is doubtful whether such premature spawning occurred in transit (unless the material was very roughly handled), as samples collected on the same ground at West Mersea, part of which were examined immediately, and part after 24 hr. storage in air, showed no significant difference in numbers of Stage I embryos, although many more advanced Stage I embryos were present after 24 hr. than immediately after collection. Also, unsegmented ova were never observed in the material examined at Liverpool, or 24 hr. after collection at West Mersea. However, the fact that most of these Stage I embryos were fertilized and actively segmenting suggests that the female Crepidula bearing them were ripe and ready to spawn.

Sea temperatures, etc. In order to investigate a possible correlation between sea temperature and the breeding of *Crepidula* records of the bottom temperature were made twice daily (early morning and early afternoon) in Thirslet Creek, near Tollesbury, from September 1946 until December 1947. From the end of February until December 1947 sea temperatures were also taken twice daily in Thornfleet (West Mersea) at 7 a.m. and 1 p.m. The daily means of the 1947 Thornfleet bottom temperatures are shown in Fig. 4. Standard sea-water thermometers (checked against an N.P.L. standard) with small buckets around the bulbs were employed in both localities. During 1947 the daily mean bottom temperatures in the two localities differed on no occasion by more than  $+2 \cdot 0^{\circ}$  F.

No salinity or pH measurements were made.

# SPAWNING IN 1946 AND 1947 ON THE BLACKWATER OYSTER BEDS

The results of the observations upon the spawning of *C. fornicata* made in 1946 and 1947 are given in Tables I and II respectively. In Table I, the numbers of females bearing Stages 2 and 3, and 4 and 5 embryos are combined, but in Table II (for 1947) each category of embryonic development is given separately. The information obtained in the two years of study is shown graphically in Figs. 2 and 3 for 1946 and 1947 respectively. With the exception of the total

TABLE I.	CREPIDULA FORNICATA.	Spawning at	Tollesbury	(THIRSLET	CREEK)	AND WEST	MERSEA
		(MERSEA QUAR	TERS) IN 1946				
				Cumulativ	e percentage	, i.e. percentag	ge .

					No. of fem	ales with		of females			
Date, 1946	Locality	No. of chains examined	No. of females	Spawn (total)	Stage 1 spawn	Stages 2 and 3 spawn	Stages 4 and 5 spawn	Stage o	Stage I	Stages 2 and 3	Mean nos. of females per chain
8. v.	Toll.	44	136	110	22	42	46	80.9	64.7	33.8	3.10
17. V.	Toll.	35	108	102	18	31	52	94.4	77.0	48.1	3.09
20. V.	W.M.	100	298	276	15	88	173	92.7	87.6	58.0	2.98
29. V.	Toll.	35	123	104	21	37	51	84.5	71.6	41.5	3.54
11. vi.	Toll.	50	184	166	26	124	16	90.2	76.1	8.7	3.68
19. vi.	W.M.	50	147	112	5	38	69	76.0	72.8	46.9	2.94
29. vi.	Toll.	50	180	138	24	73	41	76.7	57.7	22.8	3.60
10. vii.	Toll.	36	120	62	12	42	8	51.6	41.6	6.7	3.34
17. vii.	W.M.	50	151	59	18	15	26	39.0	27.2	17.2	3.02
29. vii.	Toll.	28	79	14	2	IO	2	17.2	15.2	2.5	2.82
2. ix.	Toll.	51	177	24	15	6	3	13.2	5.1	1.7	3.47
19. ix.	Toll.	50	164	3	0	3	0	1.8	1.8	0	3.38
8. x.	Toll.	30	97	0	0	0	0	0	0	0	3.23
26. xi.	Toll.	25	79	0	0	0	0	0	0	0	3.16

Toll. = Tollesbury. W.M. = West Mersea.

# TABLE II. CREPIDULA FORNICATA. SPAWNING AT WEST MERSEA (MERSEA QUARTERS) IN 1947

	No. of	N	Nos. of females bearing spawn						Cumulative percentage, i.e. percentage of females with spawn which has developed beyond					Mean nos females		
1947 examined females	Total	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage o	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	chain		
19. iii.	40	105	0	0	-				_	0	0	_	_	—		2.62
17. iv.	35	IOI	0	o	_			_		0	0	_		—	_	2.88
23. iv.	40	92	25	25	0		_			27.0	0	0		-	_	2.30
29. iv.	45	135	89	84	5	0	—	_		66.0	3.2	0		_	—	3.00
6. v.	45	112	82	52	30	0	—	_		73.0	36.6	0		_	_	2.50
14. V.	32	75	56	I	34	21	0	0	0	74.6	73.4	28.0	0	0	0	2.34
20. V.	46	105	100	15	13	13	24	27	8	95.2	81.0	68.6	56.1	33.4	7.6	2.28
4. vi.	56	121	115	7	16	21	28	22	21	95.0	90.1	76.0	58.7	35.2	17.3	2.16
16. vi.	47	III	106	19	34	16	19	3	15	95.5	78.4	47.7	33.1	16.2	13.2	2.36
25. vi.	44	78	67	I	19	9	4	4	30	85.9	84.6	60.3	48.7	43.6	38.2	1.66
2. Vii.	45	100	90	7	55	4	4	2	18	90.0	83.0	28.0	24.0	20.0	18.0	2.22
9. vii.	46	104	80	0	30	9	19	10	12	77.0	77.0	48.1	39.4	12.1	11.2	2.26
17. vii.	27	68	27	0	15	I	3	2	6	39.7	39.7	17.7	16.5	11.8	8.8	2.52
28. vii.	45	128	56	0	26	0	9	6	15	43.7	43.7	23.4	23.4	16.4	11.2	2.84
12. viii.	25	80	4	0	· 0	I	I	0	2	5.0	5.0	5.0	3.2	2.2	2.2	3.20
20. viii.	25	69	4	0	I	I	2	0	0	5.8	5.8	4.3	2.9	0	0	2.76
3. ix.	37	119	19	0	8	3	6	I	I	16.0	16.0	9.2	6.2	1.0	0.8	3.22
18. ix.	44	153	7	0	0	0	0	0	7	4.6	4.6	4.6	4.6	4.6	4.6	3.48
7. X.	26	86	0	0	0	0	0	0	0	0	0	· O	0	0	0	3.30

#### BREEDING OF CREPIDULA

percentage of females spawned in each sample, the results are given as cumulative percentages, i.e. in each sample the proportion of females carrying spawn which has developed beyond each category is plotted as a percentage of the total females present in the sample. This type of cumulative frequency distribution, because of the grouping of results, enables the temporal relationships in such biological behaviour as spawning and development to be presented more clearly. The method is similar to that employed in survival investigations in insecticide studies.





#### Significance of Results

The samples of *C. fornicata* employed in this investigation can be considered random, as (i) they were not chosen for any particular attribute, other than that the chains should be complete, i.e. broken chains were rejected, and (ii) each sample was made up of chains taken from several hauls upon the same bed.

In order that the significance of the differences between successive samples

might be assessed, the data obtained from the examination of the samples were treated statistically (Table III).

In column 6 of Table III the standard error of the percentage of females bearing spawn which has developed beyond Stage 2 (i.e. the sum of all females with embryos of the pre-veliger and later stages) for each sample in 1947 is given. By taking this stage of development for statistical treatment, the inclusion of possibly prematurely spawned females is avoided.



In column 7 are given the values of  $\chi^2$  and the corresponding probability calculated from the actual numbers of females with spawn developed beyond Stage 2 in successive pairs of samples, employing the  $2 \times 2$  Contingency Table method. No allowance has been made in this treatment for the association of females in chains, and the assessment of significance of the differences between pairs of samples is the most favourable obtainable, i.e. the assumption is that the females were sampled at random. As only complete chains were sampled at random, a stricter analysis, representing the most unfavourable condition,

in which the numbers of females in each sample were reduced in proportion to the number of chains in the sample, was employed, and the significance assessed by the method for small samples drawn from a Binomial Distribution. The values for  $\chi^2$  thus obtained are given in column 8 of Table III.

The differences which are significant upon a basis of P=0.05 are underlined in columns 7 and 8 of Table III. Use will be made of these significant differences in the discussion upon possible periodicity in spawning (see p. 59).

# Length of Breeding Period

The first sample of *Crepidula* examined in 1946 (8 May) was obtained after the breeding period had commenced, about two-thirds of the females bearing

# TABLE III. CREPIDULA FORNICATA. SIGNIFICANCE OF SPAWNING RESULTS, 1947

(I) Date	(2) No. of chains	(3) No. of	(4) No. of females with spawn passed through	(5) Percentage of females with spawn which has passed through	(6) Percentage standard error of females in '5' (Binomial)	2×2 cc	(7) ontingency table	Binomi on ba of	(8) al sampling sis of no. chains
1947	examined	females	Stage 2	Stage 2	+ or -	$\chi^2$	approx. P	$\chi^2$	approx. P
23. iv.	40	92	0			-	_		_
29. iv.	45	135	0	-	—		—		_
6. v.	45	II2	0	0	0	20.2	<0.00T	0.6	0.000
14. v.	32	75	21	28.0	7.9	29 5	<0.001	90	0.003
20. V.	46	105	72	68.6	6.9	290	0.14	131	<0.001
4. vi.	56	121	92	76.0	5.7	19.0	-014	3.05	0.09
16. vi.	47	III	53	47.7	7.3	10.0	<0.001	0.9	0.004
25. vi.	44	78	47	60.3	7.4	315	0.09	0.44	0.22
2. Vii	45	100	28	28.0	6.7	10.3	<0.001	0.10	0.014
9. vii.	46	104	50	48.1	7.4	0.30	0.000	2.90	0.09
17. vii.	27	68	12	17.7	7.4	10.9	<0.001	13.1	<0.001
28. vii.	45	128	30	23.4	6.3	0.89	0.30	2.00	0.11
12. viii.	25	80	4	5.0	4.4	12.1	<0.001	<b>9.1</b>	0.003
20. viii.	25	69	4	4.3	3.0	0.04	0.84	0.00	1.00
3. ix.	37	119	II	9.2	4.7	1.34	0.52	1.04	0.29
18. ix.	44	153	7	4.6	3.3	2.10	0.12	0.31	0.66
7. x.	26	86	ó	0		-			_
	2	0							

 $\chi^2 g = I(P = 0.05) = 3.841$ 

Values of  $\chi^2$  in heavy type indicate those significant on the p = 0.05 criterion.

spawn and of which a half contained veliger larvae. Spawning had thus begun some time previously. Subsequently, egg capsules, containing embryos in various stages of development, were present in all samples until mid-September (see Fig. 1), after which date none was seen until the following season.

In 1947 the first females bearing spawn were observed on 23 April, initial spawning having thus occurred between 17 and 23 April. All the embryos were, however, in Stage 1, and may thus have been spawned prematurely. The first females with Stage 2 embryos were observed on 29 April. From this time developing embryos were present until mid-September (18th) when empty egg cases only were observed. At Burnham-on-Crouch in 1948 and 1949, female *Crepidula* with developing embryos were observed from April until September.

Thus, on the Essex oyster beds, spawning of *Crepidula* would appear to commence in early spring, and to continue until September in most years,

giving a breeding period of about  $5\frac{1}{2}$  months' duration. The breeding period on the Essex oyster beds is therefore rather longer than that for the same species upon the New England beds as described by Conklin (1897). Orton's (1912*a*) observation that *C. fornicata* in English waters begins to spawn in March was not borne out by the 1947 observations, but, in that year, water temperatures in the Blackwater were very low (30° F. at the beginning of the month, rising to 42° F. at the end), following the abnormal weather in February, when ice was present on many parts of the east coast. In 1948, a single sample from Tollesbury (Thirslet Creek), taken on 6 April, showed that spawning had commenced a few days previously, whilst *Crepidula* began to spawn at Burnham-on-Crouch in 1950 immediately before 30 March. It would thus seem that *Crepidula*, in a warm spring, spawns on the Blackwater oyster beds at least in late March or early April. This view is supported by Prof. J. H. Orton who, in a personal communication, states that there is little doubt that spawning commences in a normal spring in March.

#### Sea Temperature and the Commencement of Spawning.

In 1946, no observations of the onset of spawning in *Crepidula* in relation to sea temperature could be made. In 1947, however, the onset of spawning was carefully followed. Initial spawning (possibly premature) took place between 17 and 23 April, whilst the first Stage 2 embryos were found in the sample collected on 29 April. During this period the mean daily sea temperature remained at a little above  $50^{\circ}$  F. until about 24 April, having risen from  $45^{\circ}$  F. to this value between 12 and 17 April, and rose to  $52^{\circ}$  F. between 24 and 30 April. Thus spawning commenced at a period when the sea temperature had risen slightly above  $50^{\circ}$  F.

No systematic observations were possible in following years, but the sea temperature at Tollesbury (Thirslet Creek) rose above  $50^{\circ}$  F. at the end of March 1948, and, as has been stated previously, a sample of *Crepidula* obtained on 6 April of that year supplied data suggesting that spawning had commenced a few days previously. No observations were made in 1949, but in 1950 spawning commenced at Burnham-on-Crouch at the end of March, when the sea temperature had risen to c.  $51^{\circ}$  F. It is therefore possible that *Crepidula* does not spawn until the sea temperature rises to, or above,  $50^{\circ}$  F.

# Temperature and Duration of Spawning Period

Although in 1947 at West Mersea initial spawning took place shortly after the sea temperature rose above  $50^{\circ}$  F., spawning ceased a considerable time before the occurrence of the autumnal homothermal epoch, the ovaries of the female *Crepidula* showing exhaustion during July. Thus, in *Crepidula* from the Blackwater beds, as in *Ostrea* (Korringa, 1947), temperature does not appear to determine the duration of the breeding period directly, and it would seem that the length of the breeding period is determined, rather, by the rate of

#### BREEDING OF CREPIDULA

exhaustion of the gonads, and the rate of regeneration of ova and sperm. Spawning in *Crepidula* does not appear to be limited by the high maximum temperatures attained during the summer, but there is some evidence that the higher summer temperatures in the Blackwater in 1947 caused a more rapid spawning, and consequently an increased rate of exhaustion of the gonads, than in 1946.

# Periodicity in Spawning

The results obtained in 1946, owing to rather infrequent sampling, do not provide definite evidence of periodic spawning, in the sense that a proportion of females in the population tend to release their ova in concert several times during the breeding period. Two well-defined maxima in the proportion of females bearing spawn of Stages 4 and 5 (i.e. veligers) occurred in the samples from West Mersea collected on 20 May and 19 June (See Table I and Fig. 2), whilst two smaller ones were recorded on 17 July and 19 August, although these are probably not significant statistically. On the latter date, however, a distinct



maximum in early stages occurred. It would thus seem that, from the beginning of May until the end of the spawning period in September, three concerted spawnings took place, one before 20 May, one immediately before 11 June (before a new moon, or during the last quarter phase) and a final one, rather distinct from the main spawning period, immediately before 19 August (at or after a new moon).

The greater frequency of sampling adopted in 1947 provides more definite evidence of concerted spawning (see Fig. 3). In this year, the first true spawning probably occurred immediately before 29 April, the first Stage 2 embryos being observed in a sample collected on this date (Table II). As the sea temperature had risen to and just above  $50^{\circ}$  some 10 days before (Fig. 4) it is unlikely that the temperature rise in itself acted as a spawning stimulus. This first spawning occurred contemporaneously with a first quarter lunar period. Spawning continued, and on 14 May, all embryos found, except in  $1\cdot3\%$  of the females, were in Stage 2 or Stage 3 of development, to be followed in the next sample on 20 May with a maximum of females bearing veliger larvae (Stages 4 and 5).

No evidence of a further concerted spawning was obtained until 16 June, when an increase in the proportion of females bearing Stages 1 and 2 embryos was recorded, followed in the next sample (25 June) by a significant peak in females with embryos developed beyond Stage 3, although the peak for females with embryos later than Stage 2 is not quite significant on the 5% level (P=0.08) (see Table III). This spawning took place at a time when the sea temperature was c. 62° F., during a slight rise, after a rapid fall from 70° F. at the beginning of June (see Fig. 4), and also when the moon was waning (new moon, 18 June).

Spawning continued over the new-moon period, a maximum of Stage 2 embryos occurring in the next sample (2 July) to be followed by a significant maximum in older embryos 7 days later. This spawning, which included c. 7% of females with Stage I embryos, took place when the sea temperature was c. 68° F., immediately before a full moon (3 July).

No further evidence of concerted spawning was recorded until the end of August, when the sea temperature was declining below  $70^{\circ}$  F., and the moon was waxing from first quarter to full. This spawning is possibly significant, although P=0.15 (Table III).

These observations are summarized in Table IV.

		spawning maximum	
Approx. date of spawning	Moon's phase	(° F.)	Remarks
Between 17 and 23 April	New moon, 21 April	50	Probably premature spawning
Between 23 and 29 April	New moon, 21 April	51.5	First true spawning
Immediately before 16 June	New moon, 18 June	62	Max. in Stage 2 embryos followed 25 June by signifi- cant peak in Stage 4 and older embryos
Immediately before 2 July	Full moon, 3 July	68	Max. in Stage 2 embryos followed 9 July by signifi- cant peak in Stage 3 and older embryos
Between 17 and 28 July (approx. 25th)	New moon, 18 July	67.5	Not significant
About 25 August	Full moon, 31 August	68	Final spawning

# TABLE IV. Possible Periodic Spawning of Crepidula FORNICATA AT WEST MERSEA, 1947

Although there is no definite indication of cyclic periodicity in spawning, the results obtained in 1947 show a marked tendency for a proportion of female *Crepidula* to spawn in concert, in a manner unassociated with temperature, or

temperature fluctuations. Spawning, however, is evidently not limited to these periods for the mimima of the incidence of females with Stage 2 (i.e. young embryos) do not touch the zero ordinate until the end of the observed breeding period.

Of the four definite spawnings, two (first and last) occurred during a firstquarter lunar period, one immediately before or at the new moon, and one just before or at the full moon. Thus, three of the four concerted spawnings can be fitted into a first-quarter lunar period, and one to a last-quarter period. Similarly, three spawnings (all except the first) occurred at times when the tidal range was increasing, whilst, although the first spawning probably took place at a period of neap tides, the intensity of spawning greatly increased as the tidal range became greater (see Fig. 3). Unfortunately, the sampling frequency was too low to provide definite evidence for or against spawning towards the end of the lunar quarter periods when the tidal range and tidal currents would increase daily. The date of commencement of spawning at Burnham-on-Crouch in 1950 (29–30 March) lends some weight to this view, as spawning commenced from 1–2 days after the occurrence of the bottom of neap tides, i.e. when the tidal range was increasing.

Lunar periodicity in spawning has been fully discussed in the past by Fox (1924), Orton (1926, 1936), Amirthalingam (1928), Battle (1930, 1932), Wheeler & Brown (1936), Korringa (1947) and others. These writers have quoted many instances of spawning of marine invertebrates at spring tides, but there are few records of spawning at neap tides or at periods when the tidal range is increasing from the minimum at neaps, although Orton (1936) states that in 1927 there was a distinct tendency for *Ostrea edulis* from the Fal and Blackwater beds to spawn upon the neap tides preceding full moon tides. Some evidence of the spawning of *Pomatoceros triqueter* during periods of neap tides at Cullercoats was obtained in 1947 (Chipperfield, 1948).

The specific inducements which cause spawning in relation to lunar phases are obscure. Briefly, the factors which may be responsible are probably either (a) nutritional or (b) concerned with intrinsic rhythms.

There has been little investigation into the possibility that cyclic changes in nutrition related to the lunar and tidal periods induce spawning, although Loosanoff & Nomezko (1946) have found that feeding of *Ostrea virginica* shows no relationship to tidal ranges or periods of light and darkness. It seems possible that hitherto unknown factors such as the 'external metabolites' of Lucas (1947) may have an important bearing on these problems. Further, in some organisms, the rhythm of spawning appears to be intrinsic, e.g. in *Convoluta* the spawning of eggs takes place during the neap tides even *in vitro*.

There is little evidence that moonlight, or its absence, or the greater illumination of the sublittoral zone at the occurrence of low water during the hours of daylight, has any effect on immersed animals as a spawning inducement (Amirthalingam, 1928). Amongst the hydrographical effects of tides are the effect of alternating hydrostatic pressures, the greatest range being produced at spring tides; the variation in water temperature due to the more rapid warming and cooling of the intertidal zone at low water; and the variations in the magnitude of the tidal currents flowing at springs and neaps. Amirthalingam (1928) feels there is little evidence that hydrostatic pressure influences spawning in *Aequipecten*, whilst Orton (1936) suggests that a decrease in hydrostatic pressure with an increase in temperature may be responsible for initiating spawning in *Ostrea* on spring tides. Korringa (1947) also favours the effect of rhythmic variations in hydrostatic pressure as spawning inducements, whilst Battle (1932) suggests that the increase in pressure at spring tides causes spawning in *Yoldia sapotilla*. The presence of pressure-sensitive organs has not, as yet, been demonstrated in marine invertebrates. Variations in the velocity of tidal currents at springs and neaps may influence spawning, either directly or indirectly, e.g. via nutritional channels.

No precise spawning stimulus or series of factors contributing to the spawning stimulus in *Crepidula* can be elicited from the observations made in 1947. Spawning in *Crepidula* appears to be independent of temperature once a value of  $50^{\circ}$  F. has been attained. Tidal currents run swiftly both in the Blackwater and the Crouch, whilst the difference between the tidal range at springs and neaps is fairly large (about 5 ft. mean). Thus either of these factors may be responsible for the tendency towards concerted spawning of *Crepidula* in 1947.

# Duration of Embryonic Life

Owing to the low sampling frequency adopted in 1946, and the commencement of observations when the spawning period was well advanced, no reliable estimate of the duration of embryonic life of *Crepidula* can be made from the data. The data obtained from the more frequent observations in 1947, however, can be utilized for this purpose.

If it is assumed that the first normal true spawning occurred between 23 and 29 April, the first female *Crepidula* bearing Stage 3 embryos (pre-veligers) were observed in the sample taken on 14 May (28%), and the first fully developed veligers (Stages 4 and 5), and the first empty egg capsules on 20 May. Of the Stage 3 embryos observed on 14 May, a number were very advanced, and, if these may be considered as the first spawned embryos, they may be represented in the sample of 20 May by the recently emptied egg capsules. Similarly, the bulk of the veliger spawn (Stages 4 and 5) in the sample of 20 May probably represent those embryos which were spawned between 29 April and 6 May. These relationships are clearly illustrated in Fig. 2.

Thus, at the beginning of the spawning period, when sea temperatures range between 50 and  $55^{\circ}$  F., the first pre-veligers appear about 14 days after spawning, and the first true veligers in 14–21 days, whilst hatching occurs in

21-28 days. This estimate of the period of incubation is of the same order as Orton's (1912 *a*) estimate that the eggs are 'protected by the mother *Crepidula* for about a month'.

The sequence of embryonic stages is less obvious later in the season, but it is possible that the spawning which occurred on about 18 June (see Table II) is represented in the sample of 28 June by the maximum of recently emptied egg capsules, whilst the peak in early veligers in the sample taken on 9 July represents those eggs spawned immediately before 2 July (see Fig. 2). Thus, during the period of higher water temperatures, development to the early veliger stage apparently takes place in about 7 days, and the hatching stage in about 10–14 days.

Thus, at the beginning of the spawning period, embryonic development in *Crepidula* would appear to take about twice as long as in June and July. The shorter duration of the veliger stages, particularly Stage 5, as compared with the younger stages, would explain the low incidence of females bearing Stages 4 and 5 embryos in the periodic samples.

# Intensity of Spawning in 1946 and 1947

The proportion of female *Crepidula* from the Blackwater bearing developing embryos and hatched egg cases (i.e. the total proportion spawned in each sample) in the 2 years are plotted in Figs. 1 and 2 for 1946 and 1947 respectively.

Even if allowances are made for the lower frequency of sampling in 1946, the gross spawning curves for 1946 and 1947 are very similar. In both years the maximum proportion of females bearing developing embryos or empty egg cases was c. 95%, or if those bearing Stage I (i.e. possibly premature) embryos are excluded as forced spawners, both maxima are reduced to about 90\%. In both years these maxima were attained in May and were maintained during early June, the proportion of spawn-bearing females decreasing rapidly in July, the zero ordinate being approached in 1947 in mid-August. In both years a further spawning occurred at the end of the principal period, in August, and was rather earlier and almost twice as heavy in 1946 as in 1947 (c. 30% females with spawn in 1946, and 15\% in 1947).

The females bearing embryos in Stages 4 and 5 (i.e. veligers) in one sample are unlikely to be represented by others in the next sample with embryos of the same stage of development, particularly during June and July, when embryonic development has been shown to be more rapid than at the beginning of the spawning period. Now, the aggregate percentage of females bearing Stages 4 and 5 embryos (i.e. veligers) in all samples from Tollesbury in 1946 is 179, or 230 if all samples from West Mersea are included except the one collected on 20 May (see Table I) only 3 days after a collection at Tollesbury, and in which, if spawning occurs more or less simultaneously throughout the Blackwater, veliger embryos arising from ova spawned at the same time are represented. The corresponding aggregate proportion for 1947 is 183%.

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In view of the rapid development of the veliger embryos the aggregates given above would suggest the probability that the majority of the fully functional females of *Crepidula* spawn at least twice in a season. Further, the fact of periodic concerted spawning would suggest multiple spawning by some females at least, particularly as exhaustion of the ovaries was not of common occurrence until July, i.e. towards the end of the main spawning period.

# Sequence of Spawning in the Chain

The spawning of female *Crepidula* in 1947, during the initial spawning period, was analysed to show the percentage without spawn with Stages 2 and 3, and Stages 4 and 5 spawn and with empty capsules in each of the first two or three positions in the chains. (Note: the first individual in the chain is taken to be the basal member which adheres to the substratum, and which is designated the 'A' position. The next individual occupies the second or 'B' position and so on.) The proportion of chains with more than three functional females (i.e. fully developed ovary, well-developed uterus and with either a very rudimentary penis or none at all) is small, and has not been considered.

Out of a total of sixty-nine chains with two females, the basal member possessed more advanced embryos in thirty-one cases, the second member bore older embryos in twenty-three cases, and in the remaining fifteen examples, both the first and the second female members of the chain possessed embryos of the same stage of development.

Only twenty-eight chains possessing three females occurred in the samples of *Crepidula* taken during the early part of the breeding season. Of these twenty-eight chains, in eleven the basal females (i.e. the female in the 'A' position) possessed more advanced embryos, in nine those in the second or 'B' position, and in three only those in the third or 'C' position. Of the remaining chains, in one the females in the 'A' and 'B' positions bore embryos in the same stage of development, and in two those in positions 'B' and 'C' did so. The number of chains with four females was too small to allow any useful analysis to be made.

The numbers involved are not very large, and the statistical error is consequently high, but in the initial spawning period, females occupying the basal or first position appear to bear somewhat more advanced embryos than those in the second position, and that these, in turn, are somewhat more advanced than in the third position. Thus, there seems to be a very slight tendency in all chains for the basal and therefore oldest female to spawn first, with the next following closely. Females in the third position appear to spawn later than those in the first two (i.e. proximal) positions, this being especially noticeable towards the end of the spawning period. It is not known whether this is due to earlier copulation, which, in any case, occurs early in the year (Orton, 1912 a). It is possible that the proximity of one or more males may delay the onset of maturity of the distal females, or that these spawn later, because they are younger females, maturing later normally.

The Final Spawning. In both 1946 and 1947, a small spawning occurred in August and September, as previously stated. The significance of this is rather obscure, for this spawning does not appear to be a further 'peak' of spawning in the main period, but one taking place after the termination of the latter. In view of Coe's (1938) work upon the sex change in *C. fornicata* from American beds, there is a possibility that the final spawning observed in 1946 and 1947 was due to the ripening of individuals which had changed their sex to become functional females during the summer. On the other hand, it is more likely that this final spawning took place in females which had spawned out earlier in the season, and which had, during the intervening period, re-developed ovaries sufficiently well to allow a further emission of ova to occur, whilst environmental conditions were still favourable to continued spawning.

# TABLE V. CREPIDULA FORNICATA. POSITION IN CHAIN AND FINAL SPAWNING, 1947

Position		Stage of embryonic development									
in chain (see p. 64)	Total no. examined	Stage 2	Stage 3	Stages 4 and 5	Stage 6	Total					
(a) 20. viii. 47	:										
С	25	0	0	0	0	0					
В	25	I	I	I	0	3					
A	13	0	I	I	0	2					
	63	I	2	2	0	5					
(b) 3. ix. 47:											
С	37	0	I	5	0	6					
В	37	4	0	0	I	5					
A	28	4	2	2	0	8					
	102	8	3	7	I	19					

If the final burst of embryos were due to the spawning of those individuals which had changed their sex during the earlier part of the season, then the presence of embryos should tend to be confined to the distal females in the chain, i.e. to those females immediately adjacent to the males.

In Table V the distribution of females bearing embryos in two samples taken in 1947 during the final spawning period is analysed.

Thus, from the table, although it is possible that some of the distal females which had spawned were those which had changed their sex during the season, there is no evidence that the final spawning is confined to any particular position in the chain, and the figures would indicate that the final spawning is a question of adequate re-development of the ovaries whilst conditions for spawning are favourable.

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### Incidence of Females with a Rudimentary Penis

During both 1946 and 1947, a number of females, with a well-developed uterus, and a yellow gonad filled with abundant oocytes but with a rudimentary (degenerating) penis, often 3–4 mm. in length, were a constant feature of the samples. These were invariably<sup>1</sup> the most distally situated females (i.e. those nearest the males), and obviously the youngest in the chain, having been the last to change from male to female.

The incidence of these females in samples examined 1947 from West Mersea between 29 April and 28 July is analysed in Table VI.

	Total	Females with rudimentary penis								
Date, 1947	females in sample	(a) Total	(b) Without spawn	(c) With spawn	(d) Percentage of total females					
29. iv.	135	9	8	I	6.7					
6. v.	II2	9	9	0	8.0					
14. v.	75	5	5	0	6.7					
20. V.	105	I	0	I	0.9					
4. vi.	121	3	3	0	2.5					
16. vi.	III	3	3	0	2.7					
25. vi.	78	I	I	0	1.3					
2. vii.	100	7	4	3	7.0					
9. vii.	104	3	3	0	2.4					
17. vii.	68	7	7	0	10.3					
28. vii.	128	2	2	0	1.6					
Total	1137	50	45 (4·0%)	(0·4%)	(1.1%)					

# TABLE VI. CREPIDULA FORNICATA, 1947. INCIDENCE OF FEMALES WITH A RUDIMENTARY PENIS

It is apparent that the mean incidence of females with a rudimentary penis is low, although it is possible that more are present at the beginning and at the end of the main spawning period than during the period of most intense spawning. The low occurrence of females with a rudimentary penis bearing spawn is very significant, and would suggest that the bulk of such females are immature, in short chains at least.

## Number of Female Crepidula in a Chain

In Tables I and II, the mean number of females per chain in each sample is recorded in the final column.

Cursory examination of these figures suggests that, in 1946, the chains collected from Thirslet Creek (Tollesbury) contain rather more females than those collected from Mersea Quarters (West Mersea), i.e. a position nearer the open sea, whilst in 1947 rather more females occurred in those chains from Mersea Quarters collected during the latter part, and after the end, of the breeding period than during the early part.

These conclusions can be subjected to a strict test, employing the method based upon the distribution of 't'.

<sup>1</sup> I am informed by Prof. Orton that this is not always applicable to long chains of *Crepidula*.

From the 1946 observations, the mean number of females in the chains from West Mersea is 2.98 (three samples), and, in chains from Tollesbury, 3.31 (eleven samples). Comparison of these samples, on the null hypothesis that they were drawn from the same population, gives a value for 't' of 2.14. As, from the tables (Fisher & Yates, 1948, p. 32) 't' for P=0.05 for 12 degrees of freedom is 2.18, it can be concluded that the probability of the Tollesbury *Crepidula* samples possessing more females per chain than the West Mersea *Crepidula* in 1946 is high, the difference between the sample means being significant on the P=0.06 level. A heavier settlement of spat, owing to the more restricted size and rate of flow of the water mass, or a lower mortality in Thirslet Creek may provide possible explanations.

In 1947 figures were similarly treated, the mean of the number of females per chain from 23 April to 17 July being compared with the mean for the period 28 July to 7 October. The sample taken on 25 June was omitted, as a large proportion of the longer chains were broken. The mean number of females per chain is 2.34 (ten samples) for the earlier period, and 3.13 (six samples) for the later period. From the comparison of these means by the normal methods a value for 't' of 5.34 was obtained. From the tables the values of 't' for 14 degrees of freedom for P = 0.05 and P = 0.001 are 2.145 and 4.140 respectively. Thus, the difference between the means is highly significant, and it would seem that, in 1947, the mean number of females per chain of *Crepidula* increased at the end of the spawning period owing, possibly, to a lower mortality at the end of the season compared with the period when spawning was intensive, or to a real increase in the number of females by the change of sex from male to female. As there was little evidence in the 1947 samples of a higher mortality during the spawning period, it is probable that the sex change is responsible for the increase in the number of females per chain.

## Settlement in 1946 and 1947 in the Blackwater

During the summer and early autumn of 1946, samples of culch and of adult *Crepidula* were examined carefully for the presence of *Crepidula* spat, but very few indeed were found, in spite of the high intensity of spawning, and the fact that abundant veligers were seen in plankton collected at West Mersea on 20 June.

In 1947, however, settlement was heavy. On 28 June, Mr French, who collected the samples at West Mersea, reported the presence of a few spat on the oyster culch, whilst a few minute *Crepidula* were taken from shells collected at Tollesbury on the 25th. By 2 July, spat was thickly clustered on old shells, particularly on the inner surfaces of dead *Crepidula* shells, and on the sheltered areas of oyster shells and culch. Few were found upon exposed surfaces of the shells and upon living *Crepidula* in the samples. A week later very large numbers of spat were present, and settlement continued until mid-August,

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thereafter declining rapidly. A small settlement, indicated by the presence of minute spat, took place in September, at a time when spat which had settled earlier were growing rapidly. Orton (1950 a) has recently discussed the variations in the spatfall of *Crepidula* on the Essex oyster beds in 1945–1947.

