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ECOLOGICAL EXPERIMENTS ON FORAMINIFERIDA

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(Text-figs. 1–6)

During 1959–60 the author undertook a seasonal study of living benthic foraminiferids at Christchurch Harbour, England (in preparation). It became apparent that certain aspects of ecology could not be studied entirely satisfactorily in the field. It was therefore resolved that experiments should be carried out on foraminiferids living under controlled conditions. Many of the experiments here described have not been duplicated and, therefore, the results must be regarded as tentative. However, common trends have been revealed in related experiments. This work was undertaken at the Laboratory of the Marine Biological Association at Plymouth during 1961–62.

Elphidium crispum (L.) was the species chosen for study as previous work had been carried out on it at Plymouth by Jepps (1942) and Myers (1942, 1943). Fairly frequent collections of fresh material were made by the motor launch 'Gammarus' (using a 'D' net) from off Drake Island and at 'White Patch' (see Marine Biol. Assoc., 1957). The sediment was placed in large, shallow, enamel trays together with sea water and food. *E. crispum* is negatively geotropic, so that within a matter of days there would be a number of individuals climbing up the sides of the tray. These were picked off with a fine paintbrush and transferred to the small plastic pill boxes in which the culturing and experiments were carried out.

EXPERIMENTS

In cultures set up during October 1961, most of the specimens were adults and showed little sign of active growth. Because of the difficulties of examining specimens in culture boxes under high magnifications, it was often not possible to tell whether specimens were alive or dead. Jepps (1942, p. 628) has noted that during feeding *E. crispum* gathers food into a sheath or 'feeding cyst' about itself. After the animal has fed, the cyst is discarded.

Feeding

Experiment 1. A culture of ten specimens was set up and fed with *Phaeodactylum tricornutum* Bohlin (cultured in sea water enriched with Miquel-Allen solution). Both the *Phaeodactylum* and the culture medium were added

as 'food', 2 ml. daily. Prior to feeding, the number of abandoned feeding cysts was counted and then they were removed. The results are shown in Table 1. The daily rate of feeding-cyst production was quite variable, covering a range of 6–11 cysts, and averaging 7·5 cysts.

TABLE 1. THE DAILY PRODUCTION OF FEEDING CYSTS BY 10 *ELPHIDIUM CRISPUM* IN SEA WATER AT APPROXIMATELY 16° C IN EXPERIMENT 1

Day	1	2	3	4	5	6	7	8	9	10	11
No. of cysts	9	10	8	←—12—→			6	9	11	10	7

Experiment 2. In a second preliminary experiment, 5 *E. crispum* were cultured in artificial sea water (Pantin, 1960; $KBr.2H_2O$ used instead of $NaBr.2H_2O$) to see if there was any difference from sea water. The results are shown in Table 2. On day 4, a large amount of food was added to cover the Christmas period (days 5–13) and the total number of cysts was counted on the 13th day. Taking the results up to day 18, the average daily rate of feeding was 4·6 cysts (which would be 9·2 for 10 specimens). From day 18, the rate of feeding fell and by the 25th day all the specimens had died.

TABLE 2. THE DAILY PRODUCTION OF FEEDING CYSTS BY 5 *ELPHIDIUM CRISPUM* IN ARTIFICIAL SEA WATER (EXPERIMENT 2)

Day	1	2	3	4	5	13	14	15	16	17	18
No. of cysts	0	6	5	5	←40+→		6	5	6	4	5
Day	19	20	21	22	23	24	25				
No. of cysts	2	3	1	0	2	2	0				

Taking the period up to the 18th day, that is, before any of the specimens died, the average daily production (4·6 for 5 specimens) was higher than that in sea water. Therefore, there was no reason to believe that artificial sea water provided an unfavourable environment.

Although it was realized that the day-to-day variation in feeding-cyst production was quite considerable (6–11 cysts in Expt. 1; 4 cysts in the first 18 days of Expt. 2), the variation was of the same order of magnitude if the results were corrected to 10 specimens (6–11 in Expt. 1; 8–12 in Expt. 2). It was therefore considered probable that the rate of feeding-cyst production could be used as a guide to the healthiness of the organism, and this was confirmed by subsequent experiments. In effect, it has been assumed that the rate of feeding is closely related to that of the metabolic activity of the animal. Although this may be regarded as presumptuous, no other measure of activity is available.

From the foregoing results it is apparent that *E. crispum* feeds on living *Phaeodactylum tricornutum*. To decide whether it would feed on dead material further experiments were undertaken.

Experiment 3. Two cultures were set up, each consisting of 10 *E. crispum*. In *A*, 4 ml. of *Phaeodactylum*, which had been irradiated beneath an ultra-violet lamp for $\frac{3}{4}$ h until it was green, was added as food. In *B*, 4 ml. of food which had been heated to 80° C. for 20 min, were added. In both instances the food was considered to be dead. After 2 days, no feeding cysts had been produced by either culture.

From this result it was thought that there were three possible explanations: (*a*) that the process of killing produced an unnatural condition in the food which was inimical to the foraminiferids; (*b*) that foraminiferids will eat only motile food; (*c*) that living *Phaeodactylum* gives off a substance which encourages foraminiferids to feed.

Experiment 4. This was designed to test the last-mentioned possibility. *Phaeodactylum* termed dead in this experiment was killed by irradiation for $\frac{3}{4}$ h. Six cultures were set up, each of 10 *E. crispum*. The daily food supply was as follows:

(*A*) the control, 4 ml. living *Phaeodactylum*; (*B*) 4 ml. dead *Phaeodactylum*; (*C*) 2 ml. living and 2 ml. dead *Phaeodactylum*; (*D*) 4 ml. dead *Phaeodactylum* plus 4 ml. culture fluid; (*E*) 4 ml. dead *Phaeodactylum* plus 4 ml. irradiated culture fluid; (*F*) 4 ml. living *Phaeodactylum* plus 4 ml. irradiated culture fluid.