# Duration of larval life in Crepidula.

Estimates of the duration of planktonic larval life based upon observations of release of larvae from, or the spawning of, the adults of sessile marine invertebrates, and of the initial settlement of spat upon appropriate substrata are notoriously inaccurate, depending, as they do, upon the assumption that the stock of larvae settling is the same as the stock which was spawned previously. Examples are well known in which, after a normal spawning, larvae developed beyond a certain stage are not seen in the plankton for a considerable period, e.g. *Verruca*. Thus, all estimates of larval life derived in this way must be interpreted with care.

Such an estimate for the duration of planktonic larval life in *Crepidula* can be obtained from the data obtained in 1947 for the Blackwater. The first hatched egg cases appeared in the sample taken on 20 May, whilst the first settled spat were observed at the end of June, thus giving a possible larval life of about 35 days.

# GROWTH OF SPAT

When a length of 3-5 mm. is reached, the young *Crepidula* become highly motile and migrate from their sheltered positions under shells, etc. and settle upon existing chains, usually taking up a terminal position, or one upon the side of an adult female. A few small individuals occasionally form short chains of two or three members. It is not known whether these chains of spat are permanent or whether the basal member can migrate, carrying the remainder of the chain. Few solitary *Crepidula* over 6 mm. in length were found on culch from the Essex oyster beds in 1947.

During this period of migration, and when permanent positions are taken up on existing chains, mortality is apparently high, for the number of small individuals (c. 6–12 mm.) observed on chains during the autumn is only a very small fraction of the total settlement on culch, etc., in the summer.

On 29 April 1947 some fifty smallest individuals from the chains in the sample were removed and measured. The range was from 7 to 26 mm. with a mean of 18 mm., which probably represents a fair growth of the previous season's spat and is in accordance with the observations of Orton (1914). Coe (1942) states that the growth rate on New England coasts is such that, at 1 year of age, *C. fornicata* are 18–28 mm. in length. Gould (1917), on the other hand, states that *C. fornicata* grew to 10–15 mm. in 2 months at Woods Hole. Such high growth rates do not appear to be attained amongst normal populations in

#### BREEDING OF CREPIDULA

the Blackwater, but no accurate information is available from measurements of individuals of unknown age in a chain owing to the impossibility of distinguishing the age groups. Investigations employing known individuals, such as Orton's (1914) constitute the only practicable method of obtaining reliable information upon growth rates, particularly of older individuals. During 1948 and 1949, a few Crepidula spat settled upon glass and Tufnol panels exposed at Burnham-on-Crouch in a general investigation of the season of settlement, rates of growth, etc., of sedentary marine organisms. These Crepidula were measured on the withdrawal of the panels. The sizes recorded lay between 10 and 22 mm.<sup>1</sup> the panels having been exposed for two months (July and August) in each year. Such information must be interpreted with care, owing to the motility of the very young spat but these figures do represent the rates of growth in shell length of individuals from the time of initial settlement under favourable conditions, upon flat surfaces. As the length of a Crepidula shell is dependent upon the size and shape of the substratum, linear dimensions constitute poor criteria of shell growth, and an increase in length can only be considered an indication of growth.

# SUMMARY

The breeding of *Crepidula fornicata* from the Blackwater oyster beds was investigated during 1946 and 1947. A few observations were also made at Burnham-on-Crouch in 1948, 1949 and 1950. The methods of sampling, presentation of the results, and of assessing the significance of the results statistically are described.

In 1946 and 1949, developing embryos were found from April until early September, giving a breeding period of about  $5\frac{1}{2}$  months' duration. The intensity of spawning was similar in both years.

In 1947, spawning commenced shortly after the sea temperature had risen to and above  $50^{\circ}$  F. Observations made in 1948 and 1950 confirmed this.

A suggestion of periodic concerted spawning of the female *Crepidula* was obtained in 1946 and 1947. The results suggest that there is an irregular periodic spawning at or immediately after neap tides.

Observations strongly suggest that most female *Crepidula* spawn at least twice in a season.

Estimates are given of the duration of embryonic and larval life. At the beginning of the breeding period, embryos develop to the pre-veliger stage in about 14 days, and to veligers in 14–21 days hatching in 21-28 days. A tentative estimate of the duration of larval life of *c*. 35 days is made. The effect of temperature on embryonic development is discussed.

The sequence of spawning in the chain is considered at the beginning and

<sup>1</sup> For example, on a glass panel exposed 12 July 1949 and withdrawn 9 September 1949, were two *Crepidula*, one 11 mm. and the other 22 mm. in length.

the end of the breeding period. There is some evidence that the older females spawn a little in advance of the younger ones.

The incidence of females (throughout the season) with a rudimentary penis is considered.

The mean number of females per chain at Tollesbury and West Mersea, and during and at the end of the spawning period, is discussed.

The settlement and growth of the spat of *Crepidula* are briefly described, and discussed.

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# STUDIES IN THE BIOLOGY OF TALITRIDAE (CRUSTACEA, AMPHIPODA): EFFECTS OF ATMOSPHERIC HUMIDITY

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# (Text-figs. 1-9)

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#### **RESISTANCE TO DESICCATION**

Although the high-water Talitridae are normally terrestrial they show little structural modification compared with aquatic amphipods. They have no obvious adaptations to limit water-loss, and their branchial method of respiration is typically aquatic. It was to be expected, therefore, that the evaporating power of the air would play a considerable part in limiting their habitats and determining their habits. The following experiments were made to try and assess the importance of this factor.

#### Apparatus and Methods

A series of experiments was carried out to measure the survival times of *Talitrus saltator* (Montagu), *Talorchestia deshayesii* (Audouin) and *Orchestia gammarella* (Pallas) at different controlled humidities. Attempts were first made to control the humidity using saturated solutions of salts. The apparatus consisted of a glass dish, 18 cm. diameter by 7 cm. deep, divided horizontally by a copper-gauze platform of 0.25 mm. mesh. Beneath the platform were three dishes of diameter 5 cm. containing the solution; above it was an 'Edney' paper hygrometer. A Perspex lid, with central, corked hole for

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introducing animals, was sealed on to the dish with plasticine. When the air in the chamber reached equilibrium with the saturated solution, as shown by a steady hygrometer reading, animals were introduced. This, however, usually caused a considerable rise in the humidity. For example, at  $15^{\circ}$  C. using saturated magnesium chloride solution, the hygrometer reading rose from the equilibrium value (58%) to 70% R.H. in 15 min. on introducing three *Talitrus* and three *Orchestia gammarella*.

Besides showing the ineffectiveness of the method, this result shows the importance of the hygrometer as a humidity check, a precaution omitted by Backlund (1945) who measured the survival times of several animals, including O. gammarella. His apparatus gave a similar surface of saturated salt solution to mine, and he introduced ten specimens at a time. His results are considered later.

In the final form of the apparatus much more constant humidities were obtained by using sulphuric acid solutions in place of saturated salt solutions; the liquid surface was greatly increased by covering the whole floor of the chamber with solution, and the number of specimens in the apparatus was limited to six at a time (two of each species). The densities of the sulphuric acid solutions were measured with a hydrometer, and the equilibrium humidities were calculated from data contained in the International Critical Tables (National Research Council, U.S.A., 1926). The experiments were carried out in a constant temperature room at  $25^{\circ}$  C. Under these conditions the greatest variation in the hygrometer reading during any experiment was a rise of  $5^{\circ}_{\circ}$  R.H. after introducing specimens into a humidity of  $36^{\circ}_{\circ}$  R.H. At higher humidities introduction of specimens caused smaller rises in the hygrometer reading, and at  $88^{\circ}_{\circ}$  R.H. and above no rise could be detected.

Adult specimens of each species were transferred direct from laboratory stocks to the constant humidity apparatus, and survival time was measured from their introduction until no movement occurred, even when the animal was touched with a covered wire (insulated flex) through the hole in the lid.

### Results and Discussion

In all three species the first noticeable effect of desiccation was a failure to co-ordinate the limb movements, and a tendency to fall on one side. Later there was a darkening of the extremities of the limbs and antennae, particularly in Orchestia. The survival times are shown in Table I. In saturated air all specimens were healthy after 24 hr. After 48 hr. those of Talorchestia deshayesii were dead, but the others were healthy. None of the species can withstand even small saturation deficiencies for more than a few hours, but Talitrus saltator always survives considerably longer than the others. This may be due to its greater size and consequent smaller surface: volume ratio, yet at all humidities the survival times of Talorchestia deshayesii and Orchestia gammarella are almost identical, in spite of the much greater size of the latter.

It was found that in air of 36% R.H., in which adult specimens of *Talorchestia* deshayesii survived 75 min., juveniles of *T. saltator* and *Orchestia gammarella* of approximately the same size as an adult *Talorchestia deshayesii* both survived only 45 min.

'If the relationship between rate of water loss...and saturation deficiency is linear and survival is limited only by water loss, then the curve of longevity against saturation deficiency is hyperbola-shaped' (Johnson, 1942). The linear relationship between saturation deficiency and the reciprocal of longevity (Fig. 1) makes it reasonable to assume that, in the species under consideration,

TABLE I. SURVIVAL TIMES OF TALITRUS SALTATOR, ORCHESTIA
GAMMARELLA AND TALORCHESTIA DESHAYESII AT DIFFERENT
CONTROLLED HUMIDITIES TEMPERATURE 25° C

TT		Survival times (hr.)							
(R.H. %)	T. saltator	O. gammarella	T. deshayesii						
100	> 48	> 48	>24						
	> 48	> 48	>24						
95	16·0	9.0	9·0						
	14·5	8.0	8·0						
88	7·5	5·25	4·5						
	7·0	4·5	3·5						
75	5∙0	2·5	2·5						
	4•75	2·5	2·5						
57	2·5 2·5	1.2	1.2 1.2						
36	1·75	1.2	1·25						
	1·5	1.0	1·25						

survival is limited only by water-loss, and that the rate of water-loss is not controlled by the animal. If this is the case, then the relationship between survival and saturation deficiency will be independent of the temperature, as has been shown to hold approximately true for several species of insect (Buxton, 1932; Johnson, 1942; Ludwig, 1945). On this assumption it is possible to compare the survival times obtained by the writer for *Orchestia gammarella* with those given by Backlund (1945). At a supposed humidity of 86% at  $20^{\circ}$  C. he obtained survivals of from 9 to 32 hr. This humidity corresponds to a saturation deficiency of 2.46 mm. Hg, under which conditions the survival time, from Fig. 1, would be 5 hr. Similar discrepancies occur at both higher and lower humidities, Backlund always obtaining much the greater survival time. This must be attributed to the method of humidity control he employed, using saturated salt solutions, which, as mentioned earlier, I found to be quite ineffective.

The animals in the above experiments had no access to food, which is normally plentiful in the habitats of all three species. Similar experiments were therefore made in which food was available, to determine whether Talitridae can utilize water in their food, or produced by its oxidation, to counteract water-loss by evaporation. Dry foods, in the form of (I) biscuit, (2) dextrose and (3) dried *Fucus* frond, were left untouched by both *Talitrus* and *Orchestia gammarella*, so that the role of metabolic water could not be determined. With moist foods it was difficult to avoid raising the humidity in the chamber on introducing the food, but no rise in the hygrometer reading occurred in the following two cases:

I. At R.H. = 95 %,  $T = 18^{\circ}$  C., a piece of *Fucus serratus* frond measuring  $6 \times 1$  cm. was provided as food. The wrack was dry to the touch, but flexible and not dried out. Three specimens of *Orchestia gammarella* were introduced. After 72 hr. one was dead, but the other two were healthy, and a considerable quantity of the wrack had been eaten.



Fig. 1. Reciprocal of mean survival time plotted against saturation deficiency. A. Talitrus saltator; O, Orchestia gammarella; O, Talorchestia deshayesii.

II. At R.H. =  $88\%_0$ ,  $T = 25^\circ$  C., a 1 cm. cube of *Drosophila* medium (Darlington & La Cour, 1942) was introduced into the chamber. Two normal specimens of *Orchestia gammarella* survived 20 and 22.5 hr. respectively. Two other specimens were introduced after first coating their mouthparts with paraffin wax to prevent them from feeding. One of these succeeded in removing the wax after 2 hr., and immediately began feeding. It was still healthy when the other died after 8 hr. All these specimens kept close to the food, usually touching it, and must have been in a local humidity appreciably higher than

the general humidity of the chamber, the survival time of the waxed specimen being the same as that at 95 % R.H. in the absence of food. Nevertheless, the greatly increased survival times of the specimens which fed suggests that water from food can play a very important part in enabling Talitridae to resist the effects of desiccation.

Field observations on the habitats and habits of the animals provide confirmation of the importance of food in this connexion. Backlund (1945) states that the humidity in the surface layers of wrack beds is at or near saturation only for a period of less than 4 hr. at night, and may fall well below 50 % R.H. by day, and that the humidity of deep layers, though always high, is not always maximal. My own observations, made by immersing a paper hygrometer in different wrack beds for periods of over 30 min., at depths of about 15 cm., have shown humidities from 85 to 90% R.H. These readings were always made when O. gammarella was present in the wrack in the vicinity of the hygrometer. The humidity of the normal habitat of O. gammarella is, therefore, such that in the absence of food the animal would die of desiccation in a few hours, and Talorchestia deshayesii, with similar resistance to desiccation, inhabits even drier situations. In another section of his work, Backlund comments on the great amounts of food consumed by Orchestia gammarella, 'but the amount of excrements is also great and most of the stuff eaten is not utilized'. I have noticed that talitrids always pass dry faeces, even when feeding on very moist food, suggesting that the amount of food eaten is determined by the animals' water requirements rather than their energy requirements.

O. gammarella is normally found among wrack on a non-sandy substratum, and Talorchestia deshayesii among, or under, wrack on a sandy substratum. Both species feed both by day and by night, and seldom wander far from the wrack, though they do come into the open more at night. A contrast is found in the behaviour of Talitrus, which feeds little by day but remains buried beneath the sand, often many yards away from the nearest wrack. At night it ranges over the beach from the lower slopes of sand dunes, well above highwater spring level, to mid-tide level or as low as the sea permits. These long periods without food by day and long migrations over the exposed beach at night probably could not be sustained by the other two species because of their much smaller resistance to desiccation.

# ORIENTATION IN HUMIDITY GRADIENTS

As talitrids are very susceptible to desiccation even at high humidities, it seemed important to investigate their behaviour when a choice of humidities is available to them, to find to what extent, and by what means, they can distinguish between moist and dry air, and to compare the reactions of the different species. Experiments were made on the same three species for which the resistances to desiccation were compared, namely Talitrus saltator, Orchestia gammarella and Talorchestia deshayesii.

#### Apparatus and Methods

To produce humidity gradients, a form of Gunn & Kennedy's 'alternative' chamber was used (Gunn & Kennedy, 1936). The apparatus measured 18 cm. diameter by 7 cm. deep, and the platform was of copper gauze of 0.25 mm. mesh. Resting on the platform were two 'Edney' paper hygrometers, one on either side of the chamber, with the sensitive paper in each case to the outside, and the edge of each instrument 1 cm. from the edge of the chamber to allow a clear path for the animals round the perimeter.



Fig. 2. Typical humidity gradient in 'alternative' chamber. Humidities at 1 cm. from edge of chamber. Temperature,  $15^{\circ}$  C.

The calibration of the hygrometers was checked at intervals of about a month over sulphuric acid solutions of measured density, and they were checked against each other at air humidity every few days so that any variation in the calibration of either instrument would have been detected. The calibration never varied by more than 2% R.H. in any month.

In the majority of cases the dishes beneath the platform contained sulphuric acid solutions of different strengths, but caustic soda and caustic potash, both solid and solution, and solid calcium chloride were used in several of the experiments with similar results to those obtained with sulphuric acid at similar humidities, showing that the animals were reacting to the humidity gradient and not to any property of the desiccating agent.

By placing the desiccating agent in two well-separated dishes an approximately linear gradient of humidity was obtained. A typical gradient is shown in Fig. 2. If each half of the floor of the chamber is covered with dishes, meeting at the boundary of the moist and dry halves, a much steeper gradient is obtained in the boundary region, with a very gradual gradient on either side (such an arrangement was used by Gunn (1937), who includes an S-shaped graph of a typical gradient under these conditions). While animals are likely to give a more obvious response to the steep gradient, the nature of the response is likely to be seen more clearly in a linear gradient where reactions will not be confined to the boundary region.

The platform was marked with a diameter, dividing the chamber into moist and dry halves, and with lines parallel to this diameter at 2 cm. intervals. With the aid of these lines the distances of animals from the diameter could easily be estimated to the nearest centimetre, such distances in the moist half being regarded as positive and in the dry half as negative, so that the position of an animal could be referred to by a number between +9 and -9.

Up to 3 hr. was required for equilibrium to become established in the chamber. Specimens were not introduced until the hygrometers had been showing steady readings for at least half an hour. Only one specimen was introduced into the chamber at one time, and numerical values of the intensity of reaction to the gradient were based on the respective times spent by the animal in each half of the apparatus. In most of the previous work on the behaviour of animals in humidity gradients using an 'alternative' chamber, several specimens have been introduced at once, and measures of intensity of reaction based on readings of the number of animals in each half (Gunn, 1937; Gunn & Cosway, 1938; Pielou & Gunn, 1940; Lees, 1943). This method gives greater uniformity of results, since each result is an average for several specimens, but it cannot be applied to Talitridae because of their high rate of waterloss. Not only does the introduction of several talitrids noticeably affect the humidities in the chamber, but, particularly at low humidities, the steepest gradients leading to the moistest air surround the animals themselves. It was found in several preliminary experiments using five to ten specimens of Orchestia gammarella that all the specimens soon formed an aggregation round any stationary individual irrespective of its position in the chamber.

Young specimens of all species jump much more frequently than do adults, making it very difficult to plot their positions accurately. For this reason specimens below a size roughly corresponding to that of sexual maturity were avoided (9 mm. for *Talitrus*, 8 mm. for *Orchestia gammarella* and 6 mm. for *Talorchestia deshayesii*, all lengths with abdomen flexed). Apart from this no conscious selection was made.

The apparatus was always illuminated from directly above, and, to allow for any visual orientation of the specimen to external objects, the chamber was turned through  $180^{\circ}$  half way through each set of observations. On first introducing specimens, they would frequently feign death for periods of over a minute or show unusually great activity with much jumping. To allow them to become acclimatized to their surroundings no observations were recorded for the first 5 min. after introduction.

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Position records (Figs. 3-6) were obtained by noting the displacement of the animal from the diameter at 15 sec. intervals (shown by open circles on the graphs), and positions of additional turning points were also recorded (shown by black circles).

As a measure of the intensity of reaction to the humidity gradient the 'excess percentage time' was used, i.e.

 $\frac{(\text{time spent in moist half}) - (\text{time spent in dry half})}{(\text{time spent in moist half}) + (\text{time spent in dry half})} \times 100,$ 

which gives a theoretical range of values from +100%, representing an animal which does not leave the moist half, to -100%, representing an animal which



Fig. 3. Position record of Talitrus saltator in humidity gradient 92-38 % R.H. at 16° C. The apparatus was turned through 180° between the upper and lower records. O, positions at 15 sec. intervals; •, positions of additional turning points.

does not leave the dry half, with 0% representing no preference. Intensities were normally calculated from observations over two periods of 8 min. each, the apparatus being turned round between the two periods. With a few, large, fast-moving specimens of Talitrus, observations were confined to two periods of 5 min. each, while with a few slow-moving specimens of Talorchestia deshayesii, observations were continued for 10 or even 12 min. in each period. In all cases the average time for one complete circuit of the chamber was less than 3 % of the total time for which observations were recorded. Intensities of reaction were calculated either from position records or, more usually, from observations on the times when the specimen crossed from one half to the other.







Fig. 5. Position record of Orchestia gammarella in humidity gradient 76-50 % R.H. at 16° C.



 Fig. 6. Position record of *Talitrus deshayesii* in humidity gradient 96–78 % R.H. at 15° C. The apparatus was turned through 180° between the upper and lower records.
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All experiments were made at temperatures between 14° and 16.5° C.

Control experiments, in which the humidity was the same on both sides of the chamber, were made for all three species. Intensities of reaction greater than twice the standard deviation in control experiments for each species were regarded as significant.

# Significant Reactions

The minimum significant intensities for *Talitrus saltator*, *Orchestia gammarella* and *Talorchestia deshayesii* are, respectively, 13, 5 and 15. The low figure for *Orchestia gammarella*, compared with the other two species, is the result of its stronger thigmo-taxis. While in control experiments this species almost invariably followed a single track round the perimeter of the chamber, the other two occasionally left the sides and wandered over the platform. The theoretical maximum deviation from zero intensity of an animal which moves round the perimeter at a uniform rate without turning is the percentage time taken for one circuit of the chamber. This value for an average O. gammarella over two 8 min. periods is estimated at about 3%, and this value was seldom exceeded in control experiments for the species. The less restricted paths followed by *Talitrus saltator* and *Talorchestia deshayesii* resulted in greater variation in controls.

# Preferred Humidities

In over 250 experiments on the three species, at humidities from 25 to 100% R.H., all the significant reactions were positive. This means that whenever talitrids show a humidity preference it is for the highest humidity available, up to, and including, saturated air. The preferred humidity is therefore the same as the optimum humidity as judged by survival times.

#### Types of Orientation

In this paper, orientation reactions are named according to the system described by Fraenkel & Gunn (1940).

All three species are thigmo-tactic, and tend to keep to the outside of the chamber with one antenna against the glass wall. This behaviour is most strongly marked in *Orchestia gammarella* which seldom leaves the outer wall, and therefore follows a restricted course. The other two species more frequently forsake the perimeter for the open platform.

Examination of position records (Figs. 3-6) shows that talitrids spend most of their time in moist air as the result of two factors: (i) the animals do not move at a constant speed, and (ii) they do not move in a constant direction.

#### Changes in Speed

The animals move more quickly in dry than in moist air. This is shown, to some extent, in all the position records, but Fig. 4 shows a specimen of *O. gammarella* whose hygro-positive reaction results entirely from this type of orienta-

tion. Movement at a constant speed round the perimeter of the chamber would give a sinusoidal graph, but the differential velocity of the specimen results in broad peaks and narrow, more pointed troughs. This orientation is an example of 'low hygro-ortho-kinesis' (Fraenkel & Gunn, 1940).

(It is probable that the stimulus in the case of talitrids and several other species which react to differences of humidity is not humidity but rate of water-loss. The conventional practice of describing such orientations in terms of humidity is followed here, but to describe them in terms of evaporation would be more correct.)

Two variants of hygro-ortho-kinesis are sometimes shown by talitrids:

(a) When the highest humidity in the chamber exceeds about 90% R.H., animals will frequently stop in the more humid regions and remain motionless for up to 30 sec. Halts in the drier regions are extremely rare and seldom exceed 5 sec. This reaction is shown by all three species, but is most common in *O. gammarella*.

(b) At lower humidities (less than about 50% R.H.) the animal may start jumping and will continue jumping until it lands in moister air, when walking is resumed. The jumping is quite random in direction, but usually three or four jumps are sufficient to take the animal out of the drier region. Though this reaction has been seen in all three species, it is most common in *Talorchestia deshayesii* and least common in *Talitrus saltator*.

Variant a is an extreme case of variation in the velocity of locomotion, while b involves variations not only in velocity but also in the type of locomotion. Both are examples of greater locomotor activity in drier air or low hygro-ortho-kinesis.

#### Changes in Direction

In Fig. 3, 5 and 6 turning reactions play a very important part in the hygropositive behaviour of the specimens; they turn more frequently when moving from moist to dry air than when moving from dry to moist air. Turns are not always made towards moister or away from drier air, in fact, in some cases, turns towards drier air are almost as frequent as those towards moister air (e.g. Fig. 5). The hygro-positive reaction results from the intervals between turns tending to be relatively shorter when the animal is moving into drier air, or, as Ullyott (1936) found with flatworms in a gradient of light intensity, 'an increase in stimulating intensity produces an increase in the rate of change of direction'.

As the animals usually follow the outer wall of the chamber, turns are usually complete reversals of direction, and the direction of locomotion is not truly random. The restrictions in the direction of locomotion are, however, not imposed by the humidity, but by the tendency of the animals to keep close to projecting objects (thigmo-taxis). As far as the humidity gradient is concerned the direction of locomotion is random. This is seen more clearly when specimens temporarily leave the outer wall of the chamber—behaviour only very occasionally seen in Orchestia gammarella, but more common in Talitrus saltator and Talorchestia deshayesii. On the open platform the undirected nature of the turning reaction is very obvious. It does not result (except by chance) in motion towards or away from the source of the stimulus (evaporation), and is not governed by the direction of the humidity gradient, but by comparisons of the intensity of the stimulus at successive times. By a 'process of alternate stimulation and adaption the animal is gradually brought to the weaker end of the gradient' (Ullyott, 1936). The orientation is clearly of the same type as that shown by Dendrocoelum in a gradient of light intensity, described by Ullyott under 'phobotaxis', and later re-named 'klino-kinesis' by Fraenkel & Gunn (1940).

In very steep humidity gradients talitrids will occasionally stop when moving into dry air, then move backwards for about 1 cm. before turning. Comparable behaviour is common in *Paramecium* when moving into unfavourable conditions, and has been included under klino-kinesis by Gunn & Walshe (1941). A similar type of reaction is frequently shown by wireworms in steep gradients of humidity, but was regarded by Lees (1943) as a directed reaction, and therefore described as 'klino-taxis'. However, his own figures show that neither the direction of recoil nor the direction of the track after turning bore any relation to the direction of the gradient, the direction of recoil being determined solely by the original path of the wireworm, and the new path frequently leading the animal back into dry air almost immediately.

True klino-kinesis as a reaction to humidity gradients is shown by the beetle, *Tenebrio molitor*, which shows a preference for dry air (Pielou & Gunn, 1940). The orientation involves 'trial movements with the antennae...followed by a fairly precise movement towards the dry side'. Nothing comparable with this type of reaction appears to occur in wireworms or talitrids whose turning reactions in humidity gradients are properly described as 'klino-kinesis'.

Normally talitrids orientate in humidity gradients by a combination of ortho-kinesis and klino-kinesis, though weaker reactions may consist solely of ortho-kinesis (as in Fig. 4). The relative importance of the two types of reaction varies considerably between different individuals in the same gradient, but no consistent differences in this respect were noticed between different species or between different sizes or sexes of the same species.

#### Intensities and Limits of Reaction

The intensities of reaction of similar specimens of the same species in the same humidity gradient often differ enormously; for example, with a gradient 98-71% R.H. at  $15^{\circ}$  C. three different specimens of *Orchestia gammarella* gave intensities of 17, 30 and 74\% excess time. These individual differences do not appear to be correlated with size, sex or previous desiccation. In spite of this lack of uniformity in the intensities of reaction of different individuals, there

is consistency in the reaction of different specimens of the same species; for if one specimen is found to react to a certain gradient then, in general, all other specimens of the species will react to the same gradient, and if no reaction is given by one specimen then all other specimens will fail to react. This makes it possible to make quite a sharp distinction between those humidity gradients to which members of each species will react and those to which they will not.



Humidity difference in chamber (R.H %)

Fig. 7. Intensities and limits of reaction of *Talitrus saltator* to humidity gradients in 'alternative' chamber. Figures beside points give intensities of reaction. Circles around points denote that two or more experiments have been made using the same humidity gradient. The broken line marks the approximate limit of significant reaction. Temperature, 14–16<sup>.5°</sup> C.

This is done in Figs. 7–9. The free-hand dotted line in each case divides those gradients to which the vast majority of reactions were significant (on the right) from those to which the vast majority were not significant (on the left). (The limits of significance for each species are given, and differences in their value are discussed, earlier.)

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The minimum humidity difference required to give a significant reaction varies at different parts of the humidity range, and the manner in which it varies differs with the species. Thus *Talitrus saltator* gives a significant reaction to a humidity difference of 4% R.H. when the highest humidity in the chamber is 90%, but humidity differences of over 10% R.H. are required to obtain a reaction when the maximum humidity is above 92% or below 60% R.H.



Fig. 8. Intensities and limits of reaction of *Orchestia gammarella* to humidity gradients in 'alternative' chamber. Temperature, 14–16<sup>.5°</sup> C. For further explanation see Fig. 7.

(Fig. 7). Orchestia gammarella is most sensitive to small humidity differences when the maximum humidity is 100% R.H., when it shows a significant reaction to a humidity difference of 3% R.H., but its sensitivity decreases steadily with decreasing humidity (Fig. 8). *Talorchestia deshayesii* reacts to a 5% R.H. difference when the maximum humidity is between 60 and 65% R.H., but its sensitivity decreases rapidly at lower, and more gradually at higher, humidities (Fig. 9).

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In all three species there is no uniform increase in intensity of reaction with increasing humidity gradient, either considering results over the whole humidity range or any section of it. This might be attributed to the large individual differences mentioned earlier, but corresponding values for *Porcellio* (Gunn, 1937) show the same feature in spite of the averaging effect of using several (five) specimens in each gradient. Values for *Agriotes*, on the other hand, increase steadily with increases in both the humidity gradient and the maximum humidity (Lees, 1943).



Fig. 9. Intensities and limits of reaction of *Talitrus deshayesii* to humidity gradients in 'alternative' chamber. Temperature, 14–16<sup>.5°</sup> C. For further explanation see Fig. 7.

Specific differences among talitrids in the humidity zone in which they are most sensitive can be related to their field behaviour to a considerable degree. *Talitrus saltator* is unable to distinguish between different humidities above 92 % R.H. This figure probably represents the lowest humidity at which it can safely spend its day-time, non-feeding period, and it seems significant that its greatest sensitivity should be shown at humidities just below this value. It is

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probable, as mentioned earlier, that the humidity reaction of talitrids depends on their ability to compare different rates of water-loss, which is governed by saturation deficiency. A relative humidity of 92% at 15° C. corresponds to a saturation deficiency of 1.02 mm. Hg, under which conditions (from Fig. 1) an adult *Talitrus* would survive over 20 hr. It is therefore suggested that *Talitrus* may use its humidity sense in determining the depth of its burrow, but the humidity in the burrow is not known.

Orchestia gammarella is the only species which inhabits the deeper layers of wrack beds where humidities are at or near saturation, and it may use its great sensitivity at high humidities to keep in this environment. To quote Backlund (1945), 'the humidity gradation is steep in the border horizon between deep layers and surface layers [of wrack beds]. Here many animals can use their humidity sense directly'.

The humidity zone in which *Talorchestia deshayesii* reacts to the smallest humidity differences lies at what must be the extreme lower limit of its environmental humidity range, but this species frequently lives in this humidity zone where mistakes in judging humidities might soon lead to desiccation, so that an efficient humidity sense is vital. The resistance of *T. deshayesii* to desiccation is almost identical with that of *Orchestia gammarella*, yet *Talorchestia deshayesii* is almost invariably found in drier situations. When the two species are found together in the same wrack bed, as frequently occurs at the mouths of estuaries, it is very noticeable that in dry weather *Orchestia gammarella* occupies the moist, deep layers, while *Talorchestia deshayesii* inhabits the dry, surface layers. *Orchestia gammarella* invades the surface layers only when the air humidity is high, as at night.

The humidity in wrack strings on sandy beaches is probably very similar to that in the surface layers of wrack banks. Here, too, *Talorchestia deshayesii* is to be found feeding by day and by night, but at night it is joined by *Talitrus saltator*. Thus, by living at the lowest humidities at which it can survive, *Talorchestia deshayesii* by day avoids competition for food with both *Orchestia gammarella* and *Talitrus saltator*.

#### Humidity Receptors

Attempts were made to locate humidity receptors in *Talitrus saltator* and *Orchestia gammarella* by amputating appendages or insulating different parts of the body from the atmosphere with wax or vaseline, and observing such specimens in humidity gradients. Specimens treated in the following ways were observed in a gradient of 95–70 % R.H.: (i) *Talitrus saltator* with antennae amputated; (ii) *Orchestia gammarella* with antennules and antennae covered with vaseline; (iii) *Talitrus saltator* with head, mouthparts, antennules and antennae covered with wax; (iv) *Orchestia gammarella* with dorsal surface covered with wax; and (v) *Talitrus saltator* with dorsal surface and thoracic legs covered with vaseline.

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All these animals showed well-marked preferences for moist air, orientating in the same manner as untreated specimens. Attempts were also made to cover the under surfaces of the animals with wax, vaseline or cellulose paint, but in all cases the specimen was either killed or completely immobilized. No doubt the covering of the branchiae had a pathological effect.

Gunn (1937) showed that covering the antennae of *Porcellio* with vaseline did not affect its humidity reactions, and suggested that receptors might be present on the thorax. Lees (1943) showed by amputation that in wireworms the antennae, labial palps and maxillary palps were all concerned in humidity reception, but he found no specific organs of reception. This, together with the discovery that the animals reacted to differences in saturation deficiency rather than relative humidity, led him to suggest that the reaction was initiated by loss of water from the head appendages, and in particular from the unsclerotized, intersegmental regions. These two animals are the only normally hygro-positive species which have been investigated previously for humidity receptors, and in neither case has any receiving organ been found. In view of this, and because I have been unable to locate the site of humidity reception in talitrids, I suggest that their reactions to humidity are brought about by evaporation from the whole body surface, and particularly from the thin-walled branchiae which probably lose water more rapidly than the rest of the body.

Organs of humidity reception have been located and identified in the hygronegative *Tenebrio* (Pielou & Gunn, 1940), and *Pediculus* (Wigglesworth, 1941). In both cases they are situated on the antennae. It has also been suggested that certain of the antennal hairs of mosquitoes are hygro-sensitive (Thomson, 1938). In all these cases the organs are thought to act like hygrometers, and in the case of the mosquito it has been shown that the intensity of the humidity reaction depends on the gradient of relative humidity and not on differences in saturation deficiency. Animals which react to humidity differences appear, therefore, to fall into two distinct classes: (a) those with hygrometer-like receptors, which react to differences in relative humidity, and (b) those with no specific receptors, which react to differences in rates of evaporation. The Talitridae fall in the second category.

This work was carried out at King's College, Newcastle-upon-Tyne, under the supervision of Prof. A. D. Hobson, whom I wish to thank for advice and encouragement.

# SUMMARY

The survival times of *Talitrus saltator*, *Orchestia gammarella* and *Talorchestia deshayesii* were measured at different controlled humidities.

All are very susceptible to desiccation, but *Talitrus saltator* survives much longer than either of the other species in unsaturated air in the absence of food. Survival times are greatly increased if moist food is available.

In humidity gradients all species select moist air, orientating by means of an ortho-kinesis and a klino-kinesis.

At 15° C., *T. saltator* reacts to the smallest gradients when the maximum humidity is 92% R.H., but is unable to distinguish between different humidities above this figure. *Orchestia gammarella* and *Talorchestia deshayesii* react to the smallest gradients when the maximum humidities are 100 and 62% respectively.

Attempts to locate humidity receptors were unsuccessful. The animals are thought to react to rate of water-loss from the whole body.

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# STUDIES IN THE BIOLOGY OF TALITRIDAE (CRUSTACEA, AMPHIPODA): VISUAL ORIENTA-TION IN *TALITRUS SALTATOR*

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## ORIENTATION BETWEEN TIDEMARKS

The nocturnal migrations of *Talitrus* over the sand surface cover considerable distances, frequently taking specimens well below mid-tide level. In spite of this, by day the species occupies burrows in a broad, but fairly well-defined, zone in the region of high-water mark. The nocturnal distribution of the animals can be attributed to a somewhat random search for food, but the question arises (worded anthropomorphically), how do the animals find their way back to the high-water region? Perhaps in an attempt to answer this question, Verwey (1929) states briefly that *Talitrus* orientates on the beach by mnemotaxis to the wind, sun and direction of the sea, and by menotaxis (compass reaction). Mnemotaxis, or orientation by memory images, has been entirely eliminated from the modern classification of orientation reactions (Fraenkel & Gunn, 1940), so that Verwey's contribution is reduced to a statement without evidence that *Talitrus* orientates by means of a compass reaction to an unknown stimulus, and no other author mentions the orientation of *Talitrus* on the beach.

When, by day, specimens of *Talitrus* are taken from their burrows and released on firm sand between tidemarks, they will, either immediately or after a period of up to 3 min. apparently random wandering, take up a straight course towards high-water mark. Typical examples are shown in Fig. 1*a*, which, like other figures in this section, was drawn from freehand records of the animals' paths. Distances were gauged by pacing. Progress is made both by walking and jumping. When an animal jumps it usually turns in the air, and is seldom still pointing towards high-water mark on alighting, but it

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immediately aligns its body with its former course before continuing. Small deviations are made towards pebbles or other objects projecting from the sand. Pebbles are not detected beyond half a metre, but the animal moves towards larger objects at greater distances, including the observer at up to 3 m. (In practice, therefore, the animals were observed from beyond this range.) Unless the *Talitrus* burrows, it soon continues on a course parallel to that previously followed. It burrows on encountering a piece of sand where the surface is broken, as at a worm-cast or a foot-print, or, eventually, on reaching the less tightly packed sand at high-water mark. A specimen has been observed to follow a straight course over 80 m. in length to high-water mark.





This orientation has been confirmed in several dozen specimens of *Talitrus* on five different sandy beaches in Northumberland. Courses usually run at right angles to high-water mark, but occasionally, though straight, they are at a slight angle to the normal. All animals released at the same point follow approximately parallel courses.

A compass reaction to the sun or wind could only take place if the animal reached its inter-tidal position by following a certain course, and returned on a reciprocal bearing. The behaviour of *Talitrus* was shown not to be an example of this type of orientation by carrying animals from their place of

capture to their release point in a covered container. Such animals orientated in the normal way. Animals collected from different points along the beach, or which had been kept in captivity for several days, all followed parallel courses from the same release point, showing that the reaction is not homing behaviour. The orientation was found to be uninfluenced by the position of the sun in the sky, or by overcast conditions, or by the direction of the wind. Strong winds would at times blow animals some distance along the beach, but on regaining their feet they would take up a course parallel to that previously followed.

That the animals did not make use of the slope of the beach was shown by releasing specimens on a raised sandbank and on an undulating surface; tracks were always towards high-water mark whether leading downhill, level or uphill. There was in no case any gradient or discontinuity of salinity, moisture or humidity that the animals could have followed, and the orientation appeared too rapid and too accurate to be due to any of these factors.

It was noticed that orientating animals always held the antennae outstretched sideways with the tips on the sand. From this it was thought possible that these appendages were acting as receptors to some directional stimulus, perhaps vibrations from the sea, but the orientation was clearly shown by animals whose antennae had been amputated.

The possibility of a visual orientation was tested by using animals whose eyes were painted with a mixture of glue and indian ink. Such specimens showed no trace of a directional orientation (Fig. 1*b*). This not only showed that the orientation was visual, but confirmed the inability of the animals to make use of the slope of the beach. Previous observations that the orientation was independent of the position of the sun showed that it was not a simple light reaction; it therefore appeared that form-vision was involved. The fact that the orientation was not destroyed in unilaterally blinded specimens (Fig. 1*c*) confirms that a light-balance between the two eyes is not involved. If such had been the case, unilateral blinding would have led to circus movements. Fig. 1*c* also gives an example of tracks which are not perpendicular to high-water mark.

Animals released from such a position that their direct route to high-water mark was barred by water, showed a normal orientation until within about 2 m. of the water, when they turned and ceased to follow a straight path (Fig. 2). This suggests that, although water can be detected at short range, the tracks of the animals towards high-water mark cannot be the result of orientation away from the sea. This is confirmed by watching orientating animals, which, when walking, follow a straight course without turning the body, yet the position of the eyes makes it quite impossible for them to see the sea.

The fact that all tracks from the same release point tend to run parallel all the way to high-water mark suggests that orientation is to some distant object, beyond high-water mark. Considering the range at which *Talitrus* detects objects on the beach (such as pebbles and man) the only suitable objects, large enough to be detected, which are normally present are sand dunes or other features composing the skyline. Orientation to the highest point on the skyline does not explain all observed cases, nor has any feature yet been detected which can be regarded as directing the orientation.

It seems, at first sight, improbable that form-vision plays an important part in directing the reactions of a nocturnal animal, yet there are very few summer nights when a silhouette of the sand dunes against the sky is not visible to man from the beach, and *Talitrus* may have better night vision than man. Some evidence for infra-red vision in *Talitrus* is provided by the fact that several



Fig. 2. Tracks of three Talitrus saltator released on a sandbank almost surrounded by water.

specimens showed the normal inter-tidal orientation in a fog which limited human vision to about 200 m., and the peaks of the nearest sand dunes were about 250 m. from the release point. Even if *Talitrus* is unable to use this orientation at night, it could still be of great importance, for the general return to the high-water region probably takes place at dawn.

The inter-tidal orientations of other talitrids have not been studied in the same detail as those of *Talitrus*, but a few observations have been made on *Talorchestia deshayesii*, Orchestia gammarella and O. mediterranea.

*Talorchestia deshayesii* appears to show similar reactions to *Talitrus*, but, owing to its small size and the necessity of standing at least 3 m. away from the specimen, it is soon lost to view, and when jumping it is influenced much more by the wind than is *Talitrus*.

The reactions of the species of *Orchestia* on a rocky beach would be likely to have important bearings on their natural behaviour, but the abundance of cover among rocks makes the observance of such reactions very difficult. Bate & Westwood (1863) state that when *O. mediterranea* is disturbed it always jumps towards the sea, but I am unable to confirm this. As far as can be observed, both this species and *O. gammarella* scatter in all directions.

Observations have been made on a number of *O. gammarella* released on sand. Specimens released within about 100 m. of rocks made straight towards the rocks. Of specimens released well away from the rocks, one consistently made straight towards the sea in several tests. This reaction was destroyed by painting the eyes. Other specimens followed courses away from the sea, which, though fairly straight, did no<sup>+</sup> run parallel.

While further observations are necessary before forming any conclusions about the visual orientation of *O. mediterranea* and *O. gammarella* in nature, it seems that *Talitrus*, and probably also *Talorchestia deshayesii*, makes use of some feature of the skyline in returning to high-water mark from between tidemarks.

#### LABORATORY EXPERIMENTS

Having obtained strong evidence that *Talitrus* makes use of form-vision in nature, laboratory experiments were designed to investigate the ability of the animals to distinguish between different shapes, and, if possible, to obtain further information about their visual responses.

Before proceeding further, some explanation of the use of the term 'telotaxis' is necessary. It has been described by Fraenkel & Gunn (1940) as 'orientation to a source of stimulus, as if it were a goal', and implies the direct attainment of orientation without a balance of intensities of stimulation. The difference between this type of reaction and balance reactions (tropo-taxis and klino-taxis) is seen when two sources of stimulation are present; an animal orientating by a balance reaction will then make between the two (assuming a positive reaction), while an animal orientating by telo-taxis will make towards one and ignore the other, though if the two sources are of similar intensity it may frequently switch its orientation from one to the other, giving a zig-zag path. Such a zig-zag path is associated with telo-taxis alone.

Fraenkel & Gunn 'restrict the term "taxis" to a reaction in which the stimulus is undifferentiated and does not involve form-vision', and, as an extension of this rule, apply the term 'telo-taxis' to certain reactions to light only. Cases in which animals react to shapes by the same orientation (e.g. *Carcinus*, *Eupagurus*) are excluded from the category on the grounds that 'the reaction is...rather too complex to be called a taxis'. Although form-vision is a more complex process than the detection of light, the orientation, in examples such as *Carcinus* and *Eupagurus*, is no more complex than photo-telo-taxis; in fact, if the stimulus were unknown, the two could not be differentiated. Telo-taxis is the description of a particular type of orientation, and as such it must include all examples of this type of orientation, whatever the stimulus. Certainly more complex forms of orientation should be excluded from telo-taxis, but it is not a description of stimuli. For these reasons I have used the term 'telo-

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taxis' in the following pages to refer to *any* reaction in which 'orientation in the direction of the source of stimulation occurs without balance' as if to a goal (Fraenkel & Gunn, 1940).

# Apparatus and Method

For investigating the visual orientation of *Talitrus*, apparatus was used as shown in Fig. 3. An animal placed on a horizontal blackboard, measuring  $6 \times 4$  ft. (1.85 × 1.23 m.), could see a vertical white screen or the projections thrown upon it at one end of the board, but was screened from direct light from the projector. The figures for the projections were painted in indian ink



Fig. 3. Arrangement of apparatus for studying visual orientation in Talitrus saltator.

on blank lantern slides. Specimens were released from under an inverted jar so that the experimenter could not influence the direction in which they faced at the time of release. As the animal crawled on the board the observer marked its track with chalk. As long as the hand and chalk were kept behind the specimen it showed no reaction to them.

Orchestia and Talorchestia are much less suitable than Talitrus for use with this apparatus. Being smaller, they are less easy to see and less easy to handle, and they show effects of desiccation more quickly. They also jump a great deal more than Talitrus so that their courses cannot be plotted as accurately. Experiments were therefore confined to Talitrus. Adult specimens of both sexes were used.

In Fig. 4 the projection on the vertical screen is shown directly above the tracks of the specimen on the horizontal blackboard. All tracks in the same figure refer to the same specimen, released at different points (marked X). In general, different figures refer to different specimens.

#### ORIENTATION IN TALITRUS

# Results and Discussion

When first removed from beneath the sand in laboratory cultures, specimens of *Talitrus* usually show a photo-negative reaction, but, if kept in the light, the orientation changes to photo-positive within 10 min. Such photo-positive animals were used in the following experiments. When confronted with an illuminated, white screen (Fig. 4a) they made towards it, but followed no very direct path, nor did the tracks end near any particular point on the screen. A similar, rather inaccurate, photo-positive reaction was shown when much of the screen was darkened so as to give a black-white, vertical boundary (Fig. 4b), and when the projection was a white square (Fig. 4c). In these three cases the animals orientate towards the light, and there is no suggestion of form-vision.

The reaction to a large, illuminated rectangle (Fig. 4d) shows quite a different orientation from the inaccurate photo-positive behaviour already seen. The tracks are more direct, and most of them are towards one or other of the upright boundaries of the rectangle, sometimes changing from one to the other to give the zig-zag path which characterizes telo-taxis. The sources of stimulus for the telo-tactic reaction are not light sources, but the vertical dark-light boundaries. The reaction cannot be explained except as a case of form-vision though the shape of an upright line is as simple a form as can be imagined. Three of the seven tracks (and part of one of the other four) are not in the direction of these objects, but only in the general direction of the screen. In these cases the specimen is showing the photo-positive behaviour seen in Fig. 4a-c.

Telo-taxis to a vertical dark-light boundary is even more clearly shown to the projection of two light squares (Fig. 4e). Here none of the reactions are to light; they are all to the shape of the upright edges. The fact that most of the tracks end at the edge of the right-hand square was probably the result of this square being slightly the more brightly illuminated.

Orientation to a more complex shape is seen in Fig. 4f, g. In both cases the animals make towards the foot of an incline. With several different specimens it was found that with the single slope (Fig. 4f) the majority of the tracks led directly to the foot of the incline when the angle *a* was between 15 and 70°, but beyond these limits the tracks were not precisely directed, but were examples of the inaccurate photo-positive orientation illustrated in Fig. 4a-c. Similarly, with the projection shown in Fig. 4g, well-directed responses were obtained when  $a_1 = a_2 = 15^\circ$  or bigger, but only occasional tracks led to the foot of the slopes when  $a_1 = a_2 = 10^\circ$ .

Comparison of Fig. 4h, *i* shows that the directed orientation to a downward taper is not given to an upward taper.

Again, using the projection of two squares shown in Fig. 4e, but with one coloured red and the other green, or red and blue, similar tracks were obtained to those using white light. While not revealing any colour preference on the

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Fig. 4. Visual orientation in *Talitrus saltator*. The upper part of each figure shows projection on vertical white screen; the lower part shows tracks of specimens released at X on the horizontal board.

part of the animals, or demonstrating colour vision, it shows that all three colours are within their visual frequency range.

It has not been possible to link the laboratory behaviour of *Talitrus* to specific shapes with its orientation on the beach, but the laboratory experiments have confirmed beyond doubt the conclusion drawn from the beach experiments that *Talitrus* is capable of form-vision and shows a positive orientation to certain shapes.

I am indebted to Prof. A. D. Hobson for advice and encouragement in this work, which was carried out at King's College, Newcastle-upon-Tyne, under his supervision.

### SUMMARY

*Talitrus saltator* placed on firm sand, between tidemarks, in daylight, usually follows a direct course to the high-water region.

The orientation is visual, and probably involves form-vision of distant objects.

Form-vision in *Talitrus* was demonstrated in the laboratory by its reaction to silhouettes. It makes towards the foot of an incline and sometimes towards a vertical, dark-light boundary.

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# PHOTOGRAPHY OF THE SEA FLOOR

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

# INTRODUCTION

During the last sixty years many attempts have been made to take photographs underwater at depths varying from just below low-water to many thousands of feet. The pioneer work of Boutan (1893) showed clearly that the essential requirements were a relatively light apparatus fitted with a good source of illumination. Ewing, Vine & Worzel (1946) gave a list of workers who have obtained underwater photographs at wading or diving depths. In their own work Ewing, Vine & Worzel have designed a number of underwater cameras with which they have taken some thousands of pictures of the sea-bottom. In their free-floating cameras the apparatus is allowed to sink to the bottom with a ballast weight. On touching the bottom a trigger sets off the camera which takes two pictures with a time interval of 30 sec. After this the ballast is released and a float brings the apparatus to the surface. In their suspended cameras the component parts are mounted, as in the free-floating cameras, on a vertical pole, with the camera near the top and a trigger at the base. Activation of the camera occurs when the trigger touches the bottom. Their most satisfactory light source proved to be the photoflash bulb. With one or other of these cameras they have obtained large numbers of clear photographs in depths ranging from 10 to 2400 ft., and have used these in studies of bottom deposits, sand ripples, and the distribution of animal life. (See also Ewing, Woolard, et al., 1946.)

In the present work an attempt has been made to design and construct an underwater camera which will serve as a tool for estimating the density of the larger bottom-living invertebrates on the trawling grounds (in depths of 50-80 m.) near Plymouth. The essential requirement was a large number of workable pictures taken at known positions in a limited area. It was decided that photo-flash bulbs would not be suitable, for although they would give a small number of high-quality pictures at each lowering of the camera, they could not be used to produce a large number of consecutive pictures at a single lowering. Up to the present it has not been possible to work with an electronic flash as light source, but Ewing, Vine & Worzel (1946) found that, except in shallow water, the cable losses are so high and the cable itself so heavy that they considered it impractical to use lengths of more than 150 ft. (c. 50 m.). It would, of course, be possible to put the supply and conversion

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unit into a pressure-proof container and lower them with the camera, but this would make the whole apparatus very inconvenient to handle at sea. It was, therefore, decided to use a battery of photoflood lamps as a source of light for the present apparatus, and this has proved relatively successful.

#### ACKNOWLEDGMENTS

I am much indebted to Mr F. J. Warren who designed and installed the electrical circuits and the control box, to Mr F. G. C. Ryder who was responsible for the construction and assembly of the underwater mechanical components, to Captain C. A. Hoodless, D.S.C., and the crew of R.V. *Sabella*, whose skill and patience have enabled the apparatus to be operated efficiently at sea, and to Dr D. P. Wilson and Mr G. M. Spooner for their guidance and assistance in photographic and statistical methods respectively.

#### DESCRIPTION OF THE APPARATUS

The arrangement of the underwater components is shown diagrammatically in Text-figs. I and 2. The camera is mounted near the top of a vertical metal tube. The lamps are arranged asymmetrically round a metal ring attached to the pole at a height of 0.65 m. from the bottom of the pole. The foot switch is mounted in the base of the pole. Between the foot switch and the attachment of the lamp ring to the pole there is a gun-metal junction box for the electrical leads.

The camera used is a Robot II fitted with a Zeiss Tessar 3.75 cm. lens. The camera switch is depressed by a solenoid mounted behind the camera and activated by the foot switch.

The camera case is of gun-metal and is fitted with  $\frac{1}{2}$  in. plate-glass window. The case is closed by the compression of a round rubber ring in the joint between the base of the case and the dome. The compression is by a 'samson' with a single  $\frac{1}{2}$  in. bolt bearing down on the summit of the dome.

In its present form the apparatus has six 500 W. photoflood lamps, each mounted in a cast aluminium alloy base and enclosed in a standard glass dome. The junction between the glass and the metal is made by grinding a surface on to the edge of the glass with carborundum powder, and then seating this ground glass surface on to the metal base with a little vaseline. The glasses are held in position by a wire 'samson', but the actual sealing is effected by the pressure of the water on the glass dome.

The foot switch consists of a 21 cm. diameter brass plate connected by a socket joint to a small sealed metal bottle containing a mercury switch. When the foot touches the bottom it is driven upwards and inverts the mercury switch which activates the solenoid in the camera case.

Power from the ship's mains (200–210 V.) is supplied to the junction box near the base of the pole by a 7-core rubber-covered cable. From the junction



Text-fig. 1. Side view of underwater photographic apparatus at the moment when a picture is taken. (Lights shown on one side of the ring only.) C, camera case; L, lamps; F, foot and foot switch.



Text-fig. 2. Photograph of the underwater camera, showing camera, ring of lamps and junction box. The foot and foot switch are out of the picture at the bottom.

box four leads go to the lamps and two connect the foot switch and the camera solenoid; the seventh core is not used.

On deck the power is led through a control box before entering the main electric cable. This box is fitted with fuses for the camera and lamp circuits and for the ship's power supply, and with switches and ammeters for the camera and light circuits. It also has a counter which records the number of pictures taken and a buzzer which rings when a picture has been taken.

# OPERATION OF THE CAMERA AT SEA

The Robot camera is loaded with a metre length of 35 mm. high-speed film and is fitted into the camera case, which is then securely closed. The ship is stopped and a lead sounding gives the depth in metres. The apparatus is then lowered on a wire warp led over the aft boom to the main winch. When the camera is 3–5 m. from the bottom the speed of lowering is reduced, the lamps are switched on and the lamp ammeter in the control box gives a reading. At the same time the camera switch in the control box is switched on, and as soon as the foot touches the bottom the camera circuit is completed, the first picture is taken and the film is automatically wound on. When this happens the camera ammeter in the control box gives a reading and at the same time the counter clicks on to record the number of the photograph, and the buzzer rings to warn the crew that bottom has been reached. The winch is stopped immediately and the apparatus is then lifted from I to 2 m. off the bottom by hand hauling on the warp.

The ship is allowed to drift for a known interval of time, and the camera is again lowered steadily to take the second picture. This procedure is repeated until the whole film has been used up (40–45 exposures). The time interval between each exposure can be varied according to the speed of drift of the ship, but it is usually of the order of 30 sec., so that a film of 40 exposures can be taken in 20 min.

One of the main advantages of this method of operation is that it is possible to plot with reasonable accuracy the location of each photograph on a largescale chart of the area. To do this it is only necessary to know the positions and times of the first and last pictures, and the time interval between each exposure.

# PRELIMINARY RESULTS

It was found that the most satisfactory method of interpreting the photographs and counting the larger invertebrates was by examining the negatives under a binocular dissecting microscope. When there were large concentrations of animals on a single frame counting was facilitated by the use of some form of grid. Using this method of interpretation in conjunction with a micrometer slide it was possible to obtain size measurements of the animals counted. The following preliminary results on the density and distribution of bottom-

# PHOTOGRAPHY OF THE SEA FLOOR

living animals in four areas near Looe and Plymouth were obtained from the examination of 479 photographs, each covering  $I m.^2$  of the bottom. The figures obtained for the size of each animal will be given in a later paper.

# Area of Station L 4 (Pl. I).

This station is mid-way between Plymouth breakwater light and the Eddystone. Photographs were taken along the following three transects:

Date	Start of transect	End of transect
7. vii. 49 24. viii. 49	<sup>1</sup> / <sub>2</sub> mile W. of L4 Rame Head, 005° T., 3.7'	<sup>3</sup> / <sub>4</sub> mile W. of L4 Rame Head, 050° T., 4.8′
8. viii. 50	Eddystone, 200° T., 3.0'	Eddystone, 200° T., 4.0'

The bottom in this area was muddy sand with small patches of gravel and shell debris, often lying in pockets. Trawl catches in recent years have repeatedly suggested that the epifauna of this area is poor, and this is confirmed from an examination of photographs representing  $83 \text{ m.}^2$  of bottom. An analysis of the numbers of each species found in this area is given in Table I, which also records comparable figures for the other three areas surveyed. The only large invertebrates recorded more than once on any transect in the L 4 area are *Asterias rubens*, *Ophiura texturata* and *Chlamys opercularis*. The figures for *Asterias rubens*, although small, suggest that this species is rather unevenly distributed at a rate of not more than one individual to every 10 m.<sup>2</sup>, while *Chlamys opercularis* is perhaps a little more abundant. The only other invertebrate found in any number is *Cellaria*, the figures given for this bryozoan denoting clumps of about 20 cm. in length. In two of the three transects there are numbers of holes, *c.* 1·3 cm. diameter, probably formed by tubicolous worms or burrowing molluscs or crustaceans.

### Area south and south-west of Eddystone.

Photographs were taken along the following two transects:

Date	Start of transect	End of transect
16. viii. 49	Eddystone 360° T., 2.0'	Eddystone $360^{\circ}$ T., $3 \cdot 0'$
23. x. 50	Eddystone 019° T., 3.2'	Eddystone $030^{\circ}$ T., $4 \cdot 3'$

The bottom in this area is clean sand, and in 1947-48 trawling records showed a rich and abundant population of *Asterias rubens* feeding on *Chlamys opercularis* (Vevers, 1949). Trawl catches in this area during 1949 and 1950 have shown a marked decrease in the numbers of these species, although the individuals of *Asterias rubens* are still large in size. A total of 122 clear photographs (=122 m.<sup>2</sup>) shows the paucity of both *A. rubens* and *Marthasterias glacialis* in 1949 and 1950 (Table I). *Chlamys opercularis* appears to be still relatively abundant, at a rate of about one individual to 2 m.<sup>2</sup>. There was no sign of *Cellaria* on these grounds, although another, unidentified, bryozoan (or ? sponge) occurred occasionally. A few specimens were recorded of unidentified burrowing sea anemones.

Locality		Area of L 4 (muddy sand with small patches of gravel)			S. and S.W. of Eddy- stone (clean sand)		S.E. of Looe Island (muddy sand and gravel with pebbles)			sand and gravel with pebbles)	
Date Mean depth (m.) Number of pictures		7. vii. 49 55	24. viii. 49 52	8. viii. 50 55	16. viii. 49 69	23. x. 50 70	30. vi. 50	7. vii. 50 50	26. vii. 50 48	5. x. 49 46	2. viii. 50 54
(each 1 m. <sup>2</sup> )		23	44	16	34	88	91	21	53	72	37
Species:											
Callionymus sp.					I	_	_	_	_		_
Asterias rubens		4	3	I		I	5	3	I	3	_
Marthasterias glacial	lis				I	2	2	_	-	2	
Porania pulvillus		_	I			<u>·</u>	_		_		_
Astropecten irregular	is	_			2	I	3	I	-		_
Palmipes membranace	eus	_					Ĩ	_	<u> </u>	_	_
Echinus esculentus						_		_		2	_
Ophiura texturata		I	3		10	7			—		I
Ophiocomina nigra										2	-
Ophiothrix fragilis						26		-		18	1280*
Pecten maximus							16	-	I	12	I
Chlamys opercularis		5	5	I	35	30	14			3	17
Turritella communis		I							-	_	<u> </u>
Eupagurus prideauxi		_	I			3	I	-	_	2	
Portunus depurator		_	—	_	_	_	I				_
Crab sp.		_	I				2			_	—
Hyalinoecia tubicola		I	_	_			I			_	
Cellaria sp.		5	23	_			2			—	-
Lepralia foliacea			_	-				5			_
Bryozoan (or sponge	)	_	_	_	6	I	6		_	_	-
Sea anemone spp.		_			2	I	5			_	_
Hydroid spp.		2	_	_	_		4	—		_	
Depressions c. 4.4 cm	n. diam.	_	_			_	5	9	27	_	—
Holes c. 1.3 cm. diar	n.	_	29	71	_	4	463	134	294	526	77

# TABLE I. NUMBERS OF ANIMALS IDENTIFIED ON UNDERWATER PHOTOGRAPHS, 1949-50.

\* The 1280 specimens of O. fragilis were actually present in 12 only of the 37 m.<sup>2</sup> interpreted.

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#### Area south-east of Looe Island.

Photographs were taken along the following three transects in 1950:

Date	Start of transect	End of transect
30. vi. 50	Looe Island 328° T., 3 <sup>.7</sup>	Looe Island 328° T., 47'
7. vii. 50	Looe Island 326° T., 3 <sup>.4</sup>	Looe Island 318° T., 40'
26. vii. 50	Looe Island 337° T., 3 <sup>.4</sup>	Looe Island 316° T., 23'

The bottom was mainly of muddy sand and gravel with pebbles in some parts. The distribution of invertebrates on the bottom was variable, only the films taken on 30 June 1950 showing any large number of individuals. On this transect starfishes (Asterias rubens, Marthasterias glacialis and Astropecten irregularis) were present in small numbers. The large scallop, Pecten maximus, was relatively abundant, when compared with its frequency on other grounds, while the queen scallop, Chlamys opercularis, was far less abundant than on the clean sand area south of Eddystone. Sea anemones and hydroids also appeared to be more numerous than on the other grounds, and in one film there is a short length showing clumps of the polyzoan Lepralia foliacea. However, the most striking feature of all three transects in this area is the high number of holes made by the infauna (c. 1.3 cm. diameter) similar to those found in the L 4 area. In addition, there were patches of ground showing smaller numbers of irregular wide-mouthed depressions about 4.4 cm. diameter. These depressions were similar to those produced at the anterior end of some polychaete burrows. Further work, including larger scale pictures, dredge or grab hauls in the area, and experiments in the laboratory will be required before the occupants of these and the smaller holes can be identified.

# Area south-west of Looe Island (Pl. I).

Photographs were taken along two transects:

Date	Start of transect	End of transect
5. x. 49 2. viii. 50	Looe Island 025° T., 4.6' Looe Island 013° T., 4.9'	Looe Island 035° T., 4.8'

The bottom deposits were, in general, similar to those in the area south-east of Looe Island, consisting of muddy sand and gravel with pebbles. The two photographic transects available for this area show quite different features. The first transect (5 October 1949), consisting of  $72 \text{ m.}^2$ , is similar to the transect taken south-east of Looe Island on 30 June 1950. It has a number of the larger starfishes, as well as *Pecten maximus* and the unidentified holes (c. 1.3 cm. diameter); it only differs in the scarcity of queen scallops and in the presence of small numbers of *Ophiothrix fragilis*.

The second transect (2 August 1950) shows no starfishes, crustaceans or polyzoan colonies, but a very large population of *O. fragilis*, with a few queen scallops and occasional patches of ground with the unidentified holes (*c.* 1.3 cm. diameter). Twelve interpreted photographs in this transect yielded a count of 1280 *O. fragilis*. But the brittle star also occurred in large numbers

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in frames other than those counted, that is, they were present in the unclouded portions of the partly clouded frames. Examination of 33 successive photographs for the presence or absence of the brittle star gave the following result, the interval between each frame in this transect being approximately 10 m.:

# TABLE II. OCCURRENCE OF OPHIOTHRIX FRAGILIS IN SUCCESSIVE PHOTOGRAPHS, 2 AUGUST 1950

Serial numbers of photographs	Occurrence of O. fragilis (+=present, -=absent, ?=doubtful)
1891-93	· +
1894-97	알려려는 것같은 것은 것으로 <u>수</u> 집을 가지 않는 것이 되었다.
1898-99	+
2000	?+
2001-15	+
2016-17	5
2018-20	+
2021-22	5
2023-24	+

Taking that part of the transect covered by pictures 1898-2015 (inclusive), a sample of 18, there were 17 squares with *O. fragilis*, and one square doubtfully occupied by the same species. If, on a simple presence and absence basis, each one of a sample of 17 squares is found occupied, then it may be shown that there is a 95% chance that at least 83% of the squares of the sampled area (i.e. 170 m.<sup>2</sup>) are occupied, or a 99% chance that at least 79% of the squares of the sampled area are occupied (since  $0.84^{17}$  gives a P of 0.95,  $0.83^{17}$  gives a P of 0.04,  $0.80^{17}$  gives a P of 0.023, and  $0.79^{17}$  gives a P of 0.018.)

However, since the number of individuals in the counted squares was large, varying from 38 to 206, the chances are even greater than this that all the squares in the sampled area were occupied, or, at any rate, that very few of the squares were unoccupied.

The total number of *O*. *fragilis* in the 12 fully interpreted and counted squares was 1280, giving a mean of  $107 \pm 14.7$  individuals to the square metre. So that a transect of, say, 150 m. would have a total of 11,550–20,550 individuals.

In all the counts of *O. fragilis* on this transect the numbers recorded are minimum figures, for it is possible that in some patches individuals may have been obscured by those lying above them.

#### DISCUSSION

Collections of animals from the sea-bed by means of dredge, otter trawl and Agassiz trawl give satisfactory qualitative samples of the epifauna. However, quantitative estimations by these methods involve errors owing to the impossibility of knowing the exact length of time during which the fishing gear has been on the bottom, and, in the case of the otter trawl, the lack of knowledge on the shape and size of the trawl mouth when in operation. Sampling of the bottom by means of grabs, such as that of Petersen (1918), or the scoop bottom

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sampler of Holme (1949) probably gives reasonably accurate results when used for the estimation of the infauna, but it is doubtful whether they give comparable results when used for the estimation of the larger epifaunal invertebrates, which are often sparsely distributed on the floor of the sea.

The use of an underwater camera to give photographs of a number of quadrats along a transect is similar in principle to the sampling methods used in extensive surveys of land vegetation (Tansley, 1923). The apparatus described in this paper is designed to obtain a succession of sample quadrats of known area at known intervals along a transect. The preliminary results from photographs of four different areas suggest that the method can be used as a competent and reasonably accurate tool, not only for the estimation of epifaunal invertebrates, which is its primary purpose, but also for counts of those infauna species which produce a tube or burrow with an opening on the surface of the sea-bed. Many photographs have been taken which show high densities of such burrowing animals. Counts of these burrows would help to confirm estimations made from the catches of bottom-sampling gear. Before this can be done, however, more information must be obtained on the shape, size and general appearance of these surface holes, so that the species producing them can be easily distinguished.

The number of photographs required to give an accurate estimate of the density of animals on a ground will clearly depend upon the nature of the bottom deposit and the type and habits of the species living there. On a poor ground, such as the L4 area, or on a ground with heterogeneous bottom deposits, such as the muddy sand and gravel area south-east of Looe Island, a relatively large number of pictures, perhaps  $300-500 \text{ m.}^2$  for 2-4 square miles in area, would be required in order to obtain a reasonably good estimate of animal density. In the latter ground there is considerable variation in the numbers and types of animals found along each of the three transects photographed.

The muddy sand and gravel area south-west of Looe Island also showed evidence of patchiness, the first transect being relatively poor in numbers of animals, while the second transect had an area which was very rich in one species only. In this latter transect, however, a small number of pictures showing homogeneous features was sufficient to enable definite conclusions to be reached on the extent of this local population of animals.

The presence of aggregations of brittle stars on localized areas of the sea-bed has long been suspected from the very large catches of these animals brought up by the dredge and Agassiz trawl. Nevertheless, the size of the aggregations, as estimated from the present photographs, is considerably greater than would be suspected from random dredge hauls. The existence of such large mono-specific concentrations of animals raises a number of problems. Blegvad (1914) found that *O. fragilis* was essentially carnivorous. It is possible, therefore, that the brittle stars photographed were feeding on an extensive bed of animals, perhaps lamellibranch or other spat. Their massing behaviour might also be

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connected with spawning, and further photographs at different times of the year might provide further evidence on this point. The species is, in fact, known to be gregarious under laboratory conditions, and it is not uncommon for young specimens to be found clinging to adults rather in the fashion of the young of brood-protecting species. Their gregarious behaviour may not, therefore, be entirely related to food and spawning, and the individuals may react in a positive way to tactile, chemical or other stimuli produced by their fellows.