The culture fluid was obtained by centrifuging a volume of *Phaeodactylum* culture and decanting off the clear fluid. Artificial sea water (salinity 34‰) was used and changed daily with the food.

TABLE 3. THE DAILY PRODUCTION OF FEEDING CYSTS IN EXPERIMENT 4

	Culture					
Day	A (control)	*B*	*C*	*D*	*E*	*F*
1	11	6	6	7	8	7
2	3	0	2	3	3	3
3	4	4	2	3	2	0
4	5	6	1	3	0	3
5	7	6	3	3	0	2
Daily average	6·0	4·4	2·8	3·8	2·6	3·0

The results (Table 3) contradict those of Expt. 3 as dead food was accepted by *E. crispum*. However, the control, fed on living food only, fared better than any of the other cultures, having a daily average of 6·0 feeding cysts. For the rest, culture *B*, having dead food only, fared better than culture *E* having both dead food and irradiated culture fluid (4·4 and 2·6 cysts daily, respectively). The presence of irradiated culture fluid definitely lowered the feeding rate because in culture *E* the daily average was low also (3·0 feeding cysts). Culture *D*, having non-irradiated culture fluid in addition to dead food showed a daily average not unlike that of culture *B* (3·8 and 4·4 feeding cysts respec-

tively). Finally, equal quantities of living and dead food as in culture *C* were as unfavourable as the combination in culture *E* (2·8 and 2·6 feeding cysts, respectively).

The main point that emerges from these results is that living food is more acceptable than dead food. It is not clear whether this points to a substance exuded by *Phaeodactylum* which might encourage *E. crispum* to feed. Certainly volatile substances are given off by marine algae (Armstrong & Boalch, 1960) and of course waste products are released. However, it could equally be taken as evidence that the method of killing is the cause of reduced feeding. It does eliminate any possibility of motile food being necessary for foraminiferids to feed.

Experiment 5. As Expt. 4 had shown that *E. crispum* would accept dead food, it was decided to test its reaction to inorganic particles of similar size to its normal food particles. Three cultures were set up, each of 10 *E. crispum*. To *A*, kaolin was added; to *B*, graphite ('Aquadag'); to *C*, fine calcium carbonate. Within one day, all the specimens in culture *A* had formed a structure which resembled a feeding cyst, and particles of the mineral were seen to be adhering to the pseudopodia. In culture *B*, 5 specimens, with graphite attached to them in the manner of a feeding cyst, were removed and placed in Bouin's fluid prior to sectioning. After 12 days, a further 3 specimens, again with graphite feeding cysts, were removed for thin-sectioning. In no case was graphite seen to have entered the chambers of the organism, demonstrating that digestion is carried on outside the test, as noted by Jepps (1942). A further point of interest is that the cohesion of the particles in the feeding cyst was sufficient to prevent the cyst from being destroyed during preparation for thin-sectioning. The sections were stained with rose Bengal to make the protoplasm pink. There was no evidence of protoplasm being responsible for holding the cyst together. The evidence points to a secretion from the foraminiferid (perhaps mucus, Jepps, 1942) since discarded feeding cysts retain their form for several days. Again, in culture *C*, feeding cysts were formed within 1 day. It could be concluded from this either that the formation of feeding cysts is not entirely restricted to feeding behaviour, or that *E. crispum* bases the choice of food particles mainly upon size. As with true feeding cysts, these inorganic cysts could be shed, leaving the foraminiferid free of debris. This discounts any possibility of the particles 'sticking' to the animal.

Experiment 6. Throughout the previous experiments, it had been noticed that the colour of the protoplasm of *E. crispum* closely matched the colour of *Phaeodactylum*. To see if this was a coincidental or true relationship, a culture of 10 specimens was fed with the green alga *Tetraselmis suecica*. This form is strongly positively phototropic and it is of interest that the foraminiferids did not follow the food to the light side of the culture. This may be taken as evidence of the random way in which they collect their food. However, the

foraminiferids were placed with the food and after 8 days they had turned yellow. After 19 days when they were again examined, they were green. Thus the protoplasmic colour appears to be closely related to that of their food.

Discussion. Sandon (1932) gave a good summary of the knowledge of the food of foraminiferids prior to that date and rightly stated that the food is typically algal. He noted that there is little selection of food by foraminiferids although some of the agglutinated forms are very selective in their choice of material for constructing their tests.

Bradshaw (1955) showed that *Rotaliella heterocaryotica* Grell would feed on both living and dead *Chlamydomonas* whereas *Rotaliella* sp. would not feed on dead *Chlamydomonas* and required living *Nitzschia*. In this study, *E. crispum* accepted dead *Phaeodactylum* although it obviously preferred living food. It would appear, therefore, that this characteristic varies from species to species. In more elaborate experiments using killed *Dunaliella* sp., Bradshaw (1961) showed that approximately 112 food cells per mm^2 were required for minimal growth and reproduction in his laboratory cultures. But, he noted that a concentration of half this value kept his specimens alive for 60 days. During this time, growth was halted and the protoplasm contracted to the earlier chambers, but normal growth and reproduction continued on the addition of abundant food. He concluded that the growth rate and reproductive efficiency appeared to increase with the continuous addition of food at least up to a concentration of 530 cells/mm^2.

Lee, Pierce, Teutchoft & McLaughlin (1961) have noted in a pilot experiment on a species of *Bolivina*, that different foods are responsible for different rates of growth under otherwise closely similar conditions. *Rhodomonas lens* and *Isochrysis galbana* neither stimulated nor inhibited growth, whereas *Nitzschia* sp. inhibited and *Nitzschia acicularis* stimulated growth. However, the authors were in some doubt as to whether equal amounts of food had been given to each culture. A particularly interesting point they raised was that microscopic and bacterial examination of their healthy cultures of various foraminiferids showed a flora common to all except *Cornuspira planorbis*.

Movement

The feeding experiments showed no selective powers in *E. crispum*. The question therefore arose as to the nature of its movement.

Experiment 7. A square tank with a line drawn centrally on the base was set up with a culture of 10 specimens and no food in a medium of artificial sea water. The specimens were lined up at 2 mm intervals along the central line. The next day there were 5 specimens on either side of the line and so too on the second day. Thus movement appeared to be random in the horizontal plane. However, it was shown that under certain conditions movement was stopped if it led into an unfavourable environment (see Expt. 9).

Discussion. Arnold (1953) conducted some interesting experiments on the movement of *Allogromia laticollaris* Arnold. He concluded that the species underwent random dispersion and that the finding of food was accidental, the latter being confirmed by the experiments here described. The experimental evidence for his concept of dispersion is not altogether convincing. He says 'the tendency is evident whenever a group of individuals is concentrated in a relatively small area'. The dispersal patterns shown by his figures 1 and 2 show an initial state where all the specimens are closely grouped together at the centre of a culture vessel. Movement by any specimen is bound to result in dispersion since movement away from the group is the only possible route. Perhaps unwittingly, he planned the experiment to show dispersion. Had he decided to place the specimens around the edge of the culture vessel, he would probably have demonstrated grouping tendencies, because apart from being able to move around the edge, the only alternative would be to move toward the centre. It seems probable that movement is typically random, a conclusion reached by Jepps in 1942 (p. 624).

The substratum

Normally the *E. crispum* taken at Plymouth is from fairly clean sands in shallow water subjected to periodic disturbance during storms. It was, therefore, of interest to determine the reaction of this species to a clayey substratum.

Experiment 8. Two cultures were set up. In *A*, dry kaolin was mixed into a thick paste with sea water and spread in a layer about 2 mm thick on the bottom of the culture box, while in *B*, ground-up talc (French Chalk) was used in the same way as in *A*. Into each culture, 10 *E. crispum* were introduced and they soon produced feeding cysts composed of a mixture of *Phaeodactylum* and kaolin (or talc). An interesting feature was that the specimens remained where they had been placed and scoured the area around themselves with their pseudopodia to gather material for their feeding cysts.

Owing to cracking of the substratum in both cultures, some specimens disappeared from sight. After 6 days, the cultures were washed clean of substratum and replenished with food and sea water. After a further 2 days, *A* had produced 14 cysts (daily average 7) and *B*, 16 (daily average 8). Thus, the experiment appeared to have had no harmful effect. Nevertheless, as the foraminiferids had been feeding on cysts composed of kaolin and food, they must have needed to feed more often to get the same nutritional value as with pure food cysts.

Experiment 9. In the next experiment a tank was set up with kaolin paste lining one half of the floor and walls, the other half being clear Perspex. Sea water and food were added above this. Into each half 5 *E. crispum* were placed. The results are shown in Table 4 and Fig. 1.

It was necessary to extend the clay substratum up the sides of the box because of the tendency of the foraminiferids to climb the sides. The results of the experiment leave little doubt that a clear substratum was preferred to one of clay. It shows that in certain circumstances, movement is not entirely random. This is perhaps related to the fact that clay/food cysts are not an efficient method of feeding.

TABLE 4. THE DAILY PRODUCTION OF FEEDING CYSTS IN EXPERIMENT 9

Day ...	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Clay half	5	4	4	*	*	3	2	4	2	3	4	3	3	*	3
Clear half	5	6	6	*	*	7	8	6	8	7	6	7	7	*	7

* Indicates no reading.

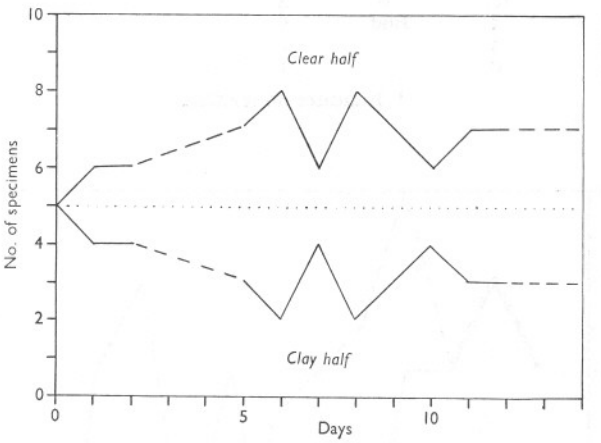

Fig. 1. Daily distribution of 10 *E. crispum* in Expt. 9.

Discussion. The only previous study known to the author of the relationship of foraminiferids to the substratum is that of Nyholm (1957) who was mainly concerned with the pseudopodial action of monothalamous forms in clayey sediment.

Salinity

Some organisms will tolerate subsaline waters (euryhaline) while others will tolerate no dilution of sea water (stenohaline). *E. crispum* is essentially a stenohaline form from its known distribution.