Although the quality of many of the photographs in the present series is by no means satisfactory, a sufficient number of them were clear enough for interpretation, and it is considered that larger scale pictures of an area smaller than  $I m.^2$  would also yield useful results.

There are still a number of technical difficulties, in particular the provision of a continuous source of illumination adequate for a long series of pictures or even for a ciné film. These difficulties must, of course, be overcome without rendering the apparatus unwieldy.

There is little doubt that photography of the sea-bed can yield much information which will be of use in estimating the numbers of bottom-living animals, not only in inshore waters, but also in the offshore trawling grounds. There is also the possibility that use could be made of television to view the sea-bottom, and film records of the television screen would render permanent the results obtained by this method.

## SUMMARY

A description is given of an underwater photographic apparatus designed to take a transect of 40-50 exposures, each 1 m.<sup>2</sup>, at depths up to at least 80 m. The camera used was a Robot II, and light was provided by six 500 W. photoflood lamps. Power was led from the ship's mains to the camera release and the lamps by a rubber-covered 15 amp. cable.

Photographs have been obtained of the sea-bottom on four different grounds near Plymouth and Looe, and from their interpretation a preliminary estimate has been made of the relative abundance of some of the larger bottom-living invertebrates in these areas. On some transects the sample photographs show a high density of unidentified holes made by members of the infauna.

On one transect a sample of 33 pictures showed a dense aggregation of the brittle star, *Ophiothrix fragilis*. This animal was present at the rate of  $107 \pm 14.7$  individuals to the square metre, and calculations have shown that the population was relatively homogeneous at this density over a distance of more than 100 m.

As the number of pictures taken at each lowering of the camera is relatively large it is considered that the photographic method described can be used to give a reasonably accurate estimate of the epifaunal invertebrates in known positions and at depth up to at least 70–80 m.

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VEVERS. PLATE I





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#### EXPLANATION OF PLATE I

Above: Photograph of I m.<sup>2</sup> of bottom near L4, showing one *Chlamys opercularis*, one *Ophiura* texturata, and also a patch of shelly gravel and (top right-hand corner) a large hole. Depth 55 m. *Below*: Photograph of I m.<sup>2</sup> of bottom, 5 miles south-west of Looe Island, showing dense mass of *Ophiothrix fragilis* lying on a bottom of muddy gravel. Depth 54 m.

# NOTES ON THE REPRODUCTION AND EARLY DEVELOPMENT OF THE CIRRATULID THARYX MARIONI (ST JOSEPH)

# By R. Phillips Dales, B.Sc., Ph.D.

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# (Text-figs. 1, 2)

The larval development of the Cirratulidae is not well known, although an account of the development of *Raphidrilus nemasoma* Mont. was published by Sokolow in 1911 (under the name of *Ctenodrilus branchiata*), and of *Audouinia tentaculata* (Montagu) by Wilson in 1936. Apart from these two publications, only scattered observations may be found in the literature, and these are listed by Wilson (1936). Unfortunately, attempts to rear the larvae of *Tharyx marioni* (St Joseph) beyond the hatching stage have failed, and as it seems unlikely that further data may be gathered for some time, such results as have been obtained are presented here.

The main points of interest in the development and reproductive habits of T. marioni are: (i) the adults are atoquous; (ii) the eggs are comparatively large and yolky, and unlike Audouinia tentaculata, the pelagic stage is omitted; and (iii) the larva on hatching is achaetous, as in other cirratulid larvae.

The worms and larval stages were collected from a small population at Chalkwell, Essex, on the north side of the Thames estuary, in fairly clean sand just above half-tide mark. The adults were buried vertically in the top few centimetres, entirely within the oxidized layer, and with the cirri exposed on the surface.

The morphology of the adults agrees well with the description given by Fauvel (1927) except that: (i) the sexually mature adults were never more than 35 mm. in length (Fauvel states the length to vary between 35 and 100 mm.); (ii) the adults were devoid of all pigment other than the red blood pigment (Fauvel describes them as 'reddish brown'); (iii) the ripe oocytes were colourless and appeared chalky white in the mass (Fauvel states that they are 'greenish').

I am indebted to Dr D. P. Wilson for kindly checking my identification.

## REPRODUCTION AND SPAWNING

It is impossible to distinguish the sexes with the naked eye. They do not differ in size, and when ripe both appear white due to the presence either of spermatozoa, or oocytes. The females, however, may be detected with a hand lens, as

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the oocytes may then be distinguished in the coelom. Males and females occurred in equal numbers. From a sample of thirty-seven worms, eighteen were females, nineteen were males.

The gametes are restricted to the mid-body region, being absent from the anterior region, and from the last few segments, in both sexes. Ripe females have about fifty genital segments, with a total number of between 1000 and 1500 oocytes.

Unfortunately spawning was not observed, but the oocytes are probably released singly into the sand. The spawning period was sharply defined, nearly all the worms spawning between 16 and 18 April 1949, about 3–5 days after full moon, when the highest tides occur at Chalkwell. There was a sharp rise in temperature at this time, which may have stimulated spawning (Fig. 1). Unspawned females quickly resorbed their oocytes after this, and a week or two later they had lost the white appearance characteristic of the sexually mature worms.



Fig. 1. Temperature of the natural habitat during spawning and early development. (Recorded by a max.-min. thermometer lying at a depth of 5 cm.)

There was little difference in size between the oocytes, either within a single female, or throughout the population. When ripe the oocytes are round, or slightly oval, and are surrounded by a fairly thick transparent layer which is adhesive at first, but which seems to lose this property when ready for spawning. They contain a large germinal vesicle, and a fine, opaque, granular cytoplasm (Fig. 2 A).

The sperm plates are rather irregular in size and shape, and vary between 20 and 50  $\mu$  across. The sperm head is acorn-shaped, and bears a tail about 40  $\mu$  in length (Fig. 2B).

#### EARLY DEVELOPMENT OF THE LARVAE

Development from artificial fertilizations, which were achieved by slitting open the adults and mixing the ripe gametes in a dish, proceeded only as far

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as late cleavage, and unfortunately no active larvae were obtained. Naturally spawned and fertilized eggs and cleavage stages were, however, collected from the sand.

A fertilization membrane was thrown off soon after artificial fertilization, and remained so close to the egg, that in many instances its presence was



Fig. 2. Ripe coelonic gametes, and developmental stages obtained from the sand. A, unfertilized oocyte (t, transparent layer); B, sperm plates, and a motile spermatozoan; C, young unhatched larva; D, hatching stage. The scale on the left applies to B only; that on the right to A, C and D.

difficult to detect until the first cleavage. Polar bodies were conspicuous, and were thrown off about an hour after fertilization. The first cleavage, which takes place at right angles to the longitudinal axis of the egg, occurred after

8-2

about 2 hr., the 4-cell stage being reached after about  $3\frac{1}{2}$  hr., whilst 6 hr. after fertilization 8-cell stages may be seen.

In the natural habitat a jelly layer is very conspicuous, and stands away from the developing larva, and although in artificially produced larvae this layer was obviously present, it did not stand away from the surface of the egg as in those stages obtained from the sand. In nature, this layer forms a capsule in which the larva is free to rotate, and is practically opaque, owing to the rich growth of diatoms and other algae which it supports, so that in practice it is necessary to dissect off this capsule in order to see the larva. It may be added that the capsules can only be distinguished with difficulty from the surrounding sand grains which are much the same in size.

About 2 or 3 days after fertilization, cilia begin to grow out through the fertilization membrane, which then becomes the larval cuticle, and the larva begins to rotate within the egg capsule (Fig. 2 C). The ciliation gradually extends down the ventral surface in a posterior direction, and well-marked bands of fine short cilia extend over the dorsal surface, the larva becoming more vigorous in its movements. Later, this ciliary movement is supplemented by muscular movement, the larva becoming highly contractile, but chaetae and other appendages are absent. Ten days after fertilization, the contractile movements are fairly vigorous and stretch the walls of the capsule, which eventually breaks open to release the larva. Late cleavage stages and gastrulae were collected from the sand on 19 April 1949, and on the 21st most of the larvae were rotating in their capsules, but no hatched larvae were found until the 28th, about 10 days after spawning.

On hatching, the larva is about  $250 \mu$  long, and has two to three segments delimited, but owing to the absence of chaetae, and to the extreme contractility of the body wall, it is difficult to determine the exact number of segments present. The larva is completely opaque, white, sausage-shaped, and with a slightly pointed anterior end. The ventral surface is uniformly ciliated, while bands of cilia extend round the dorsal surface in each segment (Fig. 2D), but the cilia are very short, and are insufficient to lift the larva off the bottom. Indeed, in spite of their well-marked muscular movements, the larvae are rather sluggish and are difficult to detect. Owing to the opacity of the body wall, no details of the gut or other internal structures could be seen.

#### DISCUSSION

The larva of this species is interesting in that it is typically bottom-living, non-pelagic, and lecithotrophic, as are the larvae of many other intertidal polychaetes, and has no pelagic stage, unlike any of the other cirratulid larvae known. Correlated with this habit are the relatively large oocytes and capsule in which the larva develops until two or three segments have been formed. The hatching larvae are similar to the early bottom stages of *Audouinia* 

*tentaculata*, described by Wilson, in the absence of chaetae, and in the extreme contractility of the body. Also, in the absence of chaetae, they resemble the corresponding stages of *Dodecaceria concharum*, figured by Mésnil and Caullery (1898). The larvae of *Audouinia tentaculata*, however, develop from much smaller eggs than those of *Tharyx marioni*, and the larvae of the former hatch in a relatively short time as ciliated planktonic trochophores. It is the metamorphosed bottom stages which may be compared with the hatching larvae of *T. marioni*, which develop from eggs one and a half times the size of those of *Audouinia tentaculata*. The development of *Tharyx marioni*, therefore, affords an interesting new example of the formation of a yolky egg and loss of an active pelagic stage.

#### SUMMARY

Males and females of *T. marioni* occur at Chalkwell in equal numbers. The species is atoquous, the large oocytes being spawned into the sand where they are fertilized. Spawning took place over a sharply defined period (16-18 April 1949) following a sharp rise in temperature, and at a time when the highest monthly tides occurred. The larva hatches about 10 days after initiation of development. It is lecithotrophic, and at first achaetous and poorly ciliated, but very contractile. These two latter characters appear to be common to all cirratulid larvae known. There is no pelagic stage.

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# OBSERVATIONS ON THE STRUCTURE AND LIFE HISTORY OF AUTOLYTUS PROLIFER (O. F. MÜLLER)

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# (Text-figs. 1-3)

## INTRODUCTION

All the specimens of *Autolytus prolifer* (O. F. Müller) described here were gathered from colonies of the hydroid *Obelia flabellata* Hincks, growing on pebbles in a shallow creek in the intertidal zone of the Chalkwell mud flats, on the north side of the Thames estuary. The syllid was found to be very plentiful on this hydroid, living in semi-transparent tubes attached to the stem, but was not found elsewhere.

The specimens agree well with previous descriptions of *Autolytus prolifer* in the number and arrangement of the pharyngeal teeth, and in the chaetae; but some of the other anatomical features show similarities with other species of *Autolytus* Grube.

Records of the occurrence of this species are scanty, due, no doubt, to lack of observation. Nevertheless, published descriptions have frequently been based on a few or even on single specimens, while here it may be pointed out that a relatively large number of worms of all ages were collected and examined.

In this paper the terms 'male form' and 'female form', or simply 'male' and 'female', have been used in place of the older terms, '*Polybostrichus*' and '*Sacconereis*' respectively.

The formation of stolons and the resulting female and male stages have been described by several previous authors (Slabber, 1778; O. F. Müller, 1788; Bruguière, 1791, table 56, figs. 8–15; Krohn, 1852; Max Müller, 1855; Keferstein, 1862; Greeff, 1866; St Joseph, 1887; McIntosh, 1908; Fauvel, 1923; de Vos, 1936; Thorson, 1946; Wesenberg-Lund, 1947), while a more general account is given by Potts (1911). Apart from de Vos, these authors appear to agree that, in the male form, only the first three segments behind the head are without natatory chaetae, whereas in the few males collected at Chalkwell six such undifferentiated segments separated the head from the metamorphosed segments. De Vos found the number of segments behind the head without natatory chaetae to be variable.

It is likely that the habit of living in tubes on hydroids is widespread among autolytoids. Wesenberg-Lund (1947) has found *Autolytus prolifer* on *Lafoëina maxima* Levinsen. McIntosh (1908) records *A. prolifer* from shells and other debris brought up by the deep-sea lines of fishermen at St Andrews (Scotland), and in tough translucent tubes under stones near lowwater mark. These worms have also been found on seaweeds (McIntosh, 1908; Thorson, 1946).

The larval development of this species has not hitherto been described, though sketches of larvae attributed to *A. prolifer* are scattered through the literature. Some of these resemble the corresponding stages described here, but others are very different. Most of the work on autolytoid development has been done on other species, e.g. Agassiz (1863) on *A. cornutus*, and Malaquin (1893) on *A. edwardsi*.

The syllids were easily collected by gathering the *Obelia* colonies. On placing these in glass dishes, most of the worms deserted their tubes and aggregated on the side of the dish nearest the light, and were then easily removed with a small brush. Some of the youngest stages obtained, however, were collected later, for although these were not found to be living in tubes, they nevertheless adhered strongly to the surface of the perisarc.

Drawings have been made with the aid of a camera lucida, and with the exception of Fig. 1 from narcotized specimens. Details were checked from material fixed in 2% formalin in sea water, after narcotizing with 8% magnesium sulphate, and mounting in benzyl alcohol or euparal, either unstained, or stained with borax carmine.

Collections were made on 1, 6 and 21 June 1949.

### IDENTITY AND STRUCTURE

The ordinary non-reproductive stages ('*animal surculare*') resemble A. prolifer (O. F. Müller) in the following features: (i) two pairs of eyes with lenses, and one pair of lateral eyespots; (ii) most (but not all) individuals with an S-shaped pharyngeal curvature; (iii) pharynx always provided with ten, equal, teeth; (iv) oblong proventriculus; (v) coloration; (vi) chaetae.

In some other characters, however, these specimens differed from the published descriptions and figures of *A. prolifer* (O.F.M.), and in some features resemble *A. auriantiacus* Clap. These differences are: (i) the median head tentacle is invariably longer than the two lateral tentacles, and may be twice as long; (ii) dorsal cirri much shorter; (iii) male form with three regions behind the head—first with six anterior segments without natatory chaetae, second with about eighteen segments; (iv) smaller size (non-reproductive form 3-7 mm., cf. Fauvel (1923), 5-15 mm.); (v) some specimens show a looped pharyngeal curvature.

Most previous authors agree that the male form has only three undifferentiated anterior segments lacking natatory chaetae (Keferstein, 1862; Malmgren 1867; Langerhans, 1879, 1880; St Joseph, 1887; McIntosh, 1908; Fauvel, 1923; Wesenberg-Lund, 1947), and that the segments bearing natatory chaetae

## OBSERVATIONS ON AUTOLYTUS

extend to the pygidium, although St Joseph notes that the terminal chaetigerous segment has shorter natatory chaetae than the others. De Vos, however, found between three and six such anterior segments, followed by twenty to twenty-seven segments bearing natatory chaetae, and that the female also varied in a similar way. On the other hand, the sexual phases described by Wesenberg-Lund all have three such anterior segments, and although the non-reproductive stages are similar to the Chalkwell specimens, the male is different in some respects. Wesenberg-Lund does not mention eye-spots (as distinct from lensed eyes), and unfortunately makes no reference to the pharyngeal armature.

The stock differs little from the drawing by Thorson (1946), except in the longer prostomial tentacle, and the much shorter parapodial cirri, but does not bear a close resemblance to drawings made by earlier workers on autolytoids ascribed to this species (Keferstein, 1862; McIntosh, 1908). Stoloniferous forms were found with a single male stolon, and forms with up to four female stolons. A single free male form was found on the hydroid, but no ripe female stages were found unattached. A specimen with a single male stolon is shown in Fig. 1A, and a dorsal view of one of the parapodia from the differentiated region in Fig. 1B. The dorsal cirrus will be seen to be much shorter than those figured by all previous authors. As well as showing the more obvious differences already described, Keferstein's male does not show a pair of anal cirri, which are clearly visible in the Chalkwell worms.

If these Chalkwell specimens are regarded as belonging to a new species, then they can only definitely be separated from A. prolifer (O.F.M.) by the sexual phase, unless the much shorter length of the dorsal cirri is regarded as a constant feature of taxonomic importance. This cannot be decided until the real affinities of the males with only three anterior segments without natatory chaetae ascribed to A. prolifer has been proved, but from the observations of de Vos (1936) it would appear that the number of segments behind the head without natatory chaetae is variable, and cannot therefore be regarded of taxonomic importance. This applies equally well to other species.

If, on the other hand, these worms are regarded as belonging to *A. prolifer*, then the pharyngeal armature alone supplies reliable separation from closely allied species such as *A. auriantiacus* Clap. It may be added that the teeth are difficult to see in *A. prolifer*, even in fixed and cleared material, and Johnston (1845, p. 146, pl. IX, figs. 3, 4; 1865, p. 192, pl. XV*a*, figs. 3, 4) thought that there were no teeth.

Other characters are variable, especially the length of the median head tentacle (Figs. I, 3D-F). Claparède (1868), McIntosh (1908), and Fauvel (1923) all show the median tentacle to be the same length as the lateral tentacles, as in *A. brachycephalus* Mar. (Marenzeller, 1874), but Viguier (1886, p. 429, pl. XXVI, figs. 13-15; pl. XXVII, fig. I) shows the median tentacle as being slightly longer, but not as long as in the specimens described here.



Fig. I. Autolytus prolifer. A, stock with a single male stolon (dorsal view); B, dorsal view of a right parapodium from the differentiated region of the male form; C, pharyngeal armature; D, chaetae. a, distal half of a natatory chaeta from a male; b-e, chaetae from non-reproduction phase.

# OBSERVATIONS ON AUTOLYTUS

The looping and coiling of the pharynx has also been regarded as a character of taxonomic importance, but in several dozen adults examined at random for this feature, it was found to be variable. Fauvel (1923) states that *A. prolifer* has an S-shaped pharynx, while that of *A. auriantiacus* is completely coiled. In the Chalkwell worms, the length of the pharynx varied, and consequently although an S-shaped loop was usually formed when the proboscis was retracted, several specimens showed a complete coil as in *A. auriantiacus* (Fauvel, 1923, p. 313, fig. 120*a*; Marenzeller, 1874, Taf. VI, fig. 1), and it may be noted that *A. auriantiacus* itself is variable, since Claparède (1868) gives a drawing with a pharynx showing a double coil (pl. 15, fig. 1). In Chalkwell worms, also, the proventriculus was situated more anteriorly than segments 7-9, but this may be correlated with the somewhat smaller size of the adults (about 5 mm., see Fig. 3F).

Representative chaetae are shown in Fig. 1D. They resemble chaetae previously figured for *A. prolifer*, but many of these drawings might equally well be attributed to other species, so that no really significant conclusions may be drawn from this character. Fauvel states (1923, p. 312), that the capillary chaetae (Fig. 1D, e, in this paper), occur singly in all segments posterior to the third chaetigerous segment, but in the Chalkwell worms they were found to occur singly in every parapodium in larvae and young worms, but were often missing from older individuals. The type shown in Fig. 1D, b was never found in young stages, but was fairly common in older worms, and in the ventral bundles of the parapodia in the males.

McIntosh (1908) gives the rather lengthy synonomy of *A. prolifer*, but in the light of the present work it is doubtful how many of the descriptions do, in fact, refer to this species.

#### REPRODUCTION AND DEVELOPMENT

The pelagic female of *A. prolifer* has already been described and figured by previous authors (Slabber, 1778; Krohn, 1852, 1855; Max Müller, 1855; Malaquin, 1890, 1893; McIntosh, 1908; Fauvel, 1923; Thorson, 1946).

There seems to be no fixed breeding season (Jensen, Johansen & Levinsen, 1904, pp. 286, 296; McIntosh, 1908, 1927; Allen, 1915; Thorson, 1946), although a spawning maximum probably occurs in late spring and early summer, as many of the records of the occurrence of specimens in the plankton refer to the period between April and July. At Chalkwell, stoloniferous forms, pelagic forms, and larvae were all found during June.

A few descriptions and figures of the larvae ascribed to *A. prolifer* are also scattered through the literature. Max Müller (1855) figures larvae from the egg-sac, and gives the size of the ripe egg as  $50 \mu$ , and a young achaetous larva with two segments as about  $200 \mu$  in length. McIntosh (1908) gives a figure of a larva which could refer to almost any syllid, and his later drawings (1927) do

not in the least resemble the larvae described here. Okada (1929), however, figures a larva rather more than  $100 \mu$  long, only slightly less advanced than the youngest stage found at Chalkwell, and although the smallest stage found here was about  $300 \mu$  in length, it showed only one more segment delimited. Okada's general remarks on the structure of syllid larvae apply to these larvae of A. prolifer.

It may be concluded that the eggs are small (100  $\mu$  or less in diameter) and develop into young achaetous larvae while still in the egg-sac suspended from the ventral surface of the female. Their pelagic life is probably not very long,

and although they appear to undergo no great morphological differentiation during this period, they increase greatly in size and may treble their length. Martinus Slabber (1778) figures larvae emerging from the egg-sac of a female, and these larvae are already composed of head, pygidium, and two achaetous segments, and correspond with the youngest stage described here. On settling, they are still achaetous, and are about  $300 \mu$  in length (Fig. 3A). The body consists of a head region formed from a prostomium bearing an akrotroch anterior to two red eye spots, and a prominent prototroch posteriorly, which apparently corresponds to the buccal segment containing the pharynx. Okada (1929) could not distinguish these two parts of the head (prostomium and buccal segment), but they were clearly visible in the Chalkwell larvae. The pharynx lies entirely within the buccal segment, and does not project backwards into the peristomial segment which remains achaetous throughout life, and at this stage bears a prominent ciliary band (Okada's 'interparatroch'). The peristomial segment is followed by the future first chaetigerous segment, in which the chaetae Fig. 2. Autolytus prolifer, young achaetous larva (ventral view) may be seen developing internally (Fig. 2 C), and which also bears a ciliary band. A further, relatively undifferentiated segment may be seen between this and the pygidium. A telotroch is not present,



a, akrotroch; in, interparatroch; p, prototroch; c, first chaetigerous segment (chaetae not yet erupted).

and does not develop until later, but the whole of the ventral surface is clothed with short cilia. Before the chaetae have been erupted from the first chaetigerous segment, two pairs of eyespots have been formed (Fig. 3), and a third pair, the most lateral in position, which remain without lenses, are added when three or four chaetigerous segments have been formed.

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Fig. 3. Autolytus prolifer. A, a slightly later stage than in Fig. 2, seen in side view, showing two pairs of eyespots, and the ventral ciliation; B, larva with two chaetigerous segments (dorsal view); C, larva with five chaetigerous segments (dorsal view); D, young Autolytus with seven chaetigerous segments (dorsal view); E, a slightly older stage with nine chaetigerous segments (dorsal view); F, young Autolytus with thirteen chaetigerous segments, showing the proventriculus (dorsal view).

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After the eruption of the chaetae from the first chaetigerous segment, further segments are added posteriorly, each segment bearing a ciliary band. A telotroch becomes clearly visible by the time two chaetigerous segments have been formed, but the ciliary band on the peristomial segment remains the most prominent tract throughout development. The ciliation is summarized in Table 1.

In larvae with two chaetigerous segments (Fig. 3B), the prostomial tentacles may be recognized. As will be seen from Fig. 3B–F, the prostomial tentacles gradually lengthen, while the dorsal cirri and the supplementary tentacles from the peristomium and the first chaetigerous segment gradually lengthen out rather later in development.

The gut remains in much the same condition for some time, but the pharynx, which at first is confined to the buccal segment, extends backwards. In larvae with two chaetigerous segments it extends into the achaetous peristomial segment, and, in larvae with five chaetigerous segments, into the first chaetigerous segment. The proventriculus is not formed until ten or eleven segments have been delimited, by which time the young *Autolytus* is over 1 mm. in length.

## TABLE I

## CILIATION OF LARVAL AUTOLYTUS PROLIFER

Head {Prostomium Buccal segment Peristomium First chaetigerous segment Second chaetigerous segment Pygidium

Akrotroch Prototroch Interparatroch Ciliary band Ciliary band Telotroch

Larval chaetae do not appear to differ appreciably from those of the adult. At first, all the chaetigerous segments may contain a chaeta of the type shown in Fig. ID, e, but these are not always present, and are often, apparently, lost. Most of the chaetae resemble those shown in Fig. ID, c, d, but those similar to Fig. ID, b were never seen in young larvae, and appear to arise later in development.

## SUMMARY

The mode of life and larval development of *Autolytus prolifer* (O. F. Müller) is described. The development does not appear to differ markedly from that described for other *Autolytus* species, although the larvae which have been described here are noticeably different from those of other species. In the present state of knowledge a detailed comparison is impossible. Most of the work done on these worms was performed about 50 to 100 years ago, and it is therefore interesting to note the general confirmation of the views of Okada on the constitution of larval syllids.

It seems likely that most of the life cycle is normally passed on a hydroid —in the locality investigated on a species of *Obelia*. There is probably no specific relationship, since these worms have been found on seaweeds and other objects.

Mucous glands are well developed, and the young larvae adhere strongly to the perisarc of the hydroid, even when unprotected by a tube. These tubes, which ramble amongst the branches of the host colony, are semi-transparent, straw coloured, and unbranched, and may be inhabited by several animals. During the winter when the hydroids die back, the worms presumably hide under stones or migrate to deeper water.

In the adults, anatomical features such as the form of the pharynx, position of the proventriculus, the relative lengths of the prostomial tentacles, and the number of segments in the male without natatory chaetae, all usually considered of taxonomic importance, have been found to be variable. Apart from the coloration, which is lost on preservation in alcohol, the number of the pharyngeal teeth alone distinguishes the species from *A. auriantiacus* Clap.

The male form collected from Chalkwell has six segments without natatory chaetae, behind the head. It is likely that the number varies between three and six.

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# ON GAMMARUS ZADDACHI OCEANICUS SEGERSTRÅLE

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# (Text-figs. 1-3)

In a survey of the British species of *Gammarus* inhabiting estuarine waters (Spooner, 1947), it was found necessary to divide *G. zaddachi* Sexton into two well-marked morphological forms, with different salinity optima, which in fact behave as distinct species. They were, however, on account of their similarity in important structural features, retained as a single species and named *G. z. zaddachi* and *G. z. salinus* respectively. At the time little could be said about a third subspecies, essentially marine in its distribution (but ranging into brackish water), which Segerstråle (1947) independently described as *G. z. oceanicus*.

It is now clear that G. z. oceanicus is, geographically, the most widespread and abundant form of the three, occurring generally on shores in shallow coastal waters all over the boreal and subarctic region of the Atlantic area, southward to Britain, Denmark and the Baltic, and eastward along the Siberian coast at least to  $140^{\circ}$  E. On the American coast it extends southward at least to New York.

G. z. zaddachi and G. z. salinus are both temperate forms, and, as far as is known, confined to the brackish waters of Europe. In the Baltic Sea all three subspecies overlap very widely.

In this further contribution on G. z. oceanicus various records of occurrence are given which help to fill in the picture already outlined by Segerstråle (1947, 1948), but which in particular establish the existence of this form as dominant along much of the coast of Scotland.

The ecological relations existing between G. z. *oceanicus* and G. *locusta* on the one hand, and between it and G. z. *salinus* on the other, can now be discussed, but important gaps in knowledge still exist.

A fortunate chance of further exploring the *Gammarus* fauna of the more inaccessible parts of the north-western British coasts was provided by Dr Dorothy C. Gibb, who, in 1948 and 1949, collected a long series of samples of the fauna associated with the brown alga *Ascophyllum nodosum* var. *mackaii*. The great bulk of this fauna was found to consist of littoral gammarids. Among these, especially in the more northern stations, *Gammarus zaddachi oceanicus* occurred with some regularity, evidently flourishing in the littoral zone where this weed, amongst others, provides cover. I am much indebted

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to Dr Gibb for the opportunity of identifying the gammarids she had collected. The value of her samples is enhanced by the detailed information of the habitats she acquired during her investigations and will publish in due course.

To various zoologists whose help I have gratefully acknowledged in presenting or lending material of *G. zaddachi*, I must now add the following: Mr J. S. Colman, Mr G. R. Forster, Mr N. A. Holme, and Dr Sven Segerstråle. It has been very instructive to compare samples of different forms from the Baltic, sent to me by Dr Segerstråle, with our own.

My special thanks are also due to Dr Isabella Gordon for the facilities afforded to me for examining the material preserved in the British Museum.

# THE CHARACTERS OF GAMMARUS ZADDACHI OCEANICUS

A description is given by Segerstråle (1947, pp. 226–9, fig. 3, a-g). The main *zaddachi* characters are seen in the form of the gnathopods (in each sex), the well-excavated lateral sinus of the head, the moderate depth of the coxal plates, the shallow rounded ventral expansion of coxal plate 4, the moderately produced epimeral plates, and in the somewhat shortened uropod 3 inner ramus. The arrangement of the antennal hair tufts is also really typical of the species, although the relative shorteness and sparseness of the hairs renders this feature much less obvious.

The hinder part of the body is relatively glabrous, as in G. z. salinus. Younger specimens of the two subspecies resemble each other closely, but can be distinguished at least by reference to the characters of antenna I. Adults are readily told apart by the setation and structure of antenna I; by the dorsal profile of the urosome; and, in life, by the body coloration (see below). From G. z. zaddachi, it is further distinguished by the lack of those features in which this relatively hairy form differs from G. z. salinus (see Spooner, 1947, pp. 20–1). G. z. oceanicus also reaches considerably larger sizes than either of the other two forms, even near the southern limit of its geographical range.

Antenna I (Fig. I). The peduncle of this limb provides the most critical diagnostic feature of oceanicus, approaching, as it does, that of G. locusta. The hair tufts on the ventral surface are sparse: on segment I there are two thin tufts (excluding the apical tuft), rarely a small third, composed of very few shortish hairs, as contrasted with at least four tufts in G. z. salinus, and one tuft (sometimes a second slight one) in G. locusta; on segment 2 there are normally three tufts, as compared with at least four in G. z. salinus and one or two in G. locusta; on segment 3 there is one strong tuft, which is often entirely lacking in G. locusta, and represented by at least two tufts in other G. zaddachi. The relative lengths of the segments approximate to ratio of 10:7.4:3.4, that is to say segments 2+3 are together a little longer than segment I (much longer in other zaddachi, and just shorter in locusta).











Fig. I. Antenna I peduncle of an adult male of (a) G. locusta, (b) G. zaddachi oceanicus, (c) G. z. salinus, (d and e) G. z. zaddachi; drawn somewhat schematically, viewed from the inner surface. The numbers written on the segments and against the accessory flagellum refer to the mean length relative to the first segment as 100. The end-points of measurements are indicated at the top of the diagram (against the peduncle segments of a). The number written above segment 3 refers to the ratio of this segment to segment 2. The numbers written below the segments represent the typical number of ventral hair tufts present in adults of various ages; those italicized being the most characteristic.

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Segment 3 is just less than half segment 2 (as in *locusta*), whereas it is a little longer than half segment 2 in other *zaddachi*. The ratios given in Fig. 1 represent typical proportions and have been calculated from the averages of several accurately measured limbs from adult males. Further reference to this diagram will show a distinct gradation of various metrical characters with *G. locusta* at the end of the series and the most intensely hairy *G. z. zaddachi* at the other. The condition in *G. z. oceanicus* is diagnostic, and is rather closer to *G. locusta* than to other *zaddachi*. The accessory flagellum length is some-



Fig. 2. Dorsal part of urosome viewed laterally, of (a) G. locusta, (b) G. zaddachi oceanicus, and (c) G. z. salinus; immature animal on left, adult male on right.

what longer in *oceanicus* than in *salinus*, possessing 9 or 10 segments in the larger males, but well short of the unusual length of this limb in typical G. *locusta*.

Urosome. In the degree of elevation mid-dorsally of urosome segments 1 and 2, G. z. oceanicus is intermediate between G. locusta and G. z. salinus (Fig. 2). The elevations are distinctly angled, but make an angle of some  $135^{\circ}$ , whereas in a comparable stage of G. locusta they are nearly right-angled. The elevations of G. z. salinus are distinctly flattened.

*Body colour*. This is of a variety of shades of brown (reddish olive to greenish or yellowish), often semi-transparent, without strong banding, at most a

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suffused darkening along the margins of the segments. The female is usually darker (as in *G. locusta* and *G. z. salinus*). Lateral orange pigment patches may be present on pleon segments I-3, but these may be lacking or composed of colourless globules. (In Finland, it seems they are usually lacking or at least colourless.) There are never spots on the mesosome segments laterally, as in *G. locusta*. Where *G. z. oceanicus* and *G. z. salinus* occur together, it is apparently possible to separate the latter by eye owing to its stronger transverse banding.

Size. Adult males normally appear to reach a length of 22-25 mm. (from rostrum to apex of telson) in Scotland, the maximum observed being 27.5 mm. Segerstråle gives maximum of 26 mm. for the Baltic, and Stephensen (1940a,b) one of 38 mm. for the Arctic, and a single exceptional male of 39 mm. from Iceland. The eggs are of similar proportions to those of other *zaddachi*.

Habitat. Being a marine coastal form, inhabiting the algal zone of shallow waters up to the mid-tide level of the shore, it is apt to occur in company with G. locusta in the more southern part of its range (see p. 138). No difference in the general habits of the two forms is yet known. Being also tolerant of some degree of dilution of the sea water and thus penetrating into estuaries, it reaches the zone inhabited by G. z. salinus, in so far as the geographical ranges overlap (see p. 137). Apart from these two, it will not normally be accompanied by any other Gammarus (s.s.) species in samples from the British Isles. Exceptions to this rule may be found in certain 'mixed' habitats, e.g. at coastal outflows of fresh water which permit the fauna of a stream to mix with that of the shore over which it flows.