Experiment 10. Six cultures, each of 10 specimens, were set up in artificial sea water of varying salinity. Food was added daily (2 ml.), adjustment being made to maintain the correct salinity where necessary by the addition of distilled water. Each day, before the food was added, the number of feeding

TABLE 5. THE DAILY PRODUCTION OF FEEDING CYSTS IN EXPERIMENT 10

Culture ...	*A* (control)	*B*	*C*	*D*	*E*	*F*
Salinity (‰) ...	35	30	25	20	15	5
Day						
1	10	10	0	0	0	0
2	12	12	4	0	0	0
3	10	10	5	0	0	0
4	10	14	5	0	0	0
5	5	11	6	End	0	0
6	5	10	5		0	0
7	5	9	8		0	0
8	10	4	8		0	0
9	6	*	7		End	End
10	12	*	4			
11	10	0	4			
12	1	End	6			
13	3		1			
14	0		1			

* Indicates no reading.

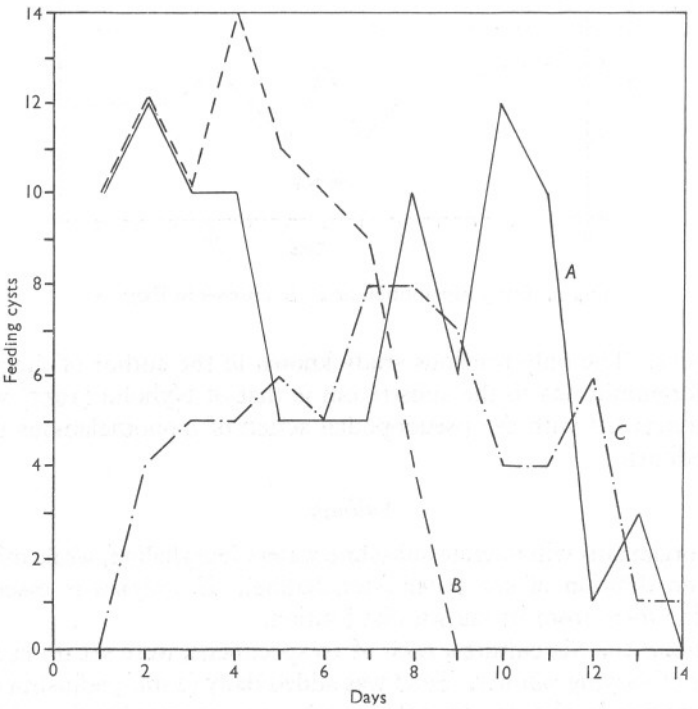

Fig. 2. Rate of feeding in culture *A* (salinity 35‰), culture *B* (salinity 30‰), and Culture *C* (salinity 25‰) in Expt. 10.

cysts was counted; they were then removed with a pipette. The results are shown in Table 5 and Fig. 2.

It will be seen that there was quite a large variation in the number of feeding cysts produced by the control, but the daily average for the 14 days was 7·1. Culture *B* (salinity 30‰) was less variable, and the average over 11 days was 8·2 feeding cysts. Culture *C* (salinity 25‰) showed a daily average of 4·6, while in cultures *D*, *E* and *F* (salinity 20‰, 15‰, 5‰, respectively) no cysts were produced at all. These results pointed to the fact that *E. crispum* thrives in waters having a salinity of 30–35‰, will survive in waters of 25‰, but *apparently* dies in water having a salinity below 25‰.

Experiment 11. An experiment was made that was essentially a duplication of no. 10, but here the calcium content of the subsaline waters was adjusted to that of normal sea water. Sea water of 35‰ salinity normally contains 400 p.p.m. calcium, which amounts to 0·4 mg/ml. The various combinations were achieved by using artificial sea water free of calcium and adding calcium

TABLE 6. THE DAILY PRODUCTION OF FEEDING CYSTS IN EXPERIMENT 11

(Duplicate of experiment in lower part)

Culture ...	*A* (control)	*B*	*C*	*D*
Salinity (‰) ...	35	25	20	16
Calcium (mg) ...	8·0	8·0	8·0	8·0
Day				
1	10	0	0	0
2	10	1	0	0
3	12	7	0	0
4	10	6	0	0
5	10	6	End	End
6	10	5		
7	8	8		
8	10	8		
9	*	*		
10	*	*		
11	0	0		
12	0	8		
13	End	6		
14		5		
15		3		
Culture ...	*A* (control)	*B*	*C*	*D*
Salinity (‰) ...	35	29	25	21
Calcium (mg) ...	8·0	8·0	8·0	8·0
Day				
1	2	1	1	0
2	5	5	2	0
3	8	10	4	End
4	7	5	2	
5	9	5		
6	2	0		

* Indicates no reading.

chloride solution in the required amount. A total of 20 ml. of water was used in each culture of 10 specimens. Food was added daily (2 ml.). The results are shown in Table 6 and Fig. 3.

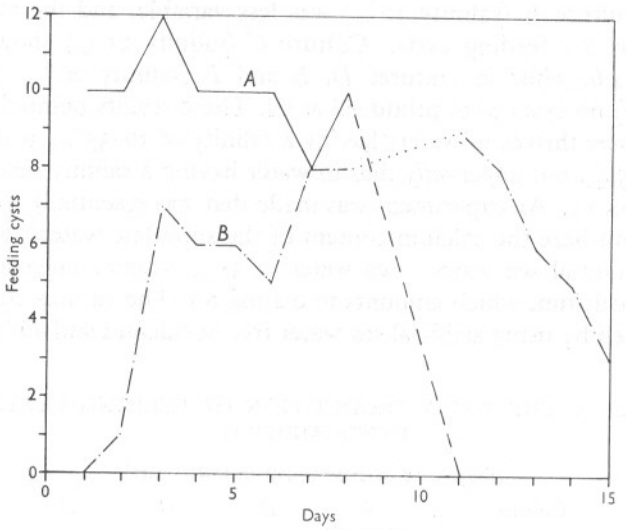

Fig. 3. Rate of feeding in culture *A* (salinity 35‰; Ca 8·0 mg./20 ml.) and culture *B* (salinity 25‰; Ca 8·0 mg./20 ml.) in Expt. 11.