### DISTRIBUTION OF GAMMARUS ZADDACHI OCEANICUS IN BRITAIN

A full list of the British localities from which G. z. oceanicus has been recognized is now given. The specimens in question have all been examined by the writer. The following abbreviations are used: G, from the collections of Dr Dorothy Gibb, all from the middle of the intertidal zone amongst Ascophyllum. A.D.H., Prof. A. D. Hobson; B.M., British Museum; coll., collected by; Coll., collection. Localities are classified under vice-counties according to the customary scheme (see, for example, Roebuck, 1921).

Scotland. W. SUTHERLAND. Loch Laxford, N. side, Loch a Chadhfi, G, 22. ix. 48, 5 33, 6 juv. (apparent inhibited development); Weaver's Bay, G, 21. ix. 48, bays on east and west side, 13, 333. Badcall Bay, N. side, G, 21. ix. 48, 2 juv. Kylestrome, G, 20. ix. 48, 2 small 33, 1 juv. W. Ross. Gairloch, Badachro Bay, G, 17. v. 48, 1 young 3. Loch Torridon, Camas Roil, G, 15. v. 48, 533, 599; Ob Mheallaidh, 8. v. 48, 533, 699. Loch Long, Conchra, G, 18. v. 48, 333, 299. Loch Duich, G, in four sites at which samples were taken regularly: site 1, in company with *Gammarus duebeni* (fresh-water seepage over shore), between 18. vi. 48 and 19. i. 49, 2133, 899, I juv.; site 2, between 18. vi. 48 and 31. v. 49, 29 33, 1099, 3 juv.; site 3, between 18. vi. 48 and 3. iii. 49, 19 33, 10 99, 7 juv.; site 4, between 18. vi. 48 and 31. v. 49, 41 33, 18 99, and 4 juv. N. EBUDES. S. Rona, vii and viii. 37, A.D.H. Coll., in stream flowing over shore, well below H.W., 3 and immature (Beadle & Cragg, 1940, as 'zaddachi hairless var.'; Spooner, 1947, p. 43). Raasay, vii and viii. 37, A.D.H. Coll., in stream over shore, 6 33, 19, II immature (Spooner, 1947, p. 43). Skye: Loch Dunvegan, G, 2. iv. 48, 2 33, 299; Loch Eishort, G, 6. iv. 48, large 3 and 9; Loch Ainort, G, single examples on two occasions (28. x. 48 and 16. xii. 48) at one of four sites regularly sampled between vi. 48 and vi. 49; Strollamus, G, at site 3 (one of three sites regularly sampled), on six dates between 26. iii. 48 and 8. vi. 49, 833 and 999. Westerness. Loch Nan Cilltean (stream inflow), G, 8. iv. 48, 3 and  $2^{\circ}_{++}$ , in company with both G. z. salinus and G. z. zaddachi. MID EBUDES. Mull: Loch Selve, G, 12. iv. 48, in two samples, 1533, 1099. ARGYLL. Loch Sunart, G, 9. iv. 48, 7 33, 499. Loch Feochan, G, I. iii. 48, 11 33, 7  $\stackrel{\circ}{\downarrow}$ . Loch Riddon, G, in two of three sites regularly sampled, a few examples only—site 1, 1. ii. 49, 1 3, 1 9; site 3, 11. xi. 48, 1 3, 21. xii. 48, 1 3, I intersex, I 9, I. ii. 49, I large J. CLYDE ISLES. Cumbrae, east of Keppel Pier, shore near L.W., 16. ii. 43, 2 large 33 (Spooner, 1947, p. 42 as salinus; specimens recently re-examined). No further examples have yet been obtained in the Millport area, where G. locusta is undoubtedly the commoner form. KINCARDINE. Muchalls, vii. 49, G. R. Forster, 22 ex. among sample of gammarids collected on the shore, including 8 mature 33, I large 9, and 13 young adults and immatures. FORFAR. Firth of Tay, among the Tees Survey collections (Alexander, Southgate & Bassindale, 1935), in sections IX and X of the estuary (Spooner, 1947, p. 43, as Segerstråle's subspecies = oceanicus; p. 52, as 'G. zaddachi saline form'.)

**England and Wales.** DURHAM. Ryhope, Norman Coll. as '*locusta*' (B.M. 1911. 11. 8. 18866–70, part), 233, 299 ovig., 1 imm., with 3 *G. locusta*. N.E. YORKS. Robin Hood's Bay, one juvenile, shore between Mill Beck and Stoupe Beck, J. S. Colman and F. Segrove (who inform me that *G. locusta* is the common marine species here, as I was able to see for myself in October 1950).

The distribution map (Fig. 3) gives a fair impression of the extent of the range of *oceanicus* in Britain. There is reasonable negative data for localities south of the areas mentioned, that on the east coast being weakest. Dr Gibb's material includes ten samples from the north and west coasts of Ireland, collected under similar conditions to those from Scotland. *G. z. oceanicus* is lacking in these as well as in such other Irish material as I have seen. The Isle of Man, too, seems to be beyond the southern limit: Mr N. S. Jones (*in litt.*) has kindly confirmed that *oceanicus* has not yet been recognized there. Robin Hood's Bay would seem to be close to the extreme southern limit on the north-east coast, as is Heligoland on the other side of the North Sea.

## DISTRIBUTION ABROAD

The essential northern character of the range of *G. zaddachi oceanicus* was shown by Segerstråle (1947, fig. 4). Additional localities in north-east Europe were given by Segerstråle in 1948; and others identified by the present writer, hitherto unpublished, are listed in Appendix I (p. 142).



Fig. 3. Distribution of *G. zaddachi oceanicus*, showing regions in which it has so far been identified, except for its eastward extension to 140° E. along the north coast of U.S.S.R. Inset, localities from which *G. z. oceanicus* has been identified in Scotland. *Note*. The exact locations of Labrador and Newfoundland samples are not known. The absence of records along much of the east coast of Scotland is simply due to lack of investigation.

It is now possible to take account of the records given by Stephensen (1940*a*, *b*, 1944), Holmes (1904), Dementieva (1931), and Gurjanova (1929, 1931, 1932, 1936), records which can, for some good reason or another, be attributed with certainty to *G. zaddachi oceanicus*. These authors have given much information on the status of *Gammarus* in northern waters, and in the light of present knowledge it is often possible to distinguish clearly which of their observations refer respectively to *G. wilkitzkii*, *G. setosus*, or *G. zaddachi oceanicus*. All necessary details are given in Appendix II (pp. 144–7).

#### G. M. SPOONER

The more complete picture now obtained of the geographic range, though still incomplete with regard to its extreme eastern and western limits, may be summarized as follows (Fig. 3).

Coasts bordering the Arctic Ocean southward from the south fringe of Franz Joseph Land ( $60^{\circ}$  E.) and north-east Spitsbergen, both areas lying on the 80th parallel. North coast of Siberia, from at least 140° E. (Brothers Laptev Sea) westward. Novaya Zemlya, at south-western end of the northern island, and west coast of the southern island. Spitsbergen, along the whole western side, where apparently the commonest gammarid. Bear Island. North coast of Russia, on the shore of the Kara Sea and of Chashskaya Bay, westward along the Kanin Peninsula and White Sea coasts. [No published data for coast between  $67^{\circ}$  and  $48^{\circ}$  E.] Murmansk coast generally, continuing along whole coast of Norway, southward to Oslo Fjord. Kattegat and all Baltic coasts south of  $63^{\circ}$  30' N. Heligoland. Great Britain, southward to coast of north-east Yorks and Firth of Clyde. (Faeroes?) Iceland, on north, east and west coasts. Greenland coasts to  $77^{\circ}$  N. on the east side and  $73^{\circ}$  N. on the west. North American Atlantic coasts including Labrador, Gulf of St Lawrence, Newfoundland, Nova Scotia, Massachusetts, and Connecticut, possibly extending farther south (see p. 140).

It occurs mainly in shallow coastal waters, captures in depths greater than 25 m. being mainly close to the shore, such as in the Norwegian fjords. There are, however, occasional records from deeper water in the open sea. In arctic and subarctic waters it is often accompanied by *G. setosus* Dement. (a species more exclusively restricted to the tidal zone and very shallow water) and sometimes by *G. wilkitzkii* Birula (a species less restricted to the immediate vicinity of the shore).

The extent to which overlapping occurs with the temperate forms G. locusta and G. zaddachi salinus is discussed below (pp. 137–9).

On many northern shores G. zaddachi oceanicus is a dominant littoral animal, and must be of great account bionomically. For example, in Iceland, it is 'one of the most frequent littoral Amphipoda, occurring almost everywhere along the coasts of Iceland....On account of its enormous frequency it is of considerable importance as food for many valuable fishes, as young gadids and plaice, but especially for the charr, when living in saltwater.' (Stephensen, 1940b; as G. locusta (L.) s.s., p. 56.)

Its hardiness and adaptability are shown by its persistence in areas such as Cheshskaya Bay, east of the Kanin Peninsula, the restricted fauna of which is discussed by Gurjanova (1929); and more especially, along the north Siberian coast, on the shore of the Nordenskiöld Sea between the meridian 120 and 140° E. The sea here has greatly reduced salinity owing to the discharge of the Lena and other Siberian rivers, and the temperature is always below zero C. The fauna of the littoral is necessarily reduced, with a tidal range of only a few inches; and the adverse effects of ice action and lack of algal cover reduce it further. The only species of littoral animal recorded, indeed, was G. 'locusta' (= zaddachi oceanicus + setosus) (Popov, 1932).

## ON GAMMARUS ZADDACHI OCEANICUS

## RELATION TO GAMMARUS ZADDACHI SALINUS

*G. zaddachi salinus* does not tolerate normal sea-water salinities, and so, in Great Britain, it is virtually confined to estuaries. It is found, however, in marine habitats when the salinity does not rise above  $31 \,^{\circ}/_{\circ\circ}$ , as in part of the Bristol Channel. In the Baltic Segerstrale (1947) has found it to be widely spread.

In the Scottish records previously given (Spooner, 1947, p. 42), that for Cumbrae should be transferred to *oceanicus*, but the Fairlie Sands and Clachan Strand records apply to typical specimens of *salinus*. Additional British localities for *G. z. salinus* are given below, which establish its occurrence in Cumberland, in the west and north of Ireland, and well up on the west and east coasts of Scotland. (As before, G refers to Dr Gibb's material.)

England and Wales. S. DEVON. R. Yealm estuary, present in both Cofflete and Newton Creeks (in 1948), but confined in them to a stretch of a few hundred yards. R. Exe estuary, present in small sample of gammarids taken near Starcross, 23. vi. 47. GLAMORGAN. Cardiff, Bristol Channel off Flatholme, coll. R. D. Purchon, 12. ix. 47, 433; in trawl 29. ix. 47, 3, 499 (conforming with occurrence on the opposite Somerset coast). CUMBERLAND. R. Esk estuary, at Eskmeals, 31. v. 50, plentiful in river channel, upstream from the railway bridge, in company with G. z. zaddachi. E. NORFOLK: Breydon, coll. R. Gurney, viii.-21, 13 (B.M. 1949.11.30.9.)

Scotland. ARGYLL. Loch Riddon, G, at two of three sites regularly sampled over a year: site 2, 11. viii. 48, 13 and 19; site 3, 30. ix. 48, 13, 299, 1 juv., 11. xi. 48, 333, 1. ii. 49, 13 (in site 3 accompanying G. z. oceanicus). WESTERNESS. Loch Nan Cilltean, G, 8. iv. 48, 3 (233 and 19 with eggs) in a sample containing also 3 G. z. oceanicus, 5 G. z. zaddachi, 7 Marinogammarus marinus, and 1 M. stoerensis, showing clear mingling of faunas of stream and shore. S. ABERDEEN. Aberdeen, coll. G. R. Forster, 1949, 233. N. ABERDEEN. Newburgh, estuary of R. Ythan, coll. G. R. Forster, vii. 49, 333, 19, 2 juv.

**Ireland.** W. DONEGAL. Mullroy Bay, G, area I (head of estuary), 833,799 (breeding), in company with about equal number of *Gammarus z. zaddachi*. N. GALWAY. Ballynakill Bay, G, north-east corner, 4 juv. with 19G. *z. zaddachi*.

There is therefore a region of considerable extent along the west coast of Scotland (at least from the Firth of Clyde to the border of West Ross (probably farther); as well as the greater part of the north-east coast of England and east coast of Scotland, where *oceanicus* and *salinus* may come in contact. The Tay estuary samples contain only *oceanicus* in the region where *salinus* would be expected, but the number of specimens is small, and no significance can be attached to the absence of the latter. More detailed investigation in this region would be of interest. That *oceanicus* and *salinus* can overlap, in the way that

*locusta* and *salinus* overlap, is shown by the samples from Loch Riddon and Loch Nan Cilltean. In the last, unique so far for Britain, all three subspecies of *G. zaddachi* occur together, as frequently may happen in collections from parts of the Baltic.

There is no evidence at all of intergrading between *salinus* and *oceanicus*, and it may be inferred that, as has been proved between the typical form of *zaddachi* and *salinus*, they cannot interbreed. For all practical purposes, therefore, the three forms can be treated as distinct species.

## OVERLAP WITH GAMMARUS LOCUSTA

Speaking broadly the marine 'niche' occupied by G. zaddachi oceanicus in the north is occupied by G. locusta (L.) in south Britain and the Low Countries southward. There is therefore special interest attached to the question of the ecological relations of the two forms in those areas in which overlap occurs. So far an adequate detailed investigation has not been made, but would certainly be valuable.

In Europe the overlap zone begins in Denmark. *G. locusta* is still the dominant form on the west coast, where indeed the existence of *G. z. oceanicus* has still to be proved. On the Baltic shores both forms are numerous, and *oceanicus* becomes dominant passing eastwards into the low salinities of the Baltic Sea. As Segerstråle (1947) shows, *locusta* reaches Finland but has a lower limit of some  $5 \cdot 5^{\circ}/_{\circ\circ}$ , while *oceanicus* persists to about the  $2 \cdot 5^{\circ}/_{\circ\circ}$  isohyaline. Along the whole coast of Norway *oceanicus* is clearly dominant, *locusta* occurring sparingly from Oslo fjord to  $70\frac{1}{2}^{\circ}$  N. (Appendix II, p. 146). In Iceland the occurrence of *locusta* has recently been proved (Segerstråle, 1950), but it is clearly overwhelmingly outnumbered by *oceanicus*. There is nothing in the data associated with these continental records to suggest any important difference in habitat, except only that *oceanicus* is rather more tolerant of reduction in salinity.

In Britain, passing northwards, all that can be said so far is that *locusta* is still the dominant form at the known southern limit of *oceanicus* (Clyde Sea Area and north-west Yorkshire), but in no great distance *oceanicus* becomes at least as frequent as *locusta*. At least this is so in the sheltered lochs of the west coast, where *oceanicus* perhaps adapts itself more readily to the variously lowered salinities. Among Dr Gibb's samples of the *Ascophyllum mackayi* fauna there were very few *locusta* at all. On the other hand, only *locusta* have so far been seen in samples from Muck (Inner Hebrides) and Barra (Outer Hebrides). There is a distinct tendency for samples to contain only one or other of the two forms, and some real segregation by habitat is suggested. It is this aspect, in particular, which requires further study.

#### THE BREEDING PERIOD OF GAMMARUS ZADDACHI OCEANICUS

In Britain most species of *Gammarus* breed in all months of the year, including *G. zaddachi zaddachi* and *G. z. salinus*. Segerstråle (1950) finds that these two forms also breed throughout the year in Finland, but points out that *G. z. oceanicus* has a resting period from the end of July to mid-November, and that during August, September and October no females could be found with broods in their pouches.

Evidence from British samples also points to a non-breeding season in the autumn for *oceanicus*. Females which are not breeding have their brood-plates reduced to an immature condition (smaller in size and lacking the fringe of finger-like processes): they are therefore at once recognized from females which are potentially capable of breeding, even if the contents of the brood pouch have been discharged or accidentally lost after preservation.

TABLE I. BREEDING IN C	GAMMARUS	ZADDACHI	OCEANICUS
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	Adult females		Approx.
Period	Developed	Undeveloped	breeding
1 Jan.–6 Feb.	8	5	60
7 Feb14 Mar.	18	Ĩ	95
15 Mar20 Apr.	23	0)	
21 Apr26 May	13	0-	100
27 May-2 July	4	0)	
3 July-7 Aug.	3	2	60
8 Aug13 Sept.	I	3	25
14 Sept19 Oct.		<u> </u>	
20 Oct25 Nov.	0	3	0
26 Nov31 Dec.	3	14	20

Table I shows the results of analysing Dr Gibb's west-Scottish material. The year is divided into ten periods. In the first column is given the number of reproductively active females observed, and in the second that of females clearly large enough to be mature, but with reduced brood plates. The latest animal with a brood was taken on 11 September, and the earliest on 4 December. But there is evidently considerable variation among individuals, and the rest period for any single individual could easily be 5 months. The data, as far as they go, indicate a non-breeding season from early September to mid-December, with its peak at the end of October. There is maximum breeding in March, April, May and June.

The onset of the non-breeding season appears to be later than in Finland by perhaps a month or 6 weeks, and renewal of reproduction also to be later by about a month.

#### GAMMARUS ZADDACHI OCEANICUS IN AMERICA

A few years ago, through the kindness of Mrs Sexton, I was able to examine some so-called *G. locusta* from eastern North America (lent by Dr C. R. Shoemaker). From sketches and measurements made at the time it was subsequently noted that the specimens belonged not to *locusta* s.s., but to *zaddachi oceanicus*. The data attached to the specimens were 'Newfoundland, 1885'.

The same belated conclusion is also true of a sample of 633 (largest 31 mm.) from 'Lac Tadoussac: July 1938: V.D.V.', lent to me in 1939 by Mr G. I. Crawford. This locality is on the Gulf of St Lawrence.

Segerstråle (1947, p. 230) has indeed drawn attention to the occurrence of G. zaddachi oceanicus on the Atlantic coast of North America, having identified one sample from Labrador and two from Massachusetts. These would previously have been regarded as 'locusta'.

More recently four additional samples of N. American 'locusta', from Nova Scotia, and the New England states (see Appendix, p. 144, for details), have been critically examined and found to be *G. zaddachi oceanicus*. These are in the British Museum Collection. All date from about 1880, and had originally been determined as *G. ornatus* Edw. on S. I. Smith's authority.

The question arises whether the true *G. locusta*, as we now undertand that species (Sexton, 1942; Spooner, 1947), occurs on the western side of the Atlantic at all. A search for clues amongst the American literature has provided a fairly certain answer: the common species which for long has passed under the name of *locusta*, and earlier on of *ornatus*, is in fact *G. zaddachi oceanicus*.

In his work on the Amphipoda of southern New England, Holmes (1904, pp. 500-1; pl. X, no. 3) gives a description, some illustrations, and a photograph of the *Gammarus 'locusta'* of that region (syn. *G. ornatus* Edw.), describing it as 'the species of amphipod decidedly most often met with in the collections from New England'. This cannot possibly be the true *G. locusta*, as is at once apparent from the photograph on his plate X (no. 3). In this the relative depth of the coxal plates, the shape of coxal plate 4, the depth of the lateral head sinus, the relative length of antenna 1 peduncle segment 2 and of the accessory flagellum, etc., are characteristic of *G. zaddachi oceanicus* as opposed to *G. locusta*. Furthermore, the drawings of the male gnathopod 3 and uropod 3 (figure on p. 501) show diagnostic features which exclude *G. locusta* and conform to *G. z. oceanicus*. Other points in the written description again agree with the latter against the former (accessory flagellum of 8 segments; the spination of male gnathopod 2 hand; no orange pigments on mesome); and there is no hint that a mixed population is being described.

There is thus as yet no evidence that G. locusta (L.) occurs on the American side of the Atlantic, and until positive evidence is forthcoming it can only be assumed that all references to G. 'locusta' on the Atlantic seaboard refer to G. zaddachi oceanicus. The validity of the name 'ornatus' will have to be seriously considered.<sup>1</sup>

<sup>1</sup> Milne-Edward's (1830) description referred to specimens from Boston, Mass. It is sufficiently detailed to exclude all species except G. zaddachi oceanicus and G. locusta s.s., hence might be held to define the former by elimination.

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(N.B. Statements referring to the occurrence of G. '*locusta*' on the Pacific coast of America should be heavily queried. South of the Behring Sea it is furthermore very doubtful whether records apply even to a related species.)

The extent to which *G. zaddachi oceanicus* ranges southward along the Atlantic shore of the U.S.A. is not known. As it is so plentiful on the Connecticut coast, it might well extend beyond New Jersey; but if its southern limit is reached as comparatively abruptly as in Britain, it may not exceed the 40th parallel of latitude.

Segerstråle (1947, fig. 3f) figures the telson of a specimen from Massachusetts, showing that two of the apical setae are delicately plumose. The first American male which I examined in detail also showed precisely the same feature but several others do not. Otherwise the possession of plumose setae (excepting the pleopods and uropod 3 rami) is a diagnostic feature of *G. setosus* Dement., in which all, or nearly all, the hairs on the urosome dorsally and on the telson are of this type.

#### SUMMARY

A number of records of *Gammarus zaddachi oceanicus* from northern Britain are given, which indicate that this form extends down the respective coasts to the Clyde Sea Area and Yorkshire.

It is an important subarctic and boreal littoral crustacean which formerly was confused with G. *locusta*. Some additional localities are quoted and some re-identifications are made from literature, when illustrations or biometric data enable this to be done.

The ecological relations between G. z. oceanicus, on the one hand, and G. z. salinus and G. locusta, on the other, are discussed.

It is emphasized that the animal which has all along been known in eastern America as G. locusta (formerly G. ornatus) is really G. z. oceanicus.

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#### APPENDIX I

LIST OF SPECIMENS OF GAMMARUS ZADDACHI OCEANICUS EXAMINED (OTHER THAN BRITISH). NEW, OR REVISED, IDENTIFICATIONS

# (G.I.C., G. I. Crawford. B.M., British Museum. Cop.Mus., Copenhagen Museum)

# **Baltic & Kattegat**

POMERANIA. Strela-Sund, near Stahlbrode, E. of Stralsund, Dr E. Schwarz, as G. locusta, 16 33,  $3^{\text{QQ}}$  (ovig.), with 2 G. z. salinus (B.M. 1933.

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11. 1. 2-10, part). S.W. SWEDEN. Bohuslän, as G. locusta, 2 imm., with 2 adult G. locusta (B.M. 97.6.1.33, part).

#### Norway

W. NORWAY. Bergen (near), Molde, 19. vi. 39, G.I.C., 13, 11 yng 33 and imm. (B.M. 1942.8.5.73-77). N. NORWAY. Tromsö, 30. vi. 39, half-tide, G.I.C, 15 advanced imm. (B.M.). Lofoten Is., Svolvaer, 26. vi. 39, mid-tide, G.I.C., 1 imm. Skaervoy, S. of Hammerfest, 2. vii. 39, half-tide, G.I.C., 1033, 699, 10 imm. (B.M., 1942.8.5.51-55). Malangen, Nordbotten, vii. 1881, Cop.Mus., 3 and 9 (with G. setosus). Porsangerfjord, Honningsvåg, 5. vii. 39, mid-tide, G.I.C., 233, 2 imm. (with G. duebeni and Marinogammarus finmarchicus) (B.M.). Porsangerfjord, Rimabukt, 10. vii. 39, L.W. to half-tide, G.I.C., large 3. Porsangerfjord, Suogalma, 6. vii. 39, L.W. to half-tide, G.I.C., 633, 899 (7 ovig.), c. 65 imm. and yng adults (B.M. 1942. 8.5.44-50). Lang Fjord, Finmark, as G. locusta, Norman Coll., 1 imm. (B.M. 1911.11.8.18852).

#### Iceland

E. ICELAND. Berufjörður, 0–3 m., 14. vii. 1900, Cop.Mus., 233 and 19(with other spp.), shore, below 'ordinary high water'. Loðmundarfjörður, 9. v. 1898, Cop.Mus., 733, 699, 2 imm. 'midtfjords i bunden, 13 m. St. 7'. S.E. ICELAND. Hornafjörður shore, 29. viii. 36, Cop.Mus., 633, 19, 19 imm. W. ICELAND. Hvammsfjörður shore, 19. vii. 1886, Cop.Mus., 633, 699, many imm.

#### Spitzbergen

As G. locusta var. zaddachi, A. Schellenberg det., 2 large 33 of 34 and 35 mm., 6 imm., with G. setosus (B.M. 1934.9.1.21-24, part).

#### Greenland

E. GREENLAND. Angmagssalik, 4 m., 10. viii. 33, Cop.Mus., 13, 19 (with G. setosus and G. wilkitzkii). Angmagssalik, Tasiusak, Cop.Mus., large 9 [nec setosus]. 'GREENLAND'. 733, 19, ex coll. Cop.Mus. S.E. GREENLAND. Lindenowsfjord, 50–75 m., 28. vii. 35, Cop.Mus., 233, 399 (not breeding) (with G. setosus). W. GREENLAND. Holsteinsborg, ex coll. Cop.Mus., 533, 699, 9 imm., (breeding actively) (with G. setosus). Holsteinsborg, N. Strömfjord, 1911, Cop.Mus., 633, 399 (with G. setosus). Waigat, Sakkrak, 1892, 533, 299, Cop.Mus. Upernavik, 12. vi. 36, Cop.Mus., 3. Davis Strait,  $63^{\circ}27'$ N,  $54^{\circ}12'$ W., E. Whymper, (B.M. 79.26 as 'locusta'), 19, not breeding, with several juvenile G. wilkitzkii.

## N. America

CANADA, QUEBEC. Lac Tadoussac, July 1938 (V.D.V.), per G.I.C., 6 large 33, largest 31 mm. NewFoundLAND, 1885, per E. W. Sexton, adult 33. Nova

SCOTIA. Off Halifax, loc. 74, 1881, U.S. Fish. Comm., 'G. ornatus Edw.', Norman Coll. as 'locusta' (B.M. 1911.11.8.18929-932), 3 33, 1  $\mathcal{Q}$ . MASSACHUSETTS. Gloucester Harbour, U.S. Fish. Comm., 'G. ornatus Edw.', Smithsonian Inst., (B.M. 80.26 as locusta), 1 3 (c. 34 mm.), 1  $\mathcal{Q}$  with brood. CONNECTICUT. New Haven, 'G. ornatus Edw.', S. I. Smith, Norman Coll. as 'locusta' (B.M. 1911.11.8.18919-928), 4 33, 11  $\mathcal{Q}$ , (most ovig.). 'N.E. AMERICA'. 'G. ornatus Edw.', S. I. Smith, Norman Coll. as locusta (B.M. 1911.8.18933-937), 2 33, 4  $\mathcal{Q}$  (not breeding).

## APPENDIX II

#### Re-identifications from the Literature

## Dementieva (1931)

At the time this paper was written all northern marine *Gammarus* were still referred to *G. locusta*, but it was realized that much variation existed. Dementieva's biometric data aimed at expressing the differences between certain selected populations in a more precise form. Her results enable these populations to be identified. She rightly concluded, incidentally, that her 'typical forms' (*zaddachi oceanicus*) and 'brackish-water forms' (*setosus*) were distinct species.

Population	Dementieva's name	Data or illustrations	Re-identification
Kola Gulf (Murmansk)	G. locusta 'typical forms'	Tables 5, 7; figs. 7a, 8a, 12a-c	G. zaddachi oceanicus Seg.
Baltic Sea	G. locusta 'typical form'	Table 7; table on p. 77 (left col.)	G. zaddachi oceanicus Seg.
Belouchia Bay (Novaya Zemlya)	G. locusta 'brackish-water form'	Tables 5, 6; figs. 7b, 8b, 12a-c	G. setosus Dement.
Zabloula Bay (Novaya Zemlya)	G. locusta 'brackish-water form'	Figs. 7c, 8c; table 6	G. setosus Dement.
Barents Sea	G. locusta 'deviating form'	Table on p. 76; fig. 9	G. wilkitzkii Birula
Baltic Sea	G. locusta 'deviating form'	Fig. 10; table on p. 77 (right col.)	G. zaddachi zaddachi Spooner
Kola Bay, etc.	G. duebenii	Figs. 11, 12 <i>a</i> , <i>b</i>	G. duebeni Lillj.

G. zaddachi oceanicus is the 'typical' form characteristic of the Kola Gulf shore, where it is not normally mixed with G. setosus. When it extends into shallow arms of the sea diluted with river water or land drainage it may overlap the range of G. duebeni. In Novaya Zemlya, G. z. oceanicus is reported from the seaward end of the bays visited, in which it tends to be replaced by G. setosus in shallower and fresher parts. In Mituschikha Bay both occurred together at the same depth. In the north Baltic, G. zaddachi oceanicus and

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G. zaddachi zaddachi occur together, as is now well known from Segerstråle's investigations. G. z. oceanicus of the Baltic are smaller than those of such typical habitats as the shore of Kola Bay, and their limbs are a little stouter.

# GURJANOVA, E., SACHS, S. & USCHAKOW, P. (1929)

The G. 'locusta' from Kola Bay, referred to in this paper may be taken to refer to G. z. oceanicus. G. setosus, as we now know, is quite scarce in this area, and G. locusta (s.s.) scarcely reaches it and is at best very rare.

## GURJANOVA, E. (1929)

G. 'locusta' is reported as 'everywhere in large numbers, littoral'. In this area, Cheshskaya Bay, conditions are rigorous and only euryhaline and eurythermal species survive. This must be chiefly G. zaddachi oceanicus, if not entirely. At most a minority may be G. setosus.

# GURJANOVA, E. (1931)

Amongst the amphipod fauna of the east Murmansk coast (Portschnicha Bay) G. 'locusta' is described as a characteristic littoral species. (G. wilkitzkii is now clearly distinguished.) Since G. setosus proves to be quite scarce on this coast, the remarks can be taken as referring to G. zaddachi oceanicus.

# GURJANOVA, E. (1932)

By the time of publication of this paper (and perhaps earlier) Gurjanova already distinguished G. setosus as separate from G. 'locusta', and refers to it as G. locusta var. mutata.

In an account of the fauna of that obscure and peculiar region off the Siberian coast known as Nordenskjöld's Sea (or the Sea of the Brothers Laptev), records are given of two of the three arctic *Gammarus*.

Gurjanova's name	Re-identification	Occurrence
G. locusta	G. zaddachi oceanicus	73° 05' N., 140° 45' E., 16.5m., 0.42° C., 2 ex 73° 20' N., 139° 08' E., 16 m., 0.45° C.,
		I ex 'also previously from ten other stations in the region of the New Siberian Islands'
G. wilkitzkii	G. wilkitzkii	72° 9′ N., 135° 45′ E., 20 m., 0·3° C.

Note that Popov (1932), in describing the conditions of life in the Brothers Laptev Sea, refers to 'G. *locusta*' being spread all along the littoral of the Siberian coast, and indeed as being the only littoral marine animal! There is virtually no tide here (a few inches at best); the temperature is always minus, and the water has greatly reduced salinity owing to the inflow of several large

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rivers, including the Lena. Presumably this must be taken to cover G. zaddachi oceanicus and G. setosus, since the latter was eventually reported from the Laptev Sea, and would be chiefly coastal.

### GURJANOVA, E. (1936)

Further records are given of *Gammarus* from the Nordenskjöld Sea and from the Kara Sea. *G. setosus* is now distinguished specifically. References to *G. 'locusta'* now refer solely to *G. zaddachi oceanicus*. It is recorded from a station  $69^{\circ} 25'$  N.,  $67^{\circ} 15'$  E. (shore of the Kara Sea).

G. setosus is reported from the Laptev Sea for the first time  $(74^{\circ} 50' \text{ N.}, 112^{\circ} 50' \text{ E.})$ .

## STEPHENSEN (1940a)

This is the first occasion on which it is recognized that differences exist between the G. locusta of temperate and south Europe, and the form which still passed under the name of locusta from the north. The full extent of difference is, however, not appreciated, and the name locusta is still retained for both. In so far as the records here quoted are Stephensen's own determination it is possible to distinguish between true G. locusta and G. zaddachi oceanicus (= Stephensen's northern form of locusta).

It is made clear that G. locusta (s.s.) ranges into Denmark, but is usually not found farther to the north. In Norway it occurs, but is scarce, and records are given for Oslo fjord; Evenskjaer; Vannøy; and Loppa  $(c. 70\frac{1}{2}^{\circ} \text{ N.})$  (p. 318). In fig. 40, II and III apply to G. locusta.

The remaining records (unless simply quotations from literature) refer to G. zaddachi oceanicus, as does fig. 40 I. The records may be summarized as follows: south and west Norway, several samples from Oslo rjord to Trondheim fjord; north Norway, numerous samples all along the coasts of mainland and islands, to the frontier; Bear Island, in one sample; west Spitsbergen, in Grieg's material; north-east Spitsbergen, at 80° 40' N.

It is clear that all along the Norway coast G. zaddachi oceanicus is far more numerous than G. locusta.

G. setosus is treated separately, under the name of G. locusta setosus. There are three records from the Norway coast, on which it is clearly very scarce; and others from west Spitsbergen; north-east Spitsbergen; Greenland and Arctic America.

G. wilkitzkii is recognized as a distinct species, but Stephensen holds that it is possibly synonymous with G. zaddachi [=G. z. zaddachi] of which it would be a large-sized Arctic representative. A discussion on this topic (pp. 334-6) points out the difficulties in finding qualitative differences 'which cannot be explained as being due to the ordinarily very great difference in size'. Stephensen, however, minimizes the real differences in the antennal setation, the significance of the relative elongation of several limb segments, and of

## ON GAMMARUS ZADDACHI OCEANICUS

the long uropod 3 inner ramus. Moreover, he entirely ignores the diagnostic propod of female gnathopod 2, which is quite unlike that of G. zaddachi. There is no question that G. wilkitzkii is separate from G. zaddachi.

## STEPHENSEN (1940b, 1944)

In these excellent accounts of the amphipod faunas of Iceland and east Greenland, all G. locusta s.s. refer to G. zaddachi oceanicus, and G. locusta setosus to G. setosus.

In Iceland, G. z. oceanicus is the form which attains an 'enormous frequency' and is recorded all round the coast. Recently the true G. locusta has been found to occur (Segerstråle, 1950); but evidently it was not seen by Stephensen, and must be very scarce, being at the extreme northern limit of its range. G. setosus occurs fairly freely round the Iceland coast, more freely than in Norway, but is clearly outnumbered by G. z. oceanicus (see 1940b, pp. 56–8 and fig. 7).