Unfortunately, the control specimens died between the 8th and 11th days, but, over the 8 days they were known to be alive, they averaged a daily production of 10 cysts. Culture *B* (salinity 25‰) produced results closely similar to that of Expt. 10, having a daily average of 4·8 cysts (cf. 4·6 in Expt. 10). Cultures *C* and *D* (salinity 20‰ and 16‰, respectively) produced no cysts. Thus, the effect of calcium in this instance appeared to be negligible, and it is pleasing to note that the results of Expts. 10 and 11 compare very favourably. However, Expt. 11 was repeated (see lower part of Table 6) and the results were lower, although showing the same trend; the average daily production of the control was 5·5, for culture *B* (salinity 29‰) 4·3 cysts, culture *C* (salinity 25‰) 2·5 cysts and culture *D* (salinity 21‰) no cysts.

Experiment 12. As Expt. 11 had shown no advantage arising from higher calcium contents in subsaline waters, in this experiment calcium was excluded as far as possible. Calcium-free artificial sea water was used, 20 ml. per culture. The only introduction of calcium was via the food. Each day the water was changed and 2 ml. food added so that no culture ever contained more than 0·8 mg calcium (Table 7).

The average daily feeding cyst production was not unlike that of Expt. 11. In view of the fact that both the control (containing calcium) and culture *B*

(calcium 'free') averaged 5·5 feeding cysts daily, it may be assumed that from the point of view of feeding, a deficiency of calcium is not important.

TABLE 7. THE DAILY PRODUCTION OF FEEDING CYSTS IN EXPERIMENT 12

Culture ...	*A* (control)	*B*	*C*	*D*	*E*
Salinity (‰) ...	35	35	30	25	20
Calcium (mg) ...	8·8	0·8	0·8	0·8	0·8
Day					
1	2	3	2	3	0
2	5	8	6	3	0
3	8	9	5	2	End
4	7	5	4	3	
5	9	5	4	1	
6	2	3	0	0	
Daily average	5·5	5·5	3·5	2·0	0

Experiment 13. A natural extension of these results was to test the reaction of *E. crispum* to a progressive lowering of salinity. Sea water was used and diluted with distilled water; 2 ml. of food were added daily. The results are shown in Table 8 and Fig. 5. The effect of reducing the salinity slowly appears to be more catastrophic than a sudden change.

TABLE 8. THE DAILY PRODUCTION OF FEEDING CYSTS IN EXPERIMENT 13

Day	Control, Salinity 37‰ Feeding cysts	Experiment Salinity (‰)	Experiment Feeding cysts
1	9	37	8
2	11	35	9
3	7	34	0
4			
5	20, av.7	33	1
6			
7	5	32	0
8	5	—	End

It will have been noticed that throughout all these salinity experiments, although there is some variation in the daily feeding cyst production at any one salinity, there is a common trend. Namely, the rate of feeding decreases with the lowering of salinity; this is an important phenomenon from an ecological viewpoint. At the end of several of the previous experiments, specimens which had been in subsaline waters for several days were returned to normal sea water. It was noticed that they quickly resumed normal feeding rates, and so they were obviously not permanently affected by the unfavourable environment.

Experiment 14. An experiment was designed to test the endurance of *E. crispum* to lowered salinities. First, 8 cultures, each of 10 specimens, were set up with food to 'fatten-up' the specimens. After three days, experimental conditions were introduced (Table 9). The cultures were kept in constant temperature rooms, Series 1 being at 8° C, the winter mean temperature for the mouth of the English Channel, and Series 2 at 16° C, the summer mean temperature. The salinities 20‰ and 15‰ were selected as *E. crispum* had

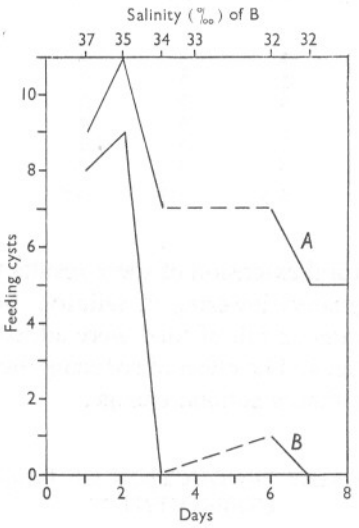

Fig. 4. Rate of feeding of the control, culture *A* (salinity 37‰), and culture *B* (salinity lowered from 37 to 32‰) in Expt. 13.

TABLE 9. THE CULTURES USED IN EXPERIMENT 14

Culture	Temperature (° C)	Salinity (‰)	
A	8	20	Series 1
B	8	20	
C	8	15	
D	8	15	
E	16	20	Series 2
F	16	20	
G	16	15	
H	16	15	

not fed at these salinities in previous experiments. Series 1 had variable amounts of artificial light but sufficient to promote the growth of the food (*Phaeodactylum*) while Series 2 had continuous artificial light. After a time, some of the cultures in Series 2 developed a white precipitate. This was thought to be calcium carbonate deposited due to removal of carbon dioxide from the water by *Phaeodactylum* during photosynthesis.