In Greenland the occurrence of G. z. oceanicus is proved on the east coast northward to Hvalrosodden  $(77^{\circ} \text{ N.})$ . Other information exists to show it occurs on the west coast to at least  $73^{\circ}$  N. (material identified by Dr Segerstråle or myself). Judging, however, from the numbers and sizes of specimens recorded, it may be concluded that G. z. oceanicus flourishes less in Greenland than in Iceland or Norway, and is considerably outnumbered by G. setosus. It is G. setosus now which attracts attention from its numbers, e.g. at Angmagssalik it 'occurs everywhere on the coast, both on rock facies and on sediment shares found at river mouths'. A difference in the bathymetric range of the two forms is noted, confirming Dementieva's (1931) records from Novaya Zemlya, namely that G. setosus in east Greenland is 'a character form of the tidal zone, only exceptionally at depths of more than a few metres', while G. z. oceanicus was taken mostly in shallow water from low-water mark downwards to about 20 m., rarely still deeper (50–75 m.) (see 1944, pp. 108– 12; fig. 8).

# THE DETERMINATION OF SILICATE IN SEA WATER

## By F. A. J. Armstrong

Experimental Officer at the Plymouth Laboratory

## (Text-figs. 1-3)

Silicon in sea water may be present in suspension, in particles of clay or sand, as a constituent of diatoms, etc., or in solution. Some silicon in solution occurs in the form of silicate. This is usually estimated by the colorimetric method of Diénert & Wandenbulcke (1923), which makes use of the yellow colour of the silicomolybdic acid which is formed when ammonium molybdate and sulphuric acid are added to the water (Atkins, 1923). The colour may be compared with that of standard solutions of picric acid (Diénert & Wandenbulcke, 1923) or potassium chromate (Swank & Mellon, 1934). The method is simple but the colour in sea water is often faint and is not easy to match visually, nor is its intensity strictly proportional to the concentration of silicate. Less colour is produced in sea water than in standard solutions made with distilled water and this 'salt error' must be allowed for (Brujewicz & Blinov, 1933; Wattenberg, 1937; Robinson & Spoor, 1936).

The work described below was done in an attempt to apply to sea water the more sensitive method in which silicomolybdic acid is formed and then reduced to molybdenum blue, a method which is now often used for determining trace amounts of silicon in other materials. It was found that this could be done, and an analytical method devised which would allow the determination of silicate in a range of concentrations from 0.1 to  $50 \mu g$ . atoms Si/l.

The method adopted is based upon the results of an investigation of the properties of silicomolybdic acid by Strickland (1950), which are summarized below.

Strickland found that when silicic acid reacts with molybdate, two forms of silicomolybdic acid may be formed, depending on the degree of acidification of the molybdate. When the normal molybdate ion is acidified with 1.45 to 1.50 equivalents of acid per g. ion of  $MoO_4^{--}$ , then the silicomolybdic acid described in the literature is the main product of the reaction. He terms this  $\alpha$ -silicomolybdic acid to distinguish it from the other form, which he calls  $\beta$ -silicomolybdic acid, produced when silicic acid reacts with molybdate acidified with more than about 2 equivalents of acid per g. ion of  $MoO_4^{--}$ . He states, further, that the presence of much electrolyte in solution decreases the ratio of  $\beta$ - to  $\alpha$ -compounds formed at a given acidity. Moreover, over a period of several hours the  $\beta$ -form changes spontaneously and irreversibly into the  $\alpha$ -form. The  $\beta$ -compound has apparently the same empirical formula as the  $\alpha$ -compound and has a similar but more intense yellow colour, the extinction for a given quantity of combined silica being over twice as great.

These findings, which I have confirmed, are consistent with reports in the literature of non-reproducibility and fading of the colour.

Strickland also investigated the reduction of these compounds by stannous chloride. In the presence of excess molybdate, both form blue compounds, but of different appearance and absorption spectra. That from the  $\alpha$ -acid, which is greenish blue, has over  $1\frac{1}{2}$  times the molecular extinction coefficient of that from the  $\beta$ -acid, which is royal blue.

For analytical use Strickland recommends preparation of the  $\beta$ -compound as more reproducible, and for this purpose it is necessary to acidify the molybdate with between 3 and 5 equivalents of acid. A concentration of molybdate, such that [Mo]=0.5 M remains after the silica has combined, is suitable for all concentrations of silicate, but the ionic strength of other ions must not exceed 0.5 M. Formation of the complex is complete in 5–10 min., and absorption measurement or subsequent reduction should not be delayed for longer than a further 15 min.

It is also known that for reduction, the stannous chloride may not be added immediately to these weakly acid solutions, or the excess molybdate will be reduced to a blue compound. If, however, the solution is first made approximately 1N with HCl, or 1.5Nwith  $H_2SO_4$ , the excess molybdate is converted rapidly (in about half a minute) into a form which will not reduce to a blue. Upon addition of stannous chloride only the silicomolybdic acid is reduced to molybdenum blue, but some of the excess molybdate is reduced to a substance of brownish yellow colour, probably a quinquevalent molybdenum compound. The amount of this increases with increasing molybdate, chloride, and stannous chloride concentrations. This compound has a measurable light absorption with the red light-filters used for measurement of absorption of molybdenum blue, and contributes to the 'reagent blank' of the method.

#### EXPERIMENTAL

Extinctions of coloured solutions were measured with the absorptiometer already described (Harvey, 1948). With it extinctions up to 0.5 can be measured to the nearest 0.001, and it will accommodate cuvettes of up to 25 cm. length. The distilled water used was prepared in a still having a silver condenser and was assumed to be silicon free.

As a basis for comparison the Diénert-Wandenbulcke method, as adapted by Atkins, was used, and series of solutions with known additions of silicon as silicate were prepared from distilled and sea water. To 100 ml. portions of these were added 2 ml. of 10% ammonium molybdate and 4 drops of 50% v/v sulphuric acid. The extinctions of the resulting yellow solutions were measured in a 20 cm. cuvette with Chance OB I (blue) and then with Ilford 60I (violet) filters in the absorptiometer. The results are shown in Fig. 1A. It will be seen that the relationship between extinction and silicon concentration is not linear, though the curvature is less with the 60I filter (which transmits light of shorter wave-length) and the sensitivity is greater. The decrease in sensitivity in sea water is also to be seen.

A test of the molybdenum-blue method was made as follows. In each of a series of 100 ml. flasks 5 ml. of 5% ammonium molybdate and 3 ml. 1N-hydrochloric acid was placed, and 25 ml. of distilled or sea water containing



Fig. 1. The relation between extinction and silicate concentration (A) with the Diénert-Wandenbulcke method; (B) with the molybdenum-blue method, (c) with the molybdenum-blue method, quantity of molybdate reduced. Silicon added (horizontal scale) in  $\mu g$ , atom Si/l. *a*, Ilford 601 filter (violet); *b*, Chance OB I filter (blue). Continuous line, distilled water; broken line, sea water.

known additions of silicon were added. After 10 min. 5 ml. of 35% v/v sulphuric acid were added, the solution allowed to stand 5 min., and 1 ml. o<sup>.1</sup> N-stannous chloride added. After a further 10 min. the extinctions of the solutions were measured in a 1 cm. cuvette, using Chance OR 1 (red) filters. These were used because they are already in use for phosphate determinations; Ilford 608 Spectrum Red filters are equally suitable. The results are shown in Fig. 1B, which displays the linearity of the relationship between extinction and silicon concentration. The sensitivity in sea water is greater, indicating that some  $\alpha$ -silicomolybdate has been formed.

The molybdenum-blue method is thus about 16 times more sensitive than the older procedure. The advantage cannot, however, be exploited by using a longer cuvette to increase extinction values unless the reagent blank (which would increase proportionally) can be decreased. With distilled water with no added silicon the extinction is 0.015, which is equivalent to about  $2 \mu g$ . atoms Si/l.

As a first step toward reducing the blank, a method of eliminating interference from phosphate and arsenate was tried. (There are usually a few parts per million of phosphate in the reagents which contribute to the blank.) In sea water the concentrations of phosphate and arsenate are normally negligible in comparison with that of silicate. An experiment showed that with the procedure given above, equal extinctions were produced by 101  $\mu$ g. of Si as silicate, 85  $\mu$ g. P as phosphate, and 235  $\mu$ g. As as arsenate. It has been shown (Denis & von Meysenbug, 1922; Bordeionu, 1927; Riehm, 1932) that oxalic (or citric or tartaric) acids will suppress interference from phosphate and arsenate. A trial showed that the addition of 2 ml. of 10% oxalic acid to the solution after acidification with sulphuric acid completely suppressed the formation of molybdenum blue from added phosphate and arsenate, leaving the extinction caused by silicate unchanged. The blank extinction decreased to 0.012.

A further decrease should result if the amounts of molybdate and stannous chloride could be reduced, and was realized by the following procedure.

In a 100 ml. flask were placed 50 ml. of the sample, and 2 ml. of a reagent made by mixing 5 vol. of 5% ammonium molybdate and 3 vol. of 1 N-hydrochloric acid. After standing 10 min., 5 ml. of 50% sulphuric acid and 2 ml. of 10% oxalic acid were added and the extinction measured in the absorptiometer, using a 4 cm. cuvette. (This gave a measure of the natural turbidity of the solution, to be subtracted from the final reading to give a true measure of the colour produced.) Ten min. after acidification 1 ml. of 0.05 N-stannous chloride was added, and the extinction measured after a further 15 min.

Results for known additions of silicon to distilled water and to a sample of sea water comparatively low in silicate are shown in Fig. 1C. The plots remain linear; the slope, indicating sensitivity, being greater for the sea water. The blank for distilled water, of 0.007, represents a concentration of 0.19  $\mu$ g.

atom Si/l. The reagent blank in sea water, which is greater than in distilled water because the high concentration of chloride causes increased formation of the yellowish quinquevalent compound, cannot be found directly unless silicatefree sea water can be obtained. The silicon content of the sample of sea water is not given by this experiment. The way in which the blank is found is described below.

As was mentioned above, the blank has two components, molybdenum blue due to silicon in the reagents, and the quinquevalent molybdenum colour due to reduction of excess molybdate. Provided that it is small the correction for silicon in the reagents is the same in distilled water and in sea water. The quinquevalent molybdenum colour is, however, greater in water containing chloride. It may be found directly by making the solution strongly acid before adding the molybdate; in 1.5 N-acid solution silicate will not combine with molybdate, and on adding stannous chloride the only colour produced is that due to reduction of excess molybdate.

Three measurements will then allow the blank correction for sea water to be calculated: (i) the extinction with silicon-free distilled water carried through the normal procedure; (ii) the extinction with distilled water when the 5 ml. 50% v/v sulphuric acid is added before the molybdate reagent, addition of oxalic acid and stannous chloride following as usual; (iii) the extinction with sea water carried through the procedure of (ii) above.

The difference between measurements (i) and (ii) is the extinction due to silicate in the reagents, and must be added to measurement (iii) to obtain the total blank correction. This is to be subtracted from extinction measurements made upon sea water.

The method at this stage of development was put into use for silicate determinations on samples of sea water, and was found to work fairly well, although the reproducibility of calibration with known additions of silicate was not entirely satisfactory. More disturbing was the occurrence of occasional high figures as if contamination had taken place. This effect was traced to attack of the resistance glass of the flasks by the sea water before addition of the molybdate reagent, and was surprisingly large. Several micrograms of silicon were dissolved in 30 min. It was found that placing the molybdate reagent in the flask first and then adding the sea water prevented this, presumably because the solution was now acid instead of faintly alkaline.

Variations in calibration figures were thought to be due to variation in the proportion of  $\alpha$ -silicomolybdate formed, and so should be reduced if this could be decreased. The difference in the slopes of the lines in Fig. 1B indicates that about 30% of the silicomolybdate formed in sea water is in the  $\alpha$ -form. An increase of the acidity of the reagent to the maximum recommended by Strickland was therefore tried, the reagent being made by mixing 2 vol. of 5% ammonium molybdate and 3 vol. of 1 N-hydrochloric acid. When 3 ml. of this reagent was used (in place of the 2 ml. previously employed) the

sensitivity in sea water was nearer to that in distilled water, and corresponded to about 12 % a-silicomolybdate formation. In subsequent routine use the calibration factors with this reagent were satisfactorily constant.

The data obtained so far were got in a laboratory at fairly constant temperature, and care had been taken to adhere to the stated timing. The usefulness of the method would be increased if it were known what latitude could be allowed, and a few experiments were done to find this out.

Fig. 2A shows the effect of temperature upon the depth of colour produced by a given quantity of silicate, the temperature being that at which the sea water and molybdate were allowed to react, the solutions being brought to  $20^{\circ}$  C. for the rest of the procedure. It is seen that the change in extinction with temperature is about 2 parts/1000/° C.

Fig. 2B shows the effect of altering the time of interaction (at  $18^{\circ}$  C.) of the sea water and molybdate, i.e. the time between addition of the sample and acidification with sulphuric acid. The maximum extinction is produced after 10–15 min.

Fig. 3 shows the development and subsequent fading of colour at temperatures of 13 and 22° C. The upper curves are of extinctions with (different) samples of sea water and represent the colour due to the combined effect of the molybdenum blue and the quinquevalent molybdenum blank, while the lower line is that of the latter alone, which was the same at both temperatures. The colour reached a maximum after 60 min. at 13° C. and after 30 min. at 22° C. and was within 1% of this maximum between about 40 and 70, and 16 and 32 min. respectively. In distilled water the colour attains its maximum within 3–5 min. and is constant for about 3 hr. at 20° C.

These times are longer than had been allowed in the earlier experiments, and have been used as a guide in subsequent work.

## **RECOMMENDED PROCEDURE**

(1) Ammonium molybdate, 5% w/v.

- (2) Hydrochloric acid, I N.
- (3) Sulphuric acid, 50% v/v.
- (4) Oxalic acid, 10% w/v.
- (5) Stannous chloride, 0.05 N.
- (6) Standard silicate solution.

The ammonium molybdate and hydrochloric acid solutions should be prepared from A.R. grade materials and silicon-free distilled water, and kept in polyethylene, ceresin, or heavily waxed bottles. The mixed reagent for use, consisting of 2 vol. molybdate and 3 vol. acid, should be freshly prepared, as it sometimes throws down a precipitate on standing for 1 or 2 days.

The stannous chloride is conveniently made just before it is required, by dilution from a stock solution of, say, 40 g.  $SnCl_2.2H_2O$  in 100 ml. 50 % v/v HCl.

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Solutions required



Fig. 2. A, The effect of temperature upon the amount of molybdenum blue formed, in seawater. Extinction in 4 cm. cuvette given by about 10  $\mu$ g, atoms Si/l. B, The effect of time upon the interaction of silicate and acid molybdate. Extinction in 4 cm. cuvette.



Fig. 3. Changes in the extinction of sea-water solutions with time. The lower line shows the quinquevalent molybdenum blank, which was the same at the two temperatures used.

The exact strength may be between 0.04 and 0.06N, and is not important provided that the same solution is used for both the blanks and the determinations.

A standard silicate solution may be prepared by fusing 0.1201 g. silica with 0.6 g. anhydrous sodium carbonate, and dissolving the melt when cold in water, and making to a volume of 100 ml. This solution contains 20  $\mu$ g. atoms Si/ml., and can be diluted serially for use. If kept in a well-stoppered polyethylene bottle, it is stable for at least a year. As a source of silica, finely powdered 'Vitreosil' is suitable. It should be heated to redness and cooled in a desiccator before weighing, and contains over 99.8% SiO<sub>2</sub>.

## Apparatus required

The absorptiometer used in these experiments is very convenient, but is only essential if the lowest silicate concentrations have to be measured. For concentrations greater than about 0.5  $\mu$ g. atom Si/l. the 'Spekker' instrument of Messrs Adam Hilger, or one of the other photoelectric absorptiometers capable of holding a 4 cm. cuvette, would serve. The method can of course be used with a visual absorptiometer or with Nessler cylinders with a small loss of precision and speed. With the latter a comparator system would be necessary because of the mixture of blue and yellow colours.

Polyethylene bottles are desirable for samples and some reagents. Ordinary (grade B) pipettes may be used for measuring out the reagents. A 50 ml. graduated cylinder is suitable for the sea-water sample.

#### Determination

Three ml. of the mixed acid molybdate reagent are placed in a 100 ml. flask and 50 ml. of the sample added. After 10 min. 5 ml. of 50% sulphuric acid and 2 ml. of 10% oxalic acid are added. The extinction of the solution in the absorptiometer is measured using deep red filters. The length of the cuvette should suit the silicon content (see below). Five minutes after acidification 1 ml. of 0.05 N-stannous chloride is added, and the extinction is measured again after a further 20-40 min. depending upon the temperature.

The difference between these two readings must be corrected for the blank which is determined as described on p. 153.

Calibration is carried out by measuring the increase in extinction produced by a known addition of silicate to a sample of sea water of low silicon content. If such a sample is not available, 3.5% w/v sodium chloride may be used without an error of more than I or 2%.

Since the extinction/concentration relationship is linear a factor may be used to convert corrected extinctions to silicon content. For example, with a 4 cm. cuvette, an extinction of 1.00 corresponds to about 27  $\mu$ g. atoms Si/l. Using an absorptiometer which measures extinctions up to 0.5, the cuvette lengths suitable for different ranges of silicon concentrations are as follows:

## SILICATE IN SEA WATER

Concentrations $(\mu g. atoms Si/l.)$	Cuvette length (cm.)
0.1-0.2	IO
0.25-12.5	4
1.0-20	I

A worked example will illustrate the method of calculation. With a 4 cm. cuvette, the following extinctions were obtained.

#### Blank

Distilled water, normal order of addition of reagents;

	Before adding stannous chloride	0.000
	After adding stannous chloride	0.006
<b>.</b>	00 11 11 6	

Distilled water, H<sub>2</sub>SO<sub>4</sub> added before molybdate:

Before adding stannous chloride 0.000 After adding stannous chloride 0.004

The extinction due to silicon in the reagents is therefore 0.006 - 0.004 = 0.002. Sea water, H<sub>2</sub>SO<sub>4</sub> added before molybdate:

> Before adding stannous chloride 0.007 After adding stannous chloride 0.013

The quinquevalent molybdenum blank for sea water is therefore 0.013 - 0.007 = 0.006, and if to this is added 0.002 for the silicon in the reagents, the total 0.008, is the blank to be subtracted from measurements on sea water.

#### Calibration

Sea water, normal addition of reagents:

Before adding stannous chloride 0.005 After adding stannous chloride 0.048

The same sea water, to which 10.0  $\mu$ g. atoms Si/l. have been added:

Before adding stannous chloride 0.005 After adding stannous chloride 0.420

The extinction due to the addition of  $10.0 \ \mu$ g, atoms Si/l. is thus (0.420 - 0.005) - (0.048 - 0.005) = 0.372, so that an extinction of 1.00 corresponds to  $10.0 \times \frac{1.00}{0.372}$  or 26.9  $\mu$ g, atoms Si/l.

#### Sample

Sea-water sample, normal order of addition of reagents:

Before adding stannous chloride 0.006 After adding stannous chloride 0.281

The extinction for the sea water, corrected for turbidity, is thus 0.281 - 0.006 or 0.275, and from this figure 0.008 must be subtracted for the total blank, leaving 0.267. The silicon content of the sample is then  $0.267 \times 26.9$  or  $7.18 \ \mu$ g. atoms Si/l.

#### Reproducibility

Replicate analyses usually agree within  $\pm 3\%$ . Five analyses of a sample of sea water gave the following results: 11.53, 11.55, 11.61, 11.61, 11.63  $\mu$ g. atoms Si/l.

### Time needed

Analysis of ten samples, with blanks and calibration, may be done in about 2 hr. Calibration is needed only occasionally.

## Storage of samples

Samples should be collected in polyethylene or heavily waxed bottles, and kept in the dark to prevent multiplication of diatoms, which may consume silicate. They should not be kept long before analysis, since suspended siliceous matter may dissolve forming soluble silicate. A series of samples gave the following figures before and after storage for 3 months:

Date of			5 F.	Si	licate (	(µg. at	oms Si	/1.)		8	
28. viii. 50 17. xi. 50	19·0 15·6	2·45 2·47	0·94 1·06	1·12 1·27	0·79 1·01	0·56 0·80	0·47 0·83	0.47 0.81	1·91 2·19	2·12 2·30	2·34 2·66
Increase (%)	-18	I	13	13	28	43	77	72	15	9	14

Dr W. R. G. Atkins (private communication) has found a similar increase in the silicate content of samples of sea water stored in polyethylene bottles for about the same time.

## SOME RESULTS

Samples from the surface at the Laboratory Stations L2 to L6 and from various depths at International Hydrographic Station E1 have been analysed by this method since March 1950, with the results given in Table I. They show high values at the surface near Plymouth, due to land drainage, decreasing with distance off-shore, although the effect is noticeable 12 miles out. They show,

Table I. Silicate Off-shore from Plymouth ( $\mu$ g. atoms Si/L.)

Station	Miles from laboratory	Depth (m.)	24 Mar.	3 May	23 May	12 June	19 July	23 Aug.	20 Sept.	23 Oc	t. 22 Nov.
L 2	2	0	_	1.03			1.98	19.0	6.13	7.61	22.0
L3	5	0		1.06			0.87	2.45	5.03	3.60	10.5
L4	8	0	_	0.87	_		0.87	0.95	3.04	2.00	4.49
L 5	12	0	—	0.94	—		0.89	1.12	1.06	1.81	4.83
L 6	17	0	_	0.62	_		0.95	0.79	1.04	I.73	3.04
ΕI	22	0	2.63	0.48	1.12		0.69	0.26	0.69	1.82	2.76
Εı	22	5	2.63	0.39	1.38		0.28	0.47	0.62	1.75	2.71
EI	22	IO	2.38	0.54	1.32	1.21	0.89	0.42	0.01	1.28	2.69
Εı	22	25	2.63	0.73	1.74		2.18	1.01	0.67	1.77	2.67
EI	22	50	2.44	0.62	2.39	1.74	2.23	2.12	1.38	1.29	2.56
Εı	22	70	2.67	0.77	2.20		2.38	2.34	1.98	1.71	2.81

too, the depletion of silicate in the upper layers at E I in the summer months, as observed here in earlier years. (Atkins, 1923–30; Cooper, 1933). On 3 May silicate was low throughout the water column. The samples had been taken after a spell of rough bright weather, during which it is probable that vertical mixing carried silicate depletion to all depths. Phosphate was also low at all depths. The change in silicate concentration only 3 weeks later is notable.

A series of samples provided by the kindness of Mr R. A. Cox and members of the Staff of R.R.S. *Discovery II* from a position  $(47^{\circ} 24' \text{ N}, 7^{\circ} 52' \text{ W})$  in the North Atlantic Ocean just off the edge of the continental shelf has

been examined, and the results are given here (Table II) because of the scarcity of observations of silicate in deep water in northern latitudes. Atkins & Harvey (1925) report 8  $\mu$ g. atoms Si/l. at 2000 m. at 37° 44′ N. 13° 21′ W., and 20 at 3000 m. at 29° 59′ N. 15° 03′ W.

## TABLE II. SILICATE IN THE NORTH ATLANTIC OCEAN, $47^{\circ}$ 24' N., $07^{\circ}$ 52' W. ON 12 May 1950. Sounding 4030 m.

Depth (m.)	Silicate (µg. atoms Si	/1.)	Depth (m.)	(μ	Silicate g. atoms Si	1.)
0	0.78		900		10.2	
IO	I.OI		1000		10.8	
20	0.52		1100		11.7	
50	1.76		1200		12.3	
100	2.32		1500		13.3	
150	2.6		2000		19.1	
200	2.6		2500		28.6	
300	3.2		3000		35.6	
400	4.8		3500		38.7	
600 800	7.1		3900		40.8	

#### SUMMARY

A sensitive molybdenum-blue method for determining silicate based on a recent account of the chemistry of silicomolybdic acid has been applied to the analysis of sea water.

Methods of eliminating interference from phosphate and arsenate, and of reducing and evaluating the reagent blank, and some effects of temperature and timing have been studied.

A method is described which may be used with a sensitive absorptiometer for silicate concentrations in the range  $0.1-50 \ \mu g$ , atoms Si/l.

Some results obtained with the method are presented, and illustrate change in silicate concentration of sea water on storage, and silicate in the water off Plymouth in 1950, and at a position in the North Atlantic Ocean.

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# AUTECOLOGY AND THE SPECIES PROBLEM IN FUCUS

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## (Text-figs. 1-6)

On the rocky shores of the British Isles three species of *Fucus* are commonly found as components of the intertidal algal belt, namely, *F. spiralis* L., *F. vesiculosus* L. and *F. serratus* L. A fourth species, *F. ceranoides* L., occurs in places subject to the influence of fresh water but, although it may be important in connexion with the interacting processes to be described below, *F. ceranoides* is omitted from the present discussion because as yet too little is known of its autecology. Under the conditions prevailing in the characteristically zoned algal flora of an undisturbed region of a rocky coast, descriptions of the three species can be applied which fairly effectively distinguish one from the others. The chief characters used for this purpose are shown in Table I.

Other characters also help in separating the three species, as, for example, methods of branching and texture of the frond, and the numbers and positions of cryptostomata; but these characters can be greatly modified by variations in environmental conditions and are difficult to estimate objectively.

## HYBRIDS BETWEEN FUCUS SPECIES

Hybrid sporelings between pairs of these species have been produced experimentally a number of times. Thuret (1854) obtained hybrid sporelings by fertilizing eggs of *F. vesiculosus* by antherozoids of *F. serratus*, but failed to make the reciprocal cross. Kniep (1925) carried out the crossing in both directions and obtained 4% development from eggs of *F. vesiculosus* and antherozoids of *F. serratus* and 2% for the reciprocal cross. He obtained much higher percentages of sporelings by crossing *F. spiralis* L. (given as *F. platycarpus* Thur.) with *F. vesiculosus*, 70 and 100% respectively using eggs of *F. spiralis* and *F. vesiculosus* with antherozoids of the opposite species. In February 1949 the present authors obtained 91% segmentation in eggs of *F. serratus* fertilized with antherozoids of *F. vesiculosus* and 99% segmentation for the reciprocal cross. No examples are on record of hybrid plants being grown

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in culture but this may be due to the difficulty of culturing sporelings of *Fucus* species under laboratory conditions.

The present authors have had some success with trial cultures of *Fucus* sporelings on sterile bricks cemented to the shore. Eggs were released on to the bricks and fertilized by releasing antherozoids over them. The eggs were planted in straight lines for ease of recognition, and the sporelings grown in the laboratory in running sea water for 1 or 2 months, by which time they could easily be seen with the naked eye. The bricks were then cemented to chosen positions on the shore and no difficulty was found in following the growth of the sporelings. On the experimental bricks it was several months before any further sporelings appeared. One difficulty with the method at present is that plantings must be carried out on a large scale to allow for loss of bricks in heavy seas.

 TABLE I. CHARACTERS USED IN THE SEPARATION OF FUCUS SPIRALIS,

 FUCUS VESICULOSUS AND FUCUS SERRATUS

Character	F. spiralis	F. vesiculosus	F. serratus
Frond: Shape Vesicles Margin	Spirally twisted None Even	Flat Present Even	Flat None Serrated
Receptacles:			
Shape	Rounded	Ellipsoidal-elongate	Extended growth
Sterile rim Inflation Sex	No dichotomies Present Inflated Hermaphrodite	I–2 dichotomies No definite rim Inflated Dioecious	Several dichotomies No definite rim Not inflated Dioecious

Since hybrid sporelings can be produced quite readily in culture, it might be assumed that they are equally readily formed in the sea, yet very few instances have been recorded of naturally occurring hybrids. Sauvageau (1909), Stomps (1911) and Kniep (1925) recorded plants with characters intermediate between F. spiralis and F. vesiculosus and assumed that these plants were hybrids. Sauvageau (1909) also described a series of plants intermediate between F. vesiculosus and F. serratus. The appearance of these plants varied with the position on the shore, approaching F. vesiculosus towards the upper limit and F. serratus towards the lower limit of distribution. Fritsch (1945, pp. 379-80) remarks, however, 'It still remains doubtful whether these and other transitional types, found at the limits of the zones occupied by the various species of Fucus, are actually hybrids or habitat forms'.

Several authors have remarked on this absence of satisfactory hybrid records (Sauvageau, 1909; Kniep, 1925; Fritsch, 1945), and the reason for their relative absence under natural conditions has been attributed to one or other of two causes. (i) Hybrids may really be absent and the suggestion has been put forward that the hybrid sporelings, although formed, find the habitat

unsuitable or are unable to compete with the parent species (Kniep, 1925). (ii) Hybrids may really be more common on the shore than is realized, but the extreme variation within the individual species makes it difficult to recognize them with certainty (Sauvageau, 1909).

Thus the three species appear to be fairly distinct from one another, and in addition to the morphological diagnostic features given, others have been used to delimit them as, for example, specific habitat requirements—position on the shore in relation to tide-levels and all that this involves in terms of exposure to desiccation, changing light intensities, temperature and other factors. *F. spiralis* is normally found in the highest position on the shore where it may be left exposed for several days at a time during neap tides; *F. vesiculosus* occupies the mid-tide zone for which it competes with *Ascophyllum nodosum*, and where it is alternately exposed and submerged for each tidal period; *Fucus serratus* occupies the lowest level, and, although part of the zone may be uncovered during the tidal period, other parts may be more or less continually covered.

Recent ecological work has shown, however, that the range of requirements for each of these species is not as limited as was previously supposed. As a result of clearance experiments carried out at different levels on the shore in the south part of the Isle of Man it was shown that removal of one species may allow extension of the ranges of others. In this connexion Ascophyllum is important because it dominates the mid-tide region and influences the ranges of all three Fucus species. An area cleared in the upper part of the Ascophyllum zone, in August 1945 at the time when Fucus spiralis was fruiting, became covered by sporelings of the latter species and these persisted and fruited the following year (Burrows, 1947). Further, truncation in December 1943 of Ascophyllum plants to a length of 10 cm. over an area of shore allowed development of sporelings of both Fucus vesiculosus and F. serratus which were present on the rock surface, but which had been prevented from developing by the presence of the Ascophyllum. The effect of such truncation on the fucoid component of the area is seen in an analysis of the populations of sample  $\frac{1}{2}$  m. quadrats taken towards the end of 1945, one with its lower edge I m. vertically below M.S.L. and the other with its lower edge on M.S.L. In each the adjacent  $\frac{1}{2}$  m. of uncut Ascophyllum area was analysed in order to obtain an estimate of the original composition of the population. Figs. I and 2 show percentage frequency distributions for lengths of plants of Fucus vesiculosus and F. serratus in the sample quadrats. Both species were initially present mainly as sporelings beneath the Ascophyllum; at the upper level-zone Isporelings of Fucus vesiculosus were more plentiful and at the lower levelzone II-those of F. serratus. In both zones the effect of the removal of the long shading fronds of Ascophyllum was to allow continued development of plants of Fucus servatus and F. vesiculosus and to allow also increase in their numbers.

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Fig. I. Growth of *Fucus vesiculosus* 20 months after cutting *Ascophyllum* frondage. A, uncut control; B, *Ascophyllum* cut to 10 cm. Percentage frequency (vertical) of various lengths of *Fucus vesiculosus* fronds (horizontal). The numbers inset give the total number of plants in the sample.



Fig. 2. Growth of Fucus serratus 20 months after cutting Ascophyllum frondage, as in Fig. 1.

## HYBRIDS ON A RECOLONIZED STRIP

The above facts emphasize the importance of competition in limiting the range of a species. Of extreme interest in this connexion were the results of an experiment carried out on the shore at Port St Mary, Isle of Man (Jones, 1948). A strip, 10 m. wide, extending from just below M.H.W.N.T. to just above M.L.W.S.T., was cleared of limpets and larger algal growth. The experiment was

designed to determine the influence on the growth of algae of the large numbers of limpets occurring on the limestone ledges which here bear only an extremely scattered algal population. The area cleared was sufficiently large to eliminate any possible influence of surrounding plants of the parent species. The subsequent development of a dense band of vegetation on the cleared strip has been described by Jones (1948), and the first stages in the succession of algal growth by Lodge (1948). An analysis of the vegetation on the strip after 3 years' growth has been carried out by the present authors. The resultant flora proved to be very different from that expected from previous conceptions of the fucoid zonation of a rocky coast. On the whole, F. serratus proved to be more plentiful on the lower part of the strip and F. vesiculosus on the upper part, but both occurred at all levels. F. spiralis formed no distinct zone but was present in small quantities at all levels down to low-water mark. In this position it was very luxuriant, the fruiting plants having a bushy appearance and a length up to 2 ft. However, the large majority of Fucus plants could not be assigned to any one species.

If, as was suggested by Kniep, it is the factor of competition with the parent species which eliminates hybrid sporelings from the shore, then in the absence of competition on a recently cleared area, the occurrence of hybrids might reasonably be expected. An analysis of sample square metres taken from various levels on the strip showed the presence of large numbers of plants which were intermediate in their characters between F. spiralis and F. vesiculosus (Fig. 3b) and between F. vesiculosus and F. serratus (Fig. 3a). For the hybrid analysis Anderson's method (1936), devised for the analysis of hybrid populations of Tradescantia, was adopted. The method is briefly as follows-given a number of readily ascertainable characters differing between two species A and B, for each of these characters an individual plant is given a score, a, b or idepending on whether, in this respect, it follows one or other species or is intermediate between the two. This qualitative scoring can be made quantitative by numerically weighting each of the letters and when the total value for each plant has been computed the indices thus found can be summarized as a frequency distribution. The importance of certain specific characters can be emphasized by appropriate weighting.

## CHARACTERS USED IN THE ANALYSIS OF HYBRIDS BETWEEN FUCUS SPIRALIS AND FUCUS VESICULOSUS

Five characters were considered as readily ascertainable and were used in the separation of F. *spiralis* and F. *vesiculosus*: (i) Vesiculation of the thallus. (ii) Spiral twisting of the thallus. (iii) Receptacle shape. (iv) Presence or absence of a sterile rim to the receptacle. (v) Hermaphrodite or dioecious condition of the receptacle.