From Table 10 and Fig. 5 it will be seen that in culture *A* having a salinity of 20‰ and temperature of 8° C, the foraminiferids did not produce any feeding cysts until the 4th day, after which a low daily production was maintained until the 15th day. Then, the water was changed to sea water and further food added. On the 17th day, cyst production was resumed. Thus, after 15 days immersion in water of 20‰ salinity, the specimens survived. Culture *E* of Series 2 was set up identical with culture *A* except in that the temperature was 16° C. Cyst production started on the 2nd day and continued at a high rate until the 4th day. It was then observed that a white precipitate had formed and no further feeding cysts were produced even after the return to normal sea water on the 15th day. It would appear that the lowered temperature aided *E. crispum* in culture *A* to withstand the lowered salinity.

TABLE 10. THE DAILY PRODUCTION OF FEEDING CYSTS IN EXPERIMENT 14

	Series 1				Series 2			
Day	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
1	0	0	0	0	0	0	0	0
2	0	0	0	0	8	8	0	0
3	0	0	0	0	9	9	0	0
4	3	6	0	0	2*	10	0	0
7	2	2	0	0	0	10*	0	0
8	4	2	0	0	0	0*	0	0
9	2	0	0	0	0	0	0	0
10	2	1	0	0	0	0	0	0
15	*x*	*y*	*x*	*y*	*x*	*y*	*x*	*y*
16	0	4	0	0	0	0	0	0
17	3	7	0	0	0	2	0	0
38	num	*x*, num	10	*x*, 3	0	*x*, 0	0	*x*, 0
39	End	3	End	0	End	3	End	0
43		10		0		3		0
44		5		0		0		0
45		4		0		1		0

* Indicates precipitation of the white material.
num, Too many to count.
x, Water changed to salinity 35‰.
y, Water replaced maintaining correct salinity.

In culture *B*, having a salinity of 20‰ and a temperature of 8° C, cyst production, as in culture *A*, commenced on the 4th day and continued almost without a break until the 38th day. The culture was then returned to normal sea water and feeding continued. Culture *F* was the analogous culture in Series 2, having a temperature of 16° C. As with culture *E*, feeding commenced on the 2nd day and the rate was high. After the precipitation of the white substance on the 7th day, no further feeding cysts were observed. Feeding was resumed at a slow rate after the water had been changed on the 15th day. On the 38th day, the culture was returned to normal sea water which led to more

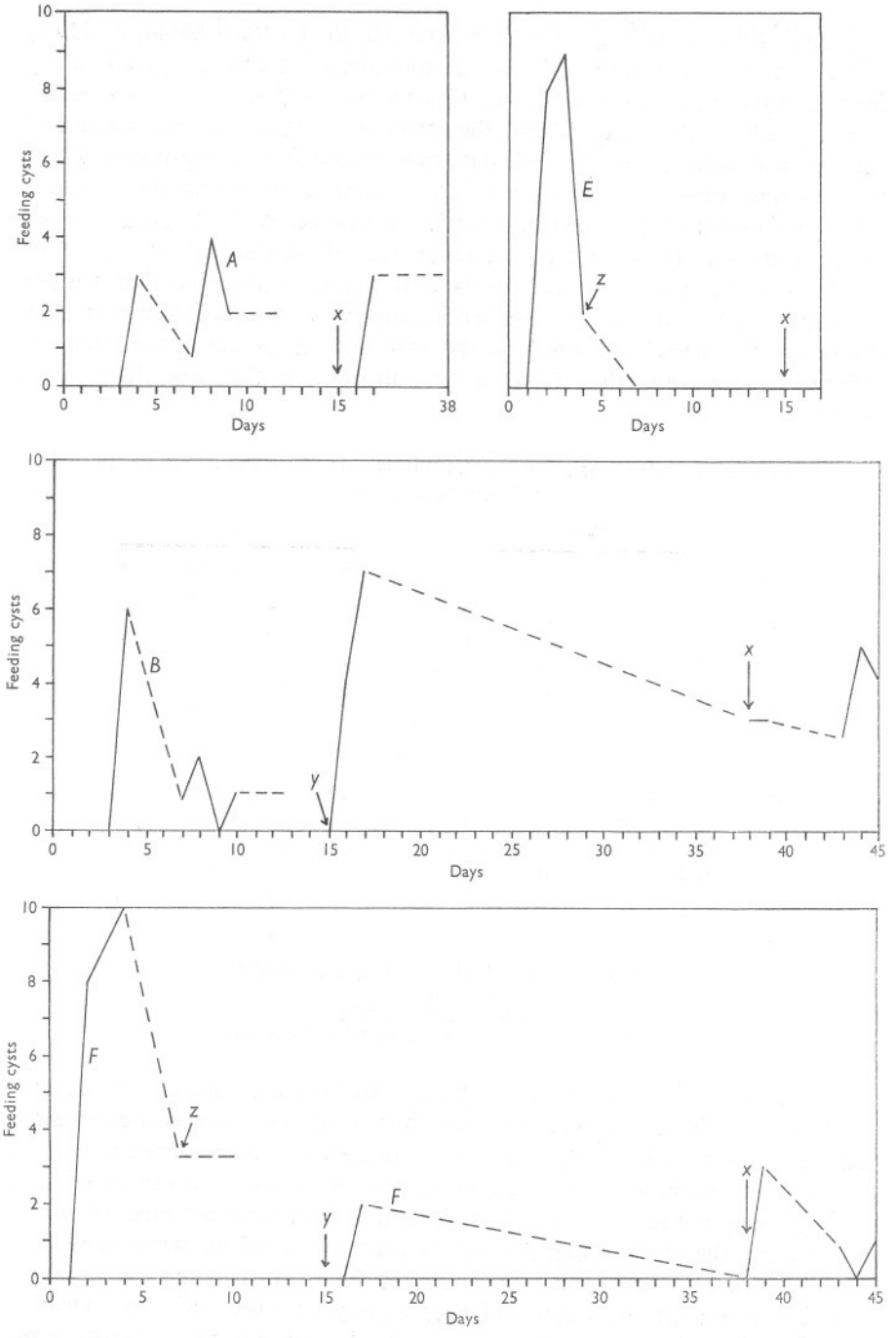

Fig. 5. Rate of feeding of cultures *A*, *B*, *E* and *F* in Expt. 14. See text for full description. (*X* indicates return to normal sea water and food; *Y* indicates water changed and food; *Z* indicates the formation of a precipitate.)

active feeding. Comparing cultures *B* and *F*, it would again appear that the low temperature of *B* had been an advantage. However, in both cultures the lowered salinity (20‰) was tolerated for 38 days.

Cultures *C* and *D* of Series 1, and *G* and *H* of Series 2 had water of 15‰ salinity. Culture *C* was the only one in which the specimens survived. No cysts were produced during the 15 days in salinity 15‰ but on the return to normal sea water, cysts were produced once more. Therefore some specimens must have survived. Thus, there seems to be little doubt that *E. crispum* can tolerate lowered salinities for considerable periods of time. Survival is better when the temperature also is low, and therefore a sudden lowering of salinity must be far more harmful in summer than in winter.

TABLE 11. AVERAGE LONGEST DIAMETER OF 10 *ELPHIDIUM CRISPUM* IN EXPERIMENT 15

	Culture		
Day	*A*	*B*	*C*
1	0·442	0·460	0·443
7	0·455	0·461	0·441
15	0·449	0·460	0·443
22	0·446	0·461	0·443
44	0·666*	0·657†	—

* Average of 7 living specimens.
† Average of 3 living specimens.

TABLE 12. THE TIMES AND QUANTITIES OF FOOD GIVEN TO EACH CULTURE IN EXPERIMENT 15

Day	Food (ml.)
1	2
7	2
15	2
22	5
30	5

Experiment 15. A final experiment was concerned with the effects of various salinities on the growth and development of juveniles of *E. crispum*. Three cultures were established, *A* having a salinity of 35‰, *B* a salinity of 30‰ and *C* a salinity of 26‰, each of 10 perfect juvenile individuals (i.e. the tests showed no deformities). The average longest diameter of the specimens in each culture was recorded (Tables 11 and 12).

From the size measurements carried out up to the 22nd day, there was no evidence of increase in size in any culture. On this day, it was noticed that the few chambers which had been added were small and of 'winter' size (Myers, 1943). This produced a notch in the outline of the test as shown in Fig. 6. To rectify this, the food added was increased to 5 ml. per culture. When the next feeding took place on the 30th day, no size measurements were made.

The second notch visible on the living tests may be related to the few days immediately preceding the 30th day, the greater supply of food on days 22 and 30 leading to the formation of larger chambers.

On the 44th day, the number of survivors was counted, there being 7 in culture *A* (salinity 35‰), 3 in culture *B* (salinity 30‰), and none in culture *C* (salinity 25‰). This is in rough accordance with the results of Expt. 14. The difference in the mean increase in size in the living specimens in salinity 35‰

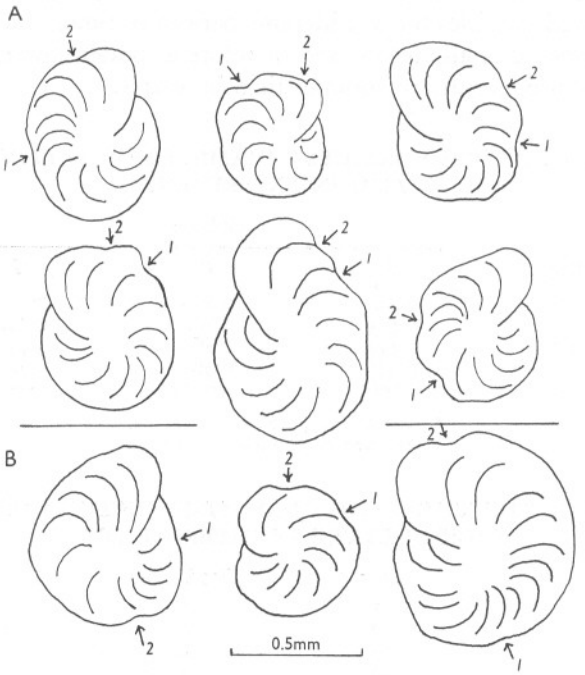

Fig. 6. Camera lucida drawings of *E. crispum* from cultures *A* and *B* of Expt. 15 showing notches in the keel produced by retarded growth during a period of food shortage.

from 0·442 to 0·666 mm, and that in salinity 30‰ from 0·460 to 0·657 mm, does not suggest any direct influence from lowered salinity. Rather, the rate of growth and resultant shape of the test is very closely related to the amount of food available.

Discussion. Bradshaw (1955, 1957, 1961) has been the main worker concerned with the effects of salinity of foraminiferids under experimental conditions. In the results of some preliminary experiments (1955) he noted that *Rotaliella heterocaryotica* Grell did not grow at salinities of 16·8–20·1‰, but active growth took place when the salinity reached 23·5‰. However, it ceased again at a salinity of approximately 37‰. Further experiments on '*Streblus beccarii* (L.) var. *tepida* (Cushman)' were concerned with the effects

of temperature and salinity on growth (1957). Salinity was seen to affect the rate of reproduction; at salinities of 20–40‰ the average time for reproduction was 23 days, but at a salinity of 13‰ it took 48 days. Salinities of 50‰ and greater retarded growth and inhibited reproduction, but did not cause death. In his most recent paper, Bradshaw (1961) determined the minimum lethal salinities of four species. The brackish water form, *Ammonia beccarii tepida* survived for 32 min in distilled water. Experiments on the rate of growth of this species showed it to be highest at a normal salinity of 34‰ and static at 8‰. Cultures kept at salinities of 8 and 10‰ for 2 weeks showed no ill effects when returned to normal sea water. He found that the forms cultured in normal salinities had smaller tests than those cultured at lower salinities.