Of these characters, nos. (i) and (v) were considered as of more importance than the others and were more heavily weighted accordingly.



Fig. 3. a, Fucus vesiculosus  $\times F$ . serratus hybrid, from the experimental strip, Port St Mary, Isle of Man. b, F. spiralis  $\times F$ . vesiculosus hybrid, with vesicles and rimmed receptacles, from Isle of Man. c, F. vesiculosus var. evesiculosus Cotton, from the outer side of the breakwater, Port Erin, Isle of Man. d, F. vesiculosus var. vadorum Aresch., from Derby Haven, Isle of Man.

## SPECIES PROBLEM IN FUCUS

Before characters can be used in a hybrid analysis it is necessary to examine the degree of variability in respect of each character shown by the parent species, because on this depends its reliability. Both species show extensive variation in relation to a number of characters and, for *F. vesiculosus* especially, a large number of varieties have been described.

## Vesiculation of the Thallus as a Diagnostic Character

Of the two characters, nos. (i) and (v) considered as of greatest importance in separating F. spiralis and F. vesiculosus, that of the hermaphrodite or dioecious condition is probably the most reliable. It may, as Stomps suggests, be the only one. There is little, if any, evidence that it is a character affected by changes in environmental conditions. Vesiculation of the thallus would appear, however, to be profoundly affected by such changes, and must therefore be considered in some detail. It appears certain from an examination of plants of F. vesiculosus found growing under varying degrees of exposure to severe wave action on the south coast of the Isle of Man, that the formation of vesicles is directly correlated with the roughness of the water washing them during the intertidal period. On very exposed areas, such as the outer side of the Breakwater at Port Erin, and at the extreme end of the Langness Peninsula, vesicles are almost entirely lacking. Fig. 3c illustrates a typical plant from the former situation, which is probably to be identified as F. vesiculosus var. evesiculosus Cotton. With increased shelter from the direct breaking force of heavy waves (and quite a small boulder effectively placed will suffice to provide it), vesicles appear and increase in numbers, becoming well developed in quiet waters. The extreme form is that distinguished as var. vadorum Aresch. which has rows of closely placed pairs of vesicles and which only occurs in the quietest inlets (Fig. 3d). The variation in form and behaviour of F. vesiculosus under varying degrees of exposure will be discussed in a separate paper: here it suffices to record merely the distribution of vesiculate and evesiculate plants in relation to degrees of exposure. Similar distributions have been described by Kylin (1907) for the west coast of Sweden, by Cotton (1912, pp. 23-6) for Clare Island, by Richard (1925) for an area on the coast of Belgium, and by Knight & Parke (1950) for the Devon, Manx and Argyll coasts. It is difficult to understand the distribution described for the Argyll coast by Moss (1948), who maintains that with increase in exposure the numbers and sizes of vesicles for F. vesiculosus increase. Moss, however, defines the exposure of the coast in terms only of proximity to the open ocean, ignoring the many other factors which are important for algal populations, as, for example, the steepness of the rock surface, the direction of the slope and the presence or absence of outlying rocks which may take the first force of open sea breakers. It is not yet known whether sporelings from evesiculate plants will produce plants forming vesicles if grown under quieter conditions, but experiments are in progress to elucidate this point. However, on a gently sloping shore such as

that of the experimental area at Port St Mary, it may be assumed that over relatively small areas environmental conditions will not vary appreciably. Since a proportion of the plants on the strip are evesiculate (Fig. 4a), and grow side by side with vesiculate plants, it may be justifiable to seek a different cause for this variation.

Two possible causes suggest themselves. Either (i) there is an evesiculate variety of F. vesiculosus which is independent of environmental conditions, or (ii) the plants are the result of crossing with an evesiculate species. However, as will be shown later, this problem probably resolves itself.

#### Spiral Twisting of the Thallus

This is another character which might depend on environmental conditions. Salt marsh forms of both F. spiralis and F. vesiculosus are frequently spirally twisted, and it has been suggested by Baker & Bohling (1916) that this is due to a more rapid growth on the lower side kept moist by continued contact with wet mud, compared with the upper side which may be exposed to drying for many days at a time. F. spiralis might tend to become twisted because it normally grows at the highest level on the shore and at neap tides may be exposed for long periods. However, similar considerations apply to this character as to that of vesiculation of the thallus and the presence of both spirally and non-spirally twisted thalli in precisely the same locality suggests a different underlying cause.

#### Receptacle Shape

Receptacle shape may show considerable variation within a single species and especially is this so for F. vesiculosus. The appearance of a receptacle is determined by a number of factors of which the most important is probably the length in relation to the breadth. The breadth of a receptacle is partly determined by the breadth of the vegetative frond, and this may be influenced by environmental conditions. With narrowing of the frond, such as occurs with increase in exposure of the plant to severe wave action, the receptacle also becomes narrow until ultimately, on very exposed shores, it becomes completely cylindrical on inflation. Dichotomy of the receptacle is uncommon in F. spiralis, whereas in F. vesiculosus it may occur once or twice in each receptacle. The degree of inflation of the receptacle may also affect its appearance and this is accentuated in the presence of water of reduced salinity. Fucoids growing at the mouths of rivers or lying in trickles of fresh water running over the shore, have very large swollen receptacles. Thus for F. vesiculosus the ellipsoidal or ovate form usually found on sheltered coasts may become more or less cylindrical with increase in exposure, or enlarged by intake of fresh water under conditions of reduced salinity. It is, nevertheless, readily distinguishable from the rounded form of F. spiralis with its very conspicuous conceptacles. A population of F. vesiculosus was, however, found in the Isle of



Fig. 4. *a*, an evesiculate plant from the experimental strip, Port St Mary, Isle of Man: *Fucus spiralis* × *F. vesiculosus* hybrid (?). *b*, plant of *Fucus* with vesicles and a serrated edge from a Lundy Island population.

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Man in one locality only, which had uniformly rounded receptacles, the plants corresponding to the description of F. vesiculosus L. var. sphaerocarpus J. G. Agardh. This population was found in a wide shallow depression on the upper part of the shore at Poyll Vaaish several miles distant from the experimental area and it seems unlikely that it would play any appreciable part in the establishment of the strip population. In using receptacle shape as a criterion in separating F. spiralis and F. vesiculosus there is to be considered the possibility of hybridization with Ascophyllum nodosum which also has somewhat rounded receptacles. Especially is this so in view of the fact that Williams (1899) obtained 50% development of hybrid sporelings using eggs of Fucus vesiculosus with antherozoids of Ascophyllum, and a plant was recorded as occurring in the Menai Straits with external characters intermediate between Ascophyllum and Fucus vesiculosus. Although this possibility was considered, it was not taken into account in the present discussion because there were no real indications that Ascophyllum had played any part in the formation of the strip vegetation. Even though conditions on the strip would now appear to be suitable for the settlement of Ascophyllum sporelings, none have yet appeared.

## Results of Analysis of Hybrids Between Fucus vesiculosus and Fucus spiralis

Fig. 5 shows the results of the hybrid analysis between F. vesiculosus and F. spiralis for four samples from the strip vegetation taken from different tide levels. The uppermost sample was taken from M.H.W.N.T. and the lowest from a little below M.S.L. The diagrams show percentage frequency distributions for plants with index numbers ranging from 0 (pure F. spiralis) to 14 (pure F. vesiculosus) The fifth histogram summarizes the distributions for the whole population. The pattern of the distribution is similar for the three upper samples: about 20% of F. spiralis, somewhat less than this of F. vesiculosus, and a range of intermediate forms between. In the lowest sample there is a marked decrease in the proportion of F. spiralis and an increase in that of F. vesiculosus. The large percentage of plants with an index number of 10 is due largely, but not entirely, to the presence of plants similar to F. vesiculosus in all respects except that they lacked vesicles. It is possible that these represent a genetically distinct variety but only cultural experiments could decide this point.

The gametes from the intermediate forms were fully formed and active.

## Forms Intermediate between Fucus vesiculosus and Fucus serratus

One great difficulty preventing the completion of hybrid diagrams for F. *vesiculosus* and F. *serratus* is the fact that although the fruiting periods of the two species overlap to some extent, they are not sufficiently coincident; only







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when all the plants are in the fruiting condition can the necessary distinctions be made. Plants intermediate between F. vesiculosus and F. serratus were found on the strip in the fruiting condition when F. vesiculosus was in fruit. This indicates either that the reproductive period of the hybrids extends over that of both parents, or that the period for some hybrids is coincident with that of F. vesiculosus while for others it may be coincident with that of F. serratus. Hybrids between F. vesiculosus and F. serratus were not as common as those between F. vesiculosus and F. spiralis, the fruiting periods of which overlap much more completely than those of the former pair of species.

## THE OCCURRENCE OF HYBRIDS UNDER NATURAL CONDITIONS

The strip at Port St Mary was a highly artificial area; an experiment of similar extent is unlikely to occur in nature and, as Anderson (1936) remarks, 'Hybridization is much more frequent in areas greatly disturbed by man than under natural conditions'. The question therefore arises as to whether, and if so how frequently, hybrids occur naturally. There is no doubt that hybrids among the three species of Fucus occur quite frequently on the open shore; many more have been found by the present authors since their existence has been recognized than had been previously noticed. Kniep (1925) suggested that the most favourable position for hybrids would be in the regions between the more sharply defined zones of the parent species, and it is in such regions that the majority of natural hybrids have been found on the Manx coasts. For example, plants with characters intermediate between those of F. vesiculosus and F. serratus occur frequently on the gently sloping rocks on the lower part of the Poyll Vaaish shore where the population is sparse and in a position in which the F. vesiculosus and F. serratus zones overlap. It is likely that the evesiculate plants mixed with F. spiralis in the upper part of the shore that are described by Knight & Parke (1950) as F. vesiculosus var. evesiculosus Cotton and evesiculate by virtue of their high position on the shore, are hybrids between F. vesiculosus and F. spiralis. Present observations do not support the suggestion that there is a relation between vesiculation and vertical distribution on the shore.

## A HYBRID POPULATION ON THE RIVER MERSEY SHORE

A second 'hybrid swarm' population has been found occurring naturally on the south bank of the River Mersey about 4 miles from Liverpool. Probably its presence in this region is due ultimately to the nature of the rock surface which is of very soft sandstone and can support only a limited size and weight of plant. Once the plants exceed a certain weight, the rock surface flakes away leaving a bare area open to recolonization. Thus the community is an open one and competition not as intense as it is on a firm rock surface. In addition, the fruiting period of *Fucus spiralis* is longer here and considerably overlaps

that of F. vesiculosus. There is no period of the year at which plants cannot be found in the full fruiting condition, though possibly the extended fruiting period is more characteristic of the hybrids than of the parent species. Fig. 6 shows hybrid analyses for F. spiralis and F. vesiculosus from square metres of algal vegetation, for four positions on the shore ranging from the region of high water down to about M.S.L. The fucoid vegetation exists as a continuous cover only in the upper part of the shore. Lower down the population is scattered and consists only of short stunted plants. The water covering the plants at high tide is so muddy that the bulk of assimilation must be carried out at low tide when the plants are exposed and the degree of exposure at M.S.L. probably represents the minimum exposure to light required for effective assimilation (Burrows, 1947). The Mersey community is not a new one and the plants show a distinct zonation. F. spiralis is limited to the top of the shore as are also the hybrids approaching F. spiralis. Lower down only the hybrids approaching F. vesiculosus persist. An interesting feature of the population is that the hybrids are much more common than the parent species. It would appear that a hybrid approaching F. vesiculosus and not the parent species is best adapted to the conditions prevailing here. There were also other features of interest. All the plants which were evesiculate and dioecious were male. Many of the apparent hybrid plants had no vegetative apices: all had formed receptacles and so set a limit to the life of the plant. Probably on the fall of the receptacles new frondage would arise by proliferation, this condition being characteristic of many of the plants in this locality.

Stomps (1911) found at Nieuport on the coast of Belgium, where the Chenal de L'Yser runs into the sea, a series of forms of F. vesiculosus and F. spiralis connecting the two species apparently precisely like the series on the River Mersey. He considered the question of the origin of these forms and ruled out hybridization on the basis of their distribution in his area. He remarked that our knowledge is still so limited that we cannot disregard a priori the possibility that the irregularities found on the shore may be due to hybridization, but he could not understand, if these aberrant individuals could so be explained, why they were more numerous in the canal region than on the sea coast.

Possibly here, as also on the River Mersey, their presence may be due to the open nature of the community. The evidence accumulated in this present investigation supports the view that such forms occur on the open coast under conditions of less acute competition from fully grown plants of the parent species, and supports Kniep's suggestion that it is this competition which prevents the establishment of hybrid plants in a fully developed open-shore zonation.

Stomps concluded from his observations that there is only one species of *Fucus* in place of the three, *spiralis*, *vesiculosus* and *ceranoides* (which he included), these being apparently habitat forms of a single species, environmental conditions deciding which hereditary characters shall be manifested in any one locality. Since over a large part of the rocky coast of Britain the characteristic fucoid vegetation is developed and in it the three taxonomic species remain distinct, there can be no advantage in disregarding this conception of them, i.e. as taxonomic species. Since up to the present it has not been possible to carry out cultural experiments on fucoids, mainly because of the difficulties of imitating intertidal conditions, the determination of the exact status of the three species must be assessed on somewhat circumstantial evidence. The results of the ecological experiment described have shown that there is no inherent sterility barrier among the three taxonomic species. This may also be true for other species of Fucus as, for example, F. ceranoides L., since hybrids between this species and F. spiralis have been reported (Sauvageau, 1908; Gard, 1910). Nothing has been found to run counter to the suggestion of Kniep (1925) that competition with the parent species prevents the development of hybrid plants in nature. Thus the barrier to complete merging of the species into one polymorphic species appears to be an ecological one, and it would appear that they are becoming isolated within specific tidal limits under the conditions prevailing in well-established open-shore vegetation. On the shore great changes in ecological conditions occur over relatively limited areas. A difference of I ft. in tide-level in the intertidal region of the shore may be equivalent to a large change in environmental conditions in terms of exposure to a drying atmosphere, changing light intensities, temperatures and the influence of water of variable salinity. It is likely that continued isolation in different parts of the intertidal region will ultimately lead to the development of other barriers, e.g. those of intersterility.

Using the ecotype terminology it would be difficult to know whether to regard these taxonomic species as ecotypes or ecospecies, since hybrid swarm populations may occur between them in nature, although, as far as we know, only rarely. The condition appears to be somewhat similar to that found for species of *Silene* among flowering plants from work carried out by Turrill and Marsden-Jones and reported by Turrill (1938). In north-west Europe ecological barriers alone keep them as distinct taxonomic units, and occasional hybrid swarm populations are found in nature.

Variation within the *Fucus* species may also occur along ecological gradients, one of which has been described, but many more probably exist, e.g. gradients of tidal depths, and reduction of the salinity of the water. Other types of variation also occur within the species which it is more difficult to connect with changes in environmental conditions. Whole populations may possibly be of hybrid origin. There has recently been found at Lough Ine in the West of Ireland a population showing the texture and serration of *F. serratus*, the spiral twisting and rounded and rimmed receptacles of *F. spiralis* and with somewhat irregular vesicular inflations. Similarly, a population exists on Lundy Island where plants possessing the vesicles of *F. vesiculosus* have

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serrated fronds characteristic of F. serratus (Fig. 4b). Species problems of this kind will probably arise for many different algae when more is known of their autecology and cytogenetical relationships.

#### ACKNOWLEDGEMENTS

We are indebted to Prof. J. H. Orton, F.R.S., and Mr N. S. Jones, who initiated the cleared-strip experiment, for permission to sample the resulting population. We also wish to thank Dr Margery Knight and Dr Mary Parke for reading the manuscript, and Mr R. B. Burrows whose co-operation made the work possible. Dr Parke kindly sent the material from Lundy Island.

### SUMMARY

Characters used to distinguish *Fucus spiralis* L., *F. vesiculosus* L. and *F. serratus* L. are listed. Cross-fertilization between pairs of these species has been observed—independently by several workers.

Ecological habitats of the three species are described and the influence of competition on zonation is indicated. A *Fucus* population occurred on an area cleared of limpets at Port St Mary, Isle of Man, in which the majority of plants could not definitely be assigned to any one *Fucus* species.

Anderson's method of hybrid analysis was applied to samples from various levels on the area. The characters used in the analysis are listed and their status briefly discussed.

A direct relationship is recorded between vesiculation of the thallus of F. vesiculosus and the degree of exposure of the habitat to severe wave action. The extreme form for very sheltered localities is F. vesiculosus var. vadorum Aresch. and for exposed situations, F. vesiculosus var. evesiculosus Cotton. Great range in vesiculation in adjacent plants on the experimental strip is therefore due to hybridity or to a varietal form of F. vesiculosus.

Spiral torsion of the thallus in relation to environmental conditions is discussed.

Variation in receptacle shape in *F. vesiculosus* and *F. spiralis* is considerable; nevertheless, the use of this character in the hybrid analysis is justified.

Hybrid analysis between *F. vesiculosus* and *F. serratus* is difficult to carry out owing to the non-coincidence of fruiting periods of the parent plants.

The occurrence of hybrids in undisturbed populations in the Isle of Man is recorded.

A 'hybrid swarm' population by the River Mersey has been analysed and its significance discussed. An attempt has been made to assess the present status of the three species of *Fucus*.

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# LITTORAL DIATOMS FROM THE SALSTONE, NEAR PLYMOUTH

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## (Text-figs. 1, 2)

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#### INTRODUCTION

The diatom flora inhabiting the muddy shores and bottoms off Plymouth has received little attention in comparison with the fauna associated with such muds. Since a large number of the microfauna depend, to a great extent, upon the diatoms as a source of food (Mare, 1942), a knowledge of the latter is therefore desirable.

The present paper is mainly concerned with the diatoms inhabiting the mud-flats at the Salstone, a small island lying in the Salcombe estuary about one and a half miles above Salcombe. The habitat is of particular interest since it is sheltered from wave action, well defined, and hardly disturbed by man. Marine conditions prevail, the small fresh-water streams seeming to exert no appreciable influence on the salinity. Very few species of diatoms belonging to fresh water have been met with during the analysis of the present samples, and since these were in negligible quantities, they are excluded from the list given below.

The tidal range at the Salstone is of the order of 15 ft., but the islet itself lies well below M.H.W.S.; its uppermost parts being covered with *Ascophyllum nodosum*. The fucoids growing on this islet, as well as on the rocky shores facing it, display the usual zonation; a more detailed account of these will be dealt with separately by one of us (A.A.A.).

The diatoms considered below have been collected by the latter author during the months of February and March 1949, mainly from the mud-flats surrounding the islet, where a *Vaucheria* community covers large areas of the flats and extends to a level below M.L.W.S. The *Vaucheria* community harbours a large number of diatoms, some of which were found in abundance at the

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time of collection. The analysis of the samples has been carried out by us together at Plön.

Among the immense number of papers about diatoms, there are only very few specially dealing with the marine littoral vegetation. The most exhaustive one is written by Hustedt (1939) about the German coast of the North Sea. On British shores, Ghazzawi (1933) made a short study of the littoral diatoms of the Liverpool and Port Erin shores. He enumerates ninetyfour species and varieties, but ten of these species are fresh-water diatoms which do not really belong to the marine flora and therefore are not mentioned in our list. Of the remaining eighty-four species, forty-three were not found in our samples, or at least not recognized with certainty, though we are convinced that further studies in the Plymouth region will considerably increase the number of species upon our mud-flats.





## SYSTEMATIC ACCOUNT OF THE SPECIES

For the identification of the species the diatom material was boiled in acid in the usual way to get rid of the organic content of the cells, and subsequently thoroughly washed, the clean frustules being mounted in hyrax. The taxonomic arrangement followed is that of Hustedt (1930 a).

#### Genus Melosira Ag.

Melosira moniliformis (Müll.) Ag. (Hustedt, 1930b, p. 231, fig. 95). Rather rare. The density of this species in certain localities on the southern coast of

England shows considerable variation between the seasons as well as between different years (Aleem, 1950b).

*M. Westii* W.Sm. (Hustedt, 1930*b*, p. 268, fig. 113). Rather frequent at M.T.L. and M.L.W.S. Occurs separately or in colonies of a few individuals. Longer chains were not met with.

*M. sulcata* (Ehr.) Kütz. (Hustedt, 1930*b*, p. 276, fig. 119). Much more frequent than the other two species. It is a cosmopolitan diatom of general distribution, also known among the plankton as well as among littoral marine sediments, both recent and fossil.

## Genus Podosira Ehr.

*Podosira Montagnei* Kütz. (Hustedt, 1930*b*, p. 281, fig. 122). Rare: a few valves were met with in samples from the *Vaucheria* community. This species is more common on rocky shores.

*P. stelliger* (Bail.) Mann (Hustedt, 1930*b*, p. 286, fig. 128). This form grows attached to algae on rocky shores. Its presence on the mud-flats is probably due to sedimentation from the plankton.

#### Genus Hyalodiscus Ehr.

*Hyalodiscus scoticus* (Kütz.) Grun. (Hustedt, 1930*b*, p. 293, fig. 133). Rare, probably also sedimented from plankton.

## Genus Sceletonema Grev.

Sceletonema costatum (Grev.) Cleve (Hustedt, 1930b, p. 311, fig. 149). Rare, sedimented from plankton.

## Genus Thalassiosira Cleve

*Thalassiosira decipiens* (Grun.) Joerg. (Hustedt, 1930*b*, p. 322, fig. 158). Rather rare; valves both of resting spores (thick-walled with coarse structure) and of vegetative cells (thinner walls) were found. Sedimented from plankton, more frequent in offshore waters near Plymouth in February–March.

### Genus Coscinodiscus Ehr.

The species of *Coscinodiscus* found on the mud-flats at the Salstone are all planktonic.

*C. excentricus* Ehr. (Hustedt, 1930*b*, p. 400, fig. 211). Frequent. This species is widely distributed in all mud sediments on the north Atlantic coasts, especially in Germany and the Norwegian fjords.

C. radiatus Ehr. (Hustedt, 1930b, p. 420, fig. 225). Rare.

C. centralis Ehr. (Hustedt, 1930b, p. 444, fig. 243). Only fragments were found.

## Genus Roperia Grun.

Roperia tessellata (Roper) Grun. (Hustedt, 1930b, p. 523, fig. 297). Rare, deposited from plankton.

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## Genus Actinoptychus Ehr.

Actinoptychus undulatus (Bail.) Ralfs (Hustedt, 1930b, p. 475, fig. 264). Rather frequent. Only specimens of small size have, however, been encountered. The species is pelagic, though frequently met with in marine sediments.

#### Genus Eucampia Ehr.

*Eucampia zoodiacus* Ehr. (Hustedt, 1930*b*, p. 772, fig. 451). Only a few valves found. Planktonic.

## Genus Triceratium Ehr.

*Triceratium alternans* Bail. (Hustedt, 1930*b*, p. 825, fig. 488). Rare, also found in colonies attached to the algae on rocky shores as well as being pelagic.

#### Genus Biddulphia Gray

Biddulphia aurita (Lyngb.) Bréb. & God. (Hustedt, 1930b, p. 846, fig. 501). Rather rare at the Salstone. It was much more frequent on the mud-flats at Whitstable, Kent (Aleem, 1950a). This species, which also occurs in a pelagic form, was produced in enormous quantities in the littoral region at Swanage, Dorset, during the spring of 1947 following a cold and rather long winter, while at the same period in the following year it was much less abundant (Aleem, 1950b). Hustedt (1939) found this species in almost all samples collected from the mud-flats on the northern coasts of Germany. The species seems not to be endemic in the mud-flats but rather deposited there.

## Genus Rhabdonema Kütz.

*Rhabdonema minutum* Kütz. (Hustedt, 1931, p. 18, fig. 548). Abundant in the *Vaucheria* community, less frequent on bare mud.

*Rh. arcuatum* (Lyngb.? Ag.) Kütz. (Hustedt, 1931, p. 20, fig. 549). Rather rare in the *Vaucheria* community.

#### Genus Striatella Ag.

Striatella unipunctata (Lyngb.) Ag. (Hustedt, 1931, p. 32, fig. 560). Rather rare in the Vaucheria community.

#### Genus Grammatophora Ehr.

Grammatophora marina (Lyngb.) Kütz. (Hustedt, 1931, p. 43, fig. 569). Not infrequent among the Vaucheria community, rather rare on bare mud.

G. serpentina (Ralfs) Ehr. (Hustedt, 1931, p. 49, fig. 577). Very rare, found only among the Vaucheria community.

#### Genus Licmophora Ag.

The species belonging to this genus were more frequent in the *Vaucheria* community than on bare mud. The intercalary bands of the cells can be taken

as a good character to distinguish the species. The septa in these bands are very short in *Licmophora flabellata* and *L. Juergensi*, a little more distinct in *L. gracilis* and *L. hyalina*, while they are very deep in *L. Ehrenbergi* and *L. paradoxa*.

L. flabellata (Carm.) Ag. (Hustedt, 1931, p. 58, fig. 581). Abundant, easily distinguished from the others by its four plate-like chromatophores.

L. gracilis (Ehr.) Grun. var. anglica (Kütz.) Peragallo (Hustedt, 1931, p. 60, fig. 583). Rather rare.

L. Juergensi Ag. (Hustedt, 1931, p. 63, fig. 586). Rather common. Longitudinal and transverse striae of the valve are distinct.

L. Ehrenbergi (Kütz.) Grun. (Hustedt, 1931, p. 70, fig. 593). Rare, distinguished by its large size and coarse striae which are slightly radiate at the apex of the valve.

L. paradoxa (Lyngb.) Ag. (Hustedt, 1931, p. 76, fig. 605). Very common, especially in the Vaucheria community.

L. hyalina (Kütz.) Grun. (Hustedt, 1931, p. 81, fig. 611). Rather frequent. The shape of the valve resembles that of a tennis-racket with a short handle. The striae are dense (about 30 in  $10 \mu$ ), they are to be distinguished only on the lower part of the valve, while the upper part appears hyaline.

#### Genus Dimerogramma Ralfs

Dimerogramma minor (Greg.) Ralfs (Hustedt, 1931, p. 118, fig. 640). Not uncommon.

## Genus Opephora Petit

Opephora marina (Greg.) Petit (Hustedt, 1931, p. 136, fig. 656). Not infrequent in the Vaucheria community.

### Genus Rhaphoneis Ehr.

*Rhaphoneis surirella* (Ehr.) Grun. (Hustedt, 1931, p. 173, fig. 679*a-c*). Rather common. Widely distributed in all mud-flats.

*R. amphiceros* Ehr. (Hustedt, 1931, p. 174, fig. 680). Frequent, the valves in this species differ considerably both in size and shape.

### Genus Synedra Ehr.

Synedra Gailloni (Bory) Ehr. (Hustedt, 1931, p. 195, fig. 690). Rather frequent.

S. tabulata (Ag.) Kütz. (Hustedt, 1931, p. 218, fig. 710a-d). Common.

var. *fasciculata* (Kütz.) Grun. (Hustedt, 1931, fig. 710*i*–*l*). Frequent. The cells in this variety tend to grow attached together in colonies like those of *Fragilaria*.

var. *acuminata* Grun. (Hustedt, 1931, p. 219, fig. 710e, f). Not infrequent, distinguished by its long, protracted, rostrate ends.

Synedra crystallina (Ag.) Kütz. (Hustedt, 1931, p. 232, fig. 719). Rather rare.

S. investiens W.Sm. (Hustedt, 1931, p. 209, fig. 699). Rare.

### Genus Thalassionema Grun.

Thalassionema nitzschioides Grun. (Hustedt, 1931, p. 244, fig. 725). Rare, sedimented from the plankton.

## Genus Thalassiothrix Cleve & Grun.

*Thalassiothrix longissima* Cleve & Grun. (Hustedt, 1931, p. 247, fig. 726). Very rare. Deposited from plankton. The species is easily distinguished by its very long and narrow valves with spiny edges.

### Genus Campyloneis Grun.

*Campyloneis Grevillei* (W.Sm.) Grun. (Hustedt, 1931, p. 321, fig. 781). Rare, more frequent on rocky shores, where it grows attached to algae such as *Polysiphonia* and *Cladophora*.

#### Genus Cocconeis Ehr.

Cocconeis scutellum Ehr. (Hustedt, 1931, p. 337, fig. 790). Very common, especially in the Vaucheria community.

var. *stauroneiformis* W.Sm. (Hustedt, 1931, p. 339, fig. 792). Frequent. The central area of the raphe-valve is enlarged into a narrow band reaching the margin.

C. dirupta Greg. (Hustedt, 1931, p. 354, fig. 809). Not infrequent in the Vaucheria community.

C. pelta A. Schmidt (Hustedt, 1931, p. 361, fig. 815). Rare. This species is similar to another species, namely C. peltoides Hustedt (1939, p. 606, figs. 23-27), which is more frequent in the mud-flats of the German coast. The latter, however, can be easily distinguished by the absence of a clear median area which is very distinct and punctate in C. pelta A.S.

C. guttata **sp.nov.** Valva elliptica,  $15\mu$  longa,  $9\mu$  lata. Raphovalva raphi directa, area axiali angusta, area centrali parva, striis radiantibus, circiter 22 in  $10\mu$ . Areovalva area longitudinali angusta, granulata, granulis in lineis radialibus dispositis, circiter 9 in  $10\mu$  (Fig. 1E, F).

Valves elliptical, length about  $15\mu$ , breadth about  $9\mu$ . Raphe-valve with straight raphe, axial-area narrow, central-area rounded, very small, striae dense, about 22 in  $10\mu$ , distinctly radiate, indistinctly punctate. Rapheless valve with rather narrow pseudoraphe, somewhat irregular, coarsely dotted; dots in slightly radiate rows, 8–9 in  $10\mu$ .

C. guttata resembles C. distans Greg. in shape and structure of the valves. The striae in the former are much denser, however, being almost twice as dense. Besides, in C. distans the puncta are included in transapical costae
which are slightly inflated round each punctum (Hustedt, 1931, p. 344, fig. 797. Peragallo, 1897–1908, pl. 3, figs. 17, 18). Such costae do not exist in our species where the large dots are distinctly separated from each other. Nevertheless, further investigations may be required to decide whether these differences are constant.

C. guttata is very rare on the mud-flats at the Salstone. Type specimen preserved in the collection of Fr. Hustedt.

#### Genus Achnanthes Bory.

Achnanthes Lilljeborgei Grun. (Hustedt, 1939, p. 609, figs. 39, 40). Very rare. The discovery of a single complete frustule of this characteristic species in our samples from the Salstone flats is very interesting since it sheds further light on our knowledge of this species. Length about  $28 \mu$ , breadth  $8 \mu$ , rapheless valve with faintly punctate costae, 9 in  $10 \mu$ . The raphe-valve is thinner, with denser striae, 15 in  $10 \mu$ , which are almost perpendicular to the median line. Raphe straight, axial area very narrow, no central area.

This is perhaps the second time in which the two real valves which belong to this species have been found together, the first was by Hustedt (1939) from the mud flats on the German coast. The specimens from these two localities are identical.

The rapheless valve of this species as figured by Cleve (1892, pl. 3, fig. 19) and Peragallo (1897–1908, pl. 2, fig. 5, right fig.!) is so characteristic that the specimen found by us undoubtedly belongs to this species. On the other hand, the valve with raphe which has been drawn both by Cleve (fig. 20) and by Peragallo (fig. 5, left fig.!) apparently do not belong to this species, as has been pointed out by Hustedt (1939), but rather to *Navicula palpebralis* f. *Vahliana* (Grun.) Hust., which seems to be generally distributed on mud-flats in the European north Atlantic coasts. The statement by Cleve (1895) that the axial area of the lower (=raphe-) valve of *Achnanthes Lilljeborgei* is 'lanceolate narrow' applies more to this *Navicula*.

There can be no doubt that the raphe-valve which we found attached to the rapheless valve in our material is the true valve of this *Achnanthes*.

A. Hauckiana Grun. (Hustedt, 1931, p. 388, fig. 834). Rare. Brackish water species of general distribution on European coasts.

A. brevipes Ag. (Hustedt, 1931, p. 424, fig. 877*a*-*c*). Only a few valves were met with in the sediment.

var. *parvula* (Kütz.) Cleve (Hustedt, 1931, p. 426, fig. 877*f*-*i*). Rare. Both the species and its variety are more common on rocky shores in the spray zone (Aleem, 1950*b*).

A. longipes Ag. (Hustedt, 1931, p. 427, fig. 878). Very common in the *Vaucheria* community in the lower littoral region. The zonal distribution of this species at the Salstone is similar to that described by Aleem (1950*b*) from other localities.

#### Genus Diploneis Ehr.

Diploneis bombus Ehr. (Hustedt, 1931, p. 704, fig. 1086). Rather rare.

*D. aestuari* Hustedt, (Hustedt, 1939, p. 612, figs. 41, 42). Rather frequent, first known from the German coast. Valve elliptical, slightly constricted, length about  $15 \mu$ , breadth about  $6 \mu$ , transapical striae about 18 in  $10 \mu$ .

# Genus Amphipleura Kütz.

Amphipleura rutilans (Trent.) Cleve (Hustedt, 1931, p. 720, fig. 1093*a*, *b*). Frequent, filaments were found growing on the mud at M.T.L.

# Genus Stauroneis Ehr.

Stauroneis salina W. Smith (Hustedt, 1930a, p. 258, fig. 414). Frequent, the species has a wide distribution in the mud-flats.

#### Genus Navicula Bory.

Since only a comparatively small number of species of this very large genus were found, we prefer to give them here in alphabetical order.

*Navicula abrupta* Greg. (Cleve, 1895, p. 61, A.S. Atl. pl. 3, figs. 1, 2). Frequent, some of the specimens found are smaller than is cited in the literature. The smallest valves found are about  $30 \mu$  long and  $17 \mu$  broad.

*N. aleemi* Hustedt, **sp.nov.** Valva lineari-lanceolata, apicibus subobtusis, non protractis, circiter  $26\mu$  longa et  $6\mu$  lata. Raphe directa, area axiali angusta, in media parte valvarum lanceolate amplificata, area centrali modice lata. Striis in media parte valvarum leniter radiantibus, 20-24 in  $10\mu$ , ad polos leniter convergentibus, densioribus, circiter 30 in  $10\mu$  (Fig. 1D).

Valve linear-lanceolate with slightly convex margins and rather obtusely rounded ends, about  $26 \mu$  long and  $6 \mu$  broad. Raphe straight, embedded in a distinct longitudinal median rib. Axial area narrow, towards the central nodule lanceolately enlarged. Central area moderately large, elliptical lanceolate. Striae slightly radiate, at the ends of the valve slightly convergent, round the central area 20–24 in 10 $\mu$ , becoming denser towards the ends, till 30 in 10 $\mu$ . Longitudinal lines inconspicuous.

This species belongs to the group of the *Naviculae lineolatae*, but is distinguished from the other species by its distinct longitudinal median rib, the shape of the areas and by its dense structure.

Rare, found on the mud flats at the Salstone, type specimen in the collection of Fr. Hustedt.

N. arenaria Donk. (Donkin, 1871–2, p. 56, pl. 8, fig. 5). Not rare. More frequent on sandy flats (Hustedt, 1939, p. 622).

*N. avenacea* (Bréb.) (Cleve, 1895, p. 15). Rare, only a few valves belonging to this species have been found.

*N. britannica* **sp.nov.** Valva lineari-lanceolata, apicibus acutis, circiter  $39\mu$  longa et  $8\mu$  lata. Raphe directa, poris centralibus approximatis, area axiali

angustissima, area centrali nulla. Striis transapicalibus fere perpendicularibus vel leniter radiantibus, circiter 20 in  $10\mu$ , striis longitudinalibus directis, circiter 24 in  $10\mu$  (Fig. 1C).

Valves linear-lanceolate with convex margins and acute ends,  $39 \mu$  long and  $8 \mu$  broad. Raphe straight, with very approximate central pores, axial area very narrow, no central areas. Striae perpendicular to the median line or very slightly radiate, about 20 in  $10 \mu$ , crossed by distinct straight longitudinal lines, about 24 in  $10 \mu$ .

This species also belongs to the *Naviculae lineolatae*, especially to the group of N. *ramosissima* Ag. and its allied forms. It is distinguished by its very approximate central pores and its denser structure.

Found on the mud-flats of Salstone, but rare. Type specimen in the collection of Fr. Hustedt. The species was also found previously by one of us (A.A.A.) at Swanage.

N. cancellata Donk. (Cleve, 1895, p. 30). Frequent, easily recognized by its strong and distinctly lined ribs and slightly constricted frustules in girdle view.

N. crucigera (W. Smith) Cleve (Hustedt, 1930*a*, p. 270, fig. 439). Frequent. N. cryptocephala Kütz. (Aleem, 1949, p. 436, fig. 8*a*, *b*). Frequent, widely distributed in mud-flats.

var. veneta Kütz. (Aleem, 1949, fig. 8c, d). Frequent.

N. digitoradiata (Greg.) A. Schmidt (Hustedt, 1930a, p. 301, fig. 518). Frequent.

*N. flanatica* Grun. (Hustedt, 1939, p. 628, figs. 86–90). Rather frequent, distinguished by its distinct lanceolate axial area and by its coarse longitudinal lines.

N. forcipata Grev. (Cleve, 1895, p. 65). Frequent.

var. densestriata A. Schmidt, (p. 66). Rather frequent, valves  $50-60 \mu$  long,  $15 \mu$  broad, striae 22 in  $10 \mu$ .

N. gregaria Donk. (Hustedt, 1930*a*, p. 269, fig. 437). Frequent, distinguished from N. cryptocephala mainly by its less radiate striae and the presence of clear longitudinal lines.

N. (Schizonema) grevillei Ag. (Cleve, 1894, p. 152). Rare.

*N. groschopfi* Hustedt (Hustedt, 1939, p. 631, figs. 102, 103). Frequent, first described by Hustedt from mud-flats on the German coast. The species is characterized by its small size and dense striae (20 in  $10\mu$ ) and rhombic-lanceolate values.

*N. lyra* Ehr. (Cleve, 1895, p. 63). Rare, widely distributed on mud-flats. Most of our specimens belong to var. *intermedia* Peragallo (1897–1908, pl. 23, figs. 6–10), which we have not separated.

*N. ostrearia* Turp. (Peragallo, 1897–1908, pl. 7, fig. 22). Common. This *Navicula* is easily distinguished in the living state by its blue colour and hyaline structure. It grows associated with mussels and shells in the *Fucus* zone.

N. palpebralis Bréb. f. Vahliana (Grun.) (Hustedt, 1939, p. 635. Schmidt, 1874, pl. 2, fig. 21). Rather frequent, widely distributed on mud-flats.

N. plicata Donk. (Cleve, 1894, p. 154). Not frequent. The valves in this species are in most cases linear-lanceolate with more or less convex margins, but not linear alone as described by Cleve, and Peragallo (1897–1908, pl. 8, fig. 15). Besides, the striae are more radial than is shown in Peragallo's figure.
N. (Schizonema) ramosissima Ag. (Cleve, 1894, p. 26). Frequent. (Fig.

IA, B).

var. mollis (W. Smith) (Aleem, 1949, p. 432, fig. 6f, g). Frequent. N. salinicola Hustedt (Hustedt, 1939, p. 638, figs. 61-69). Frequent.

# Genus Pinnularia Ehr.

*Pinnularia ambigua* Cleve (Cleve, 1895, p. 94). Rare. As Hustedt (1939, p. 641) has already pointed out, this species should perhaps be transferred into the genus *Amphora*. The frustules are distinguished by a rather larger number of intercalary bands and are mostly asymmetrical to the apical plane as in the species of the genus *Amphora*. The valves are not always symmetrical to the apical axis, but more or less asymmetrical. We leave this species at present in *Pinnularia* until further studies of these two genera are undertaken.

#### Genus Trachyneis Cleve

*Trachyneis aspera* (Ehr.) (Cleve, 1894, p. 191). Rare, also known from other mud-flats of the European coasts.

# Genus Scoliopleura Grun.

Scoliopleura tumida (Bréb.) Rabh. (Heiden in Schmidt (1874–1944), pl. 262, figs. 1–4, 6). Frequent.

#### Genus Scoliotropis Cleve

Scoliotropis latestriata (Bréb.) Cleve (Heiden in Schmidt (1874–1944), pl. 261, figs. 4, 5). Rather frequent.

## Genus Caloneis Cleve

Caloneis liber (W. Smith) (Cleve, 1894, p. 54). Rare.

## Genus Gyrosigma Hass.

Gyrosigma balticum (Ehr.) Rabh. (Hustedt, 1930*a*, p. 224, fig. 331). Not infrequent, easily distinguished by its more or less long linear valves and distinct striae.

G. Spenceri (W. Smith) Cleve (Hustedt, 1930*a*, p. 225, fig. 336). Rather frequent.

G. fasciola (Ehr.) (Cleve, 1894, p. 116). Frequent, characterized by its long protracted ends.

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Fig. 2. A, Gyrosigma fasciola var. sulcata Grun. × 1200. B, G. tenuissimum (W. Smith) Cleve. × 1200. C, G. distortum (W. Smith) Cleve. × 1200. D, Pleurosigma maroccanum Cleve. × 600. E, part of same. × 1200. F, P. subhyalinum Hust. & Al, n.sp. × 1200. F.H. ad nat. del.

var. *sulcata* Grun. (Cleve, 1894. Van Heurck, 1880–5, pl. 21, fig. 7). More frequent than the typical form, from which it is distinguished by the stronger longitudinal striae (Fig. 2A).

G. distortum (W. Smith) Cleve (Hustedt, 1930a, p. 224, fig. 334). Not rare. The ends of the valves found are less protracted and more obtuse than described in the literature. (Fig. 2 C).

G. littorale (W. Smith) (Cleve, 1894, p. 116). Rather rare. A very characteristic species, distinguished especially by the distant longitudinal lines.

G. tenuissimum (W. Smith) (Cleve, 1894, p. 117). Rather rare. Transapical striae 20 in  $10\mu$ , longitudinal ones much denser, scarcely to be seen. (Fig. 2B).

# Genus Pleurosigma W. Smith

Pleurosigma angulatum (Quek.) W. Smith (Cleve, 1894, p. 40). Rare.

P. aestuari Bréb. (Cleve, 1894, p. 42). Frequent.

P. Normani Ralfs (Cleve, 1894, p. 40). Rather rare.

*P. naviculaceum* Bréb. (Cleve, 1894, p. 36). Rather frequent. The structure is coarser in the middle part of the valves, becoming denser towards the ends. In this respect it agrees with *P. Normani* and it seems that *P. Normani* comprises only the larger specimens of *P. naviculaceum*. In this paper we leave the two species separate until Hustedt's studies of the genus for the publication in the *Kieselalgen* are finished.

*P. subhyalinum* **sp.nov.** Valva sigmoidea, apicibus acutis, circiter  $105 \mu$  longa,  $11 \mu$  lata. Raphe sigmoidea centrali, area axiali nulla, area centrali parvissima. Structura delicatissima, striis obliquis 32–36 in  $10 \mu$ , sub angulo  $45-50^{\circ}$  decussatis, striis transapicalibus inconspicuis, 28-30 in  $10 \mu$ . (Fig. 2F).

Valves slender, hyaline, sigmoid with acute ends, length about  $105 \mu$ , breadth  $11 \mu$ . Raphe sigmoid, central, axial area absent, central area very small. Structure very delicate, only to be resolved in a medium with high refractive index, oblique striae 32-36 in  $10 \mu$ , crossing each other with an angle of  $45-50^{\circ}$ . Transapical striae inconspicuous, 28-30 in  $10 \mu$ .

The structure of the valve in this species is perhaps akin to P. hyalinum Grun. (Cleve & Grunow, 1880, p. 52), later cited by Cleve (1894, p. 42) in a note with P. javanicum Grun. However, neither Grunow nor Cleve has given any figure of this species and concerning the number of striae they state only that these are 'too delicate for measuring'.

It has been the experience, however, in dealing with the species belonging to *Pleurosigma* to take into consideration two important characters, namely: the exact shape of the valve with its raphe, and the number of striae. But since neither of these conditions has been satisfied in *P. hyalinum* Grun., we find ourselves unable to refer our specimen to this species and we prefer to keep it separate.

P. obscurum W. Smith (Cleve, 1894, p. 43). Rather rare.

P. formosum W. Smith (Cleve, 1894, p. 45). Not rare, but mostly in fragments.

*P.* (*Rhoicosigma?*) maroccanum Cleve (Cleve, 1894, p. 42). (*Rhoicosigma maroccanum* Cleve in Peragallo, 1893, p. 32, pl. 9, fig. 22). Very rare. Cleve considers this a doubtful species, requiring further examination. There exists only one figure of it made by Peragallo, which was copied by the same author (1897–1908, pl. 36, fig. 1). For these reasons and because of the fact that we found only a single valve in our samples which we are sure belongs to this species, we prefer to give a figure and a short description of our specimen.

Valve distinctly sigmoid with rather acute ends, length  $250 \mu$ , breadth  $30 \mu$ ! Raphe more sigmoid than the valve (therefore strongly excentric towards the ends!). Oblique striae about 17 in  $10 \mu$ , transapical striae about 19 in  $10 \mu$ . Central area very small (Fig. 2 D, E).

The valve is slightly bent in the transapical axis as in species belonging to the genus *Rhoicosigma*. On the other hand, in this latter subgenus, unlike *Pleurosigma*, the two valves are different in shape. Since the second valve in *P. maroccanum* seems not to be known (at least not mentioned in the literature), it is doubtful whether the two valves belonging to the same frustule of *P. maroccanum* differ at all.

#### Genus Amphiprora Ehr.

Amphiprora paludosa W. Smith (Hustedt, 1930a, p. 339, fig. 624). Rather frequent.

A. pulchra var. pulchella (Peragallo, 1897–1908, pl. 37, figs. 4, 5). Rare.

A. sulcata O'Meara (Peragallo, 1897–1908, pl. 38, figs. 1–3). Rather rare.

# Genus Tropidoneis Cleve

*Tropidoneis Vanheurcki* Grun. (Peragallo, 1897–1908, pl. 40, figs. 12, 13). Frequent.

*T. vitrea* W. Smith (Peragallo, 1897–1908, pl. 41, figs. 7, 8). Frequent. The last two species are generally distributed in mud-flats.

#### Genus Auricula Castr.

Auricula dubia Peragallo (Peragallo, 1897–1908, pl. 42, figs. 8–11). Only a single specimen found.

#### Genus Amphora Ehr.

#### Subgenus Amphora Cleve

Amphora proteus Greg. (Cleve, 1895, p. 103). Frequent, widely distributed in all marine coasts.

A. marina (W. Smith) Van Heurck (Cleve, 1895, p. 103). Rather frequent, this species is similar to the previous one, with which it may be united.

# Subgenus Halamphora Cleve

Amphora coffaeiformis Ag. (Hustedt, 1930*a*, p. 345, fig. 634). Very common. Also common on other mud-flats as well as on the rocky shore. The species is very variable. Many small species of *Amphora* have been described which probably belong to this species.

var. *acutiuscula* (Kütz.) (Hustedt, 1930*a*, p. 346). Rather frequent. Structure coarser than in the species. Both the species and its variety are of wide distribution both in marine coast lines and in salt lakes and ponds. They are able to stand a rather wide variation of pH.

A. costata W. Smith (Cleve, 1895, p. 122). Rare.

A. turgida Greg. (Cleve, 1895, p. 123). Rare.

# Subgenus Oxyamphora Cleve

Amphora lineolata Ehr. (Hustedt, 1930a, p. 346, fig. 636). Frequent.

A. hyalina Kütz. (Cleve, 1895, p. 127). Very rare, distinguished by its semicircular valve in girdle view, structure hyaline.

A. arcus Greg. (Cleve, 1895, p. 127). Not rare.

A. ostrearia Bréb. (Cleve, 1895, p. 129). Common. Similar to A. arcus, but distinguished by its narrow stauros.

A. laevis var. laevissima Greg. (Cleve, 1895, p. 130). Rare.

#### Subgenus Psammamphora Cleve

Amphora ocellata Donk. (Cleve, 1895, p. 133). Rare.

## Subgenus Cymbamphora Cleve

Amphora angusta (Greg.) (Cleve, 1895, p. 135). Rather frequent.

#### Genus Gomphonema Ag.

Gomphonema exiguum Kütz. (Cleve, 1894, p. 188). Rare.

## Genus Rhopalodia O. Müller

*Rhopalodia musculus* (Kütz.) O. Müll. (Hustedt, 1930*a*, p. 392, fig. 745). Not rare, but more common in brackish water.

#### Genus Cylindrotheca Rabh.

*Cylindrotheca gracilis* (Bréb.) Grun. (Hustedt, 1930*a*, p. 393, fig. 746). Rather frequent, this species could easily be overlooked because of its hyaline structure. It belongs to the Nitzschiaceae and is characterized by its spirally twisted keels.

#### Tryblionellae

# Genus Nitzschia Hass.

Nitzschia punctata (W. Smith) Grun. (Van Heurck, 1880-5, pl. 57, figs. 19, 20). Frequent.

var. coarctata Grun. (Hustedt in Schmidt, 1874–1944, pl. 330, fig. 16). Frequent.

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N. acuminata (W. Smith) Grun. (Hustedt, 1930a, p. 401, fig. 764). Rather frequent.

N. apiculata (Greg.) Grun. (Hustedt, 1930a, p. 401, fig. 765). Frequent.

# Dubiae

Nitzschia commutata Grun. (Hustedt, 1930a, p. 405, fig. 774). Rare.

*N. littorea* Grun. (Van Heurck, 1880–5, pl. 59, fig. 21). Rather rare, found only in the *Vaucheria* community. Characterized by the very distant median keel-puncta.

*N. dubiiformis* Hustedt (Hustedt, 1939, p. 658, figs. 111–113). Very common in the mud-flats. First described from the German coast, where it is also very frequent, especially on the sandy flats. It is characterized by its hyaline valves, dense striae and by its very variable number of keel-puncta which vary from 10 to 18 in  $10\mu$ , the two median ones are more distant than the others.

## Panduriformes

Nitzschia panduriformis Greg. (Hustedt in A.S. Atl. pl. 331, figs. 19–21). Rather rare. Much more frequent on warmer coasts.

N. constricta (Greg.) Grun. (Van Heurck, 1880–5, pl. 58, fig. 8). Rather common.

## Lanceolatae

Nitzschia aequorea Hustedt (Hustedt, 1939, p. 663, fig. 120). Rather rare, characterized by its typical lanceolate valves, acute ends, small size and dense striae, which are about 35 in  $10 \mu$ .

#### Sigmoideae

Nitzschia sigma (Kütz.) W. Smith (Hustedt, 1930a, p. 420, fig. 813). Common.

## Spathulatae

Nitzschia angularis W. Smith (Van Heurck, 1880–5, pl. 62, figs. 11–14). Not infrequent.

N. distans Greg. (Peragallo, 1897-1908, pl. 73, fig. 3). Common.

## Dissipatae

Nitzschia macilenta Greg. (Peragallo, 1897–1908, pl. 72, figs. 1, 2). Rare. N. socialis Greg. (Peragallo, 1897–1908, pl. 72, figs. 7, 8). Rather frequent.

# Pseudonitzschiae

Nitzschia pungens Grun. (Cleve & Möller, 1882, Diat. No. 307, Cleve, 1897, p. 24, pl. 2, fig. 23). Rare. This species has hitherto been only known from Japan, and apart from the above two references there is no other mention of it in the literature. Cleve describes a variety *atlantica* from the Kattegat

with somewhat denser striae (15 in  $10\mu$ ), but our specimens agree more with the species than with this variety (length  $100\mu$ , breadth  $4.5-5\mu$ , striae about 10 in  $10\mu$ ).

The species is akin to *Pseudonitzschia sicula* Castr., *P. migrans* Cleve and *P. seriata* Cleve figured in Peragallo (1897–1908, pl. 72, figs. 25–29) under the group Pseudonitzschia. The constriction of cell is not yet exactly known in this group, in which no keel-puncta are to be seen and that is perhaps the reason why some of the species included have been referred to *Synedra*.

Further studies are required in order to show the taxonomic position of this group and to elucidate the relation between the different species and until this is done we retain the original nomenclature of Grunow.

# Nitzschiellae

*Nitzschia closterium* (Ehr.) W. Smith (Hustedt, 1930*a*, p. 424, fig. 822). Very common. Also widely known in the plankton. It was rather common in the plankton off Plymouth in March.

*N. longissima* (Bréb.) Ralfs (Schmidt, 1874–1944, pl. 335, figs. 1, 2). Not infrequent on the mud-flats.

# Genus Surirella Turp.

Surirella ovalis Bréb. (Hustedt, 1930a, p. 441, figs. 860, 861). Rare.

S. gemma Ehr. (Van Heurck, 1880–5, pl. 74, figs. 1–3). Frequent.

S. fastuosa Ehr. (Peragallo 1897–1908, pl. 58, figs. 5–7). Rather rare.

S. caspia Brun (Brun, 1891, p. 45, pl. 13, fig. 6). S. cardaria Brockmann (Brockmann, 1914, p. 70, fig. 11). Rare; only in the Vaucheria community.

This is an interesting species of which little is known. Apart from the figure given by Brun, his description of this species is quite insufficient. The only other mention, according to our knowledge, is a record by Meister (1932, p. 24) among a list of diatoms from the mouth of Belawan river in Sumatra. In the collection of Hustedt is to be found the original material of *Surirella caspia* Brun from the Caspian Sea, as well as that of *S. cardaria* Brockmann from the mouth of the Weser. These two species are identical, and we quote Brockmann's species as a synonym of *S. caspia* Brun and give the following description of the latter:

Cells with heteropolar apical axis, slightly twisted round this axis. Valves more or less broadly ovate to nearly orbicular, with obtusely rounded ends,  $50-120 \mu$  long,  $36-60 \mu$  broad. Ales broad, with distinct projection, primary alar canals broader than the windows, 10-20 in  $100 \mu$ . Below the alar bordercanal (see Appendices I and II), the primary alar canals are divided by short ribs into two to five secondary alar canals. Surface of the valve distinctly undulated. The crests of these undulations end on the ales in broad and flatly rounded arcs, while towards the median line the furrows are broader than the crests. Median area more or less developed, though not sharply defined. Transapical striae coarse, about 12 in  $10 \mu$  near the border of the valve. Short spines are scattered all over the surface of the valve, specially on the crests.

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# Genus Campylodiscus Ehr.

*Campylodiscus Thureti* Bréb. (Peragallo, 1897–1908, pl. 57, figs. 8, 9). Very rare, only one specimen was found in the *Vaucheria* community. More frequent on warmer coasts.

# DISCUSSION

The species recorded in this paper are similar to those inhabiting mud-flats at Whitstable, Kent (Aleem, 1949). Many are also recorded by Hustedt (1939) from mud-flats on the German coast. Such marine mud-flats are, as a rule, rich in species belonging to the Raphidae, particularly those of the genera *Amphora*, *Gyrosigma*, *Navicula*, *Nitzschia*, *Pleurosigma* and *Tropidoneis*. These possess a well-developed raphe and are capable of moving freely within the substratum (Aleem, 1950*a*). They are to be found on the flats all the year round, though in varying degrees of abundance in the different seasons.

The mud-flats also comprise, in less significant quantities, other species which are recruited from three different sources, namely: (i) attached forms from the rocky shore, (ii) pelagic forms from the plankton, (iii) fresh-water forms from rivers and lakes. The last of these is much less significant than the others. Among those recruited from the rocky shore and found on the flats at the Salstone are species of *Cocconeis, Achnanthes, Licmophora, Grammatophora* and *Rhabdonema*. They are mostly colonial forms which grow attached by gelatinous stalks to rocks or as epiphytes on algae. Such forms were represented on the bare mud by only a few detached individuals, owing to lack of a proper substratum. A number of them, however, were much more frequent as epiphytes on seaweeds, especially in the *Vaucheria* community at M.L.W.S. The following diatoms are the principal species recognized from this community during the period of collection:

Achnanthes longipes	L. flabellata
Amphora coffaeiformis	L. Juergensi
A. hyalina	Opephora marina
Cocconeis scutellum	Synedra Gailloni
Grammatophora marina	S. tabulata
Licmophora gracilis var. anglica	

Among those recruited from the plankton and to which reference has been made in this paper are the following species:

> Actinoptychus undulatus Coscinodiscus centralis C. excentricus C. radiatus Eucampia zoodiacus Hyalodiscus scoticus

Nitzschia pungens Roperia tessellata Sceletonema costatum Thalassionema nitzschioides Thalassiosira decipiens Thalassiothrix longissima

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They are mostly centric forms, rare on the mud-flats, and their presence here accidental. Most of them are more frequent in the plankton off Plymouth during March. No species of the genera *Chaetoceros* or *Rhizosolenia* were met with, neither as vegetative cells nor as resting spores, in our samples from the mud-flats, although these two genera were richly represented in the Plymouth plankton.

Apart from these, there are species which thrive equally well both in the plankton and in the littoral region. A conspicuous example of these is *Nitzschia closterium* which is found in appreciable quantities on all mud-flats, in littoral and supra-littoral pools as well as among the plankton. It is also known as a bottom form able to live in less well-oxygenated conditions (Mare, 1942). *Melosira moniliformis, M. sulcata* and *Biddulphia aurita* are also examples which are equally abundant both in the littoral region and in the plankton. It is more probable, however, that the origin of such species is in the littoral region and that later on they are recruited into the plankton, where they still retain the capacity to multiply.

#### SUMMARY

A systematic study of the marine diatoms inhabiting the mud-flats at the Salstone, South Devon, is given. Altogether 132 species and 9 varieties, belonging to 45 genera are mentioned. Among these the following are described as new species: *Cocconeis guttata*, *Navicula aleemi* Hust., *N. britannica*, *Pleurosigma subhyalinum*.

Four other little-known diatoms, namely, Achnanthes lilljeborgei, Nitzschia pungens, Pleurosigma maroccanum and Surirella caspia, have also been found in the same locality and a detailed description of these is given. The Salstone diatoms are in essential respects similar to those found in other European north Atlantic mud-flats.

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#### APPENDIX I

It has become necessary with the advance of our knowledge of diatoms due to modern microscopy to introduce some new terms describing certain configurations in the structure of the valve. Some such terms have already been proposed in the German literature by R. Lauterborn, O. Müller and Fr. Hustedt for which no equivalent translations have yet been given. We therefore propose the following translations of such German terms as we have used in this respect in our paper.

'Raphenschale' (sensu Hustedt, 1930a, p. 188)=raphe-valve.

'raphenlose Schale' (sensu Hustedt, 1930a, p. 188) = rapheless valve.

- 'Flügelrandkanal' (*sensu* Lauterborn, 1896, p. 15 = Querkanal) = alar border canal.
- 'Fenster' (*sensu* O. Müller, 1903, p. 24) = windows (for the intervening spaces between the alar canals of *Surirella*, also equivalent to the keel puncta in *Nitzschia*).

'Wellenberge' (sensu Lauterborn, 1896, pp. 14, 15) = crests.

'Wellentäler' (sensu Lauterborn, 1896, pp. 14, 15) = furrows.

The last two terms describe the undulations of the valve of *Surirella*. The word 'crest' had already been adopted by others, while 'Wellentäler' was formerly translated by 'hollows'. We prefer the term 'furrows' which seems to be more appropriate for the structure in question, and we suggest that the word 'depressions' might better be applied to the space between the median line and margin (in the valve of *Surirella*) in apical direction.

'Schleifenköpfe' (sensu O. Müller, 1903, p. 25) = arcs.

'Primärkanäle'=primary canals (see appendix II).

'Sekundärkanäle' = secondary canals (see appendix II).

'sekundäre Fenster' = secondary windows (see appendix II).

# APPENDIX II

# Additional note on the construction of the ales in Surirella

# By Fr. Hustedt

Lauterborn (1896, pp. 14–16, pl. 1, figs. 8–12) made an intensive study of the structure of the ales in *Surirella calcarata* Pfitz. Most other species of this genus, specially the fresh-water forms, have ales identical with those of the latter species. Since many marine species of the genus deviate from it in structure, it is necessary to give here some additional remarks.

In many species the crests on the surface of the valve are very enlarged towards the basis of the ales, so that the alar canals become very broad too. In such cases narrow ribs or small windows project from the inner wall of the border canal into these enlarged alar canals in varying degrees, thus dividing the mouth of the alar canals into more or less numerous small openings. I have called the original alar canals 'primary canals', the smaller ones inside the latter 'secondary canals', and the spaces left between two adjacent canals 'secondary windows'.

# ABSTRACTS OF MEMOIRS

# RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

# FLOOR OF THE ENGLISH CHANNEL

# By W. B. R. King

# Geol. Mag., Vol. LXXXVII, 1950, pp. 383-4

Cores of the solid rock as well as the superficial cover have been obtained with a modified Stetson corer worked from the R.V. *Sabella* of the Marine Biological Association. Three areas have been sampled: (1) near the Eddystone, where New Red Sandstone has been proved over a wide area, while Chalk was found 18 miles south of the lighthouse; (2) mid-Channel, between Isle of Wight and Cherbourg, where Chalk was proved in the northern parts and Wealden sands and Jurassic clays in the central area, with Chalk again to the south; (3) an area crossing the Hurd Deep, W.N.W. of Guernsey to about mid-Channel, where the bed rock proved to be mostly Chalk. The research is continuing. W.B.R.K.

#### Two New Pycnogonids from Bermuda

# By Marie V. Lebour

#### Proc. Zool. Soc. Lond., Vol. 118, 1949, pp. 929-32

Two new species of pycnogonid are described, *Anoplodactylus tenuirostris* and *Parapallene bermudensis*. The first a small species from the Reach, probably related to *A. petiolatus*, the second, one specimen only, from open water about 100 feet depth, near the bottom. M.V.L.

## Some New Decapod Crustacea from Bermuda

#### By Marie V. Lebour

#### Proc. Zool. Soc. Lond., Vol. 118, 1949, pp. 1107-17

A new species of Discias and three species of Periclimenes are described.

The *Discias* was captured near the bottom, 100 ft. or more, in open water. Only three species are so far known and this new one is peculiarly interesting, the specialization of the first and second legs being unusually marked, but

# ABSTRACTS OF MEMOIRS

the mandible and maxilla showing a more primitive condition. The larvae of *Periclimenes iridescens* n.sp. were hatched from the egg and are interesting in being intermediate between *P. diversipes* and the typical *Periclimenes* (*Ancylocaris*) larvae described by Gurney.

It was found that the specific name of *P. (Ancylocaris) bermudensis* n.sp. was preoccupied and this was altered to *rhizophorae*. M.V.L.

#### Some Euphausids from Bermuda

#### By Marie V. Lebour

# Proc. Zool. Soc. Lond., Vol. 119, 1949, pp. 823-37

A collection of euphausids is described, following the work of Gurney (1947) and filling in certain gaps in this work. For the first time a nauplius of *Stylocheiron* is recognized, having hatched from the egg of *S. carinatum*. Certain variations in pleopod succession are noted in various species. M.V.L.

# NOTES ON SOME LARVAL DECAPODS (CRUSTACEA) FROM BERMUDA

#### By Marie V. Lebour

# Proc. Zool. Soc. Lond., Vol. 120, 1950, pp. 369-79

The larvae of *Panulirus argus* were hatched out and the first stage figured. Notes on later stages are given and descriptions of two larval scyllarids. Late stages of *Petrolisthes armatus* are described and two interesting zoeae of raninids. No species of adult *Ranina* has as yet been recorded from Bermuda. Some crab zoeae hatched from the egg are described—*Portunus spinimanus*, *Lobopilumnus agassizii* and *Grapsus grapsus*. M.V.L.

### SWIMMING OF DOLPHINS

#### By G. A. Steven

#### Science Progress, No. 151, 1950, pp. 524-5

Some years ago, while the author was serving in a ship on passage with another vessel in tow, a unique opportunity arose of obtaining exact observations of the time taken for dolphins to swim a known distance and so calculate their speed. It was found that some animals were capable of almost exactly 20 knots. The number of tail beats in animals swimming at 9 knots were also noted. The counts varied from 23 to 27 beats in 10 sec. Certain interesting characteristics of dolphin wakes are also described. G.A.S.

# ABSTRACTS OF MEMOIRS

# DIFFERENTIATION OF THE SEA-URCHIN EGG FOLLOWING REDUCTION OF THE INTERIOR CYTOPLASM IN RELATION TO THE CORTEX

# By Sven Horstadius, I. J. Lorch and J. F. Danielli

# Exp. Cell. Res., Vol. 1, 1950, pp. 188-93

The central cytoplasm of eggs of *Echinus esculentus* L. and *Psammechinus miliaris* (Gmelin) was reduced by suction before fertilization. The total cytoplasmic reduction amounted to about 50%.

In 20 eggs which started development no deviations in either animal or vegetal direction were observed. The resulting plutei were normal in every respect except size.

The results are contrary to Dalcq's concept of the differential localization of gradients at the centre and periphery of the sea-urchin egg. J.F.D.

# The Mechanics of the Blood Vascular System of Ascidiella Aspersa

# By C. A. Haywood and H. P. Moon University College, Leicester

#### Journ. Exp. Biol., Vol. 27, 1950, pp. 14-28

The blood vascular system of *A. aspersa* is considered with particular reference to the well-known periodic reversal of the heart beat. It is emphasized that the problem involves two considerations: (I) why the heart stops beating periodically, and (2) why having stopped it should then reverse. The first consideration is taken in some detail and a mechanical model of the blood vascular system is considered mathematically. It is shown that a 'back pressure' similar to that postulated by La Hille arises, and that the heart should exhibit the periodic cessation of beating. A relation is deduced connecting the time between successive reversals with the velocity of the heart beat, and is shown to be capable of direct experimental verification. In this the ascidian is brought to various temperatures as a means of varying the velocity of the heart beat. Allowing for the variation of the viscosity of the blood with the temperature it is found that the relation is satisfied within certain limits, the significance of which is discussed.

Various criticisms of La Hille's 'back pressure' theory are shown to be invalid and examples are given of other closed contractile systems containing fluid which show reversal. H.P.M.

# BOOK REVIEW

# THE SEA AND ITS MYSTERIES

#### By John S. Colman

#### G. Bell and Sons, Ltd. 1950. Price 12s. 6d. net.

The most remarkable feature of this book is the facility with which Mr Colman puts into simple language so many recent physical and chemical discoveries about the sea. To make easy, and yet accurate, reading of details of wave motion, winds, ocean currents and the like and to sustain interest for page after page on these and other technical matters, usually considered difficult to expound, is no mean feat. The style is pleasant, the facts abundant and well assembled, the illustrations adequate and there are few blemishes. The result is a pleasurable book that should go far to stimulate interest in the science of the sea.

It is difficult to pick out any part for special commendation, each reader will have his or her own preference, but the chapter on coral reefs probably thrilled the reviewer more than any of the others. In a vivid piece of writing Mr Colman brings the wind-driven waves of the Pacific crashing through his pages and he adds all the colour and beauty of the Great Barrier Reef as well, or so it seems to one who has never been there. Mr Colman's own familiarity with so much of the sea the world over is very evident throughout; so often his writing is from first-hand experience and it is that above all else that makes the book live. It deserves success. D.P.W.

# THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888. The cost of the building and its equipment was £12,000 and, since that date, a new library and further laboratory accommodation have been added at an expenditure of over £23,000.

The Association is maintained by subscriptions and donations from private members, scientific societies and public bodies, and from universities and other educational institutions; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the Laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. An account of the Laboratory and the scope of the work undertaken there will be found in Vol. xv (p. 735) and Vol. xxvII (p. 761) of this *Journal*.

The Laboratory is open throughout the year and its work is carried out under the supervision of a Director and with a fully qualified research staff. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology and physiology. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat and these also collect the specimens required in the Laboratory.

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#### TERMS OF MEMBERSHIP

f s. d.

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the Laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the Laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the Library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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The Council of the Marine Biological Association wish it to be understood that they do not accept responsibility for statements published in this *Journal* excepting when those statements are contained in an official report of the Council.

# CAMBRIDGE UNIVERSITY PRESS LONDON: BENTLEY HOUSE, N.W.1 NEW YORK: 51 MADISON AVENUE CANADA AND INDIA: MACMILLAN

Printed in Great Britain at the University Press, Cambridge (Brooke Crutchley, University Printer)