PALAEOECOLOGICAL CONCLUSIONS

In relation to the great diversity of foraminiferid types, our knowledge of their feeding habits is negligible. Very few species have been cultured in the laboratory and few experiments on feeding have been carried out. So far, it is known that both living and dead representatives of unicellular algae are acceptable as food, while Buchanan & Hedley (1960) have demonstrated experimentally that the pseudopodia of *Astrorhiza limicola* Sandahl are able to adhere to small living animals and that these are digested extracellularly. It has been a matter for speculation that bacteria play an important role in the food supply. Only Bradshaw (1961) and Lee *et al.* (1961) have attempted to use antibiotics to remove bacteria. Not surprisingly, an antibiotic which will kill bacteria may also adversely affect the foraminiferid. Thus, at present, nothing further can be said about their part in the food supply.

Myers is the only author who has attempted to relate the growth and reproduction of a foraminiferid to the phytoplankton available in the sea. He found that *E. crispum* grows fast and reproduces when the spring outburst of phytoplankton occurs. In this respect foraminiferids are similar to other small animals which depend on microscopic plant life for food. In the present experiments and those described by Bradshaw (1961) it can be seen that foraminiferids will survive for considerable periods when the food supply is insufficient. This is marked by a contraction of the protoplasm to the earlier chambers and a cessation of growth. This is important from the ecological and palaeoecological points of view. It means that foraminiferids can withstand a shortage of food for some weeks at least. When food becomes abundant they immediately resume active feeding and then start to grow. Bradshaw (1957) has demonstrated that they will quickly and regularly reproduce when conditions remain favourable. Conditions are more likely to be unfavourable for part of the year in temperate and arctic regions (particularly temperature) than in tropical regions. Thus Myers (1942) observed continuous growth and

a completion of the life cycle in 1 year in *E. crispum* in the Java Sea because of the more amenable conditions.

The relationship of foraminiferids to salinity changes is of great interest to palaeoecologists, but is unfortunately a subject about which very little is known. The main effects of salinity on a foraminiferid should be concerned with the chemical composition of the water and osmosis. The latter is most relevant to the experiments under discussion. It is generally assumed that microscopic marine animals are isotonic with sea water and they are therefore described as poikilosmotic. Freshwater animals are hypotonic to their environment and are described as homoiosmotic. Brackish-water animals may be either homoiosmotic or poikilosmotic. However, an homoiosmotic state implies that the organism must be capable of osmoregulation. *E. crispum* is a stenohaline marine organism and therefore should be poikilosmotic. It would be expected that exposure to a subsaline medium would result in an increase in its turgidity leading ultimately to death. The experiments here described refute this. In Expt. 14, a period of 38 days at a salinity of 20‰ was survived apparently without harm. The main effect shown by reduced salinities in these experiments has been a reduction in the rate of feeding. Lowered temperatures were found to be advantageous in surviving a period of reduced salinity. This may be due to the fact that osmotic pressure and temperature are related. Taking the data of Expt. 14, the osmotic pressure of salinity 20‰ (chlorinity 11·1‰) is 14·19 atmospheres at 25° C (Robinson, 1954). Using the conversion factor

$$1+\frac{t-25}{298}$$

where t (°C) is another temperature, the result is 13·76 atmospheres at 16° C and 13·38 atmospheres at 8° C. However, the difference is very small in the present experiment although in comparing tropical and arctic conditions it would be much more significant (14·33 atmospheres at 30° C and 13·24 atmospheres at 5° C). It is, of course, possible that the main effect of lowering the temperature is not so much concerned with lowering the osmotic pressure as with slowing down the metabolic processes of the foraminiferids.

The only experiment described here concerning growth is Expt. 15. It is suggested from the results that the size of the chambers added on to *E. crispum* is related to the amount of food available. During the experiment when food was short, the newly added chambers were small and consequently the largest diameter did not increase (see Table 11). Much apparently conflicting evidence has been put forward to explain differences of overall size. They range from the obvious shortage of food, through deficiencies of oxygen and excesses of phosphates, to unfavourable salinities and unfavourable temperatures.

Myers (1943) observed that in populations of *E. crispum* living in Plymouth Sound, those in the sublittoral zone grew faster and larger than those in the

littoral zone and considered this to be related to the amount of food available. Boltovskoy (1954) suggested that a deficiency of food was responsible for the paucity of the population, the small size of the specimens, and for irregularities in the tests of foraminiferids along the Patagonian shelf. If inorganic particles of similar size to the food particles are widely accepted as food (Expt. 5) the wasted effort may partly account for the smaller size of the foraminiferids living on mud bottoms. Said (1953) noted that the foraminiferids of Great Pond, Massachusetts, were smaller than those in Vineyard Sound and thought it was probably due to lower oxygen concentrations. However, the Pond was also subject to salinity and temperature variations and the bottom was mud. Howes (1939) put forward the suggestion that if there was an abnormal phosphate content in the body fluids of coelomate animals, this would cause a change in the calcium metabolism, perhaps leading to smaller shells.

In 1904, Metcalf described the occurrence of dwarf specimens of *Neritina virginica* in salt ponds in Jamaica (see Metcalf, 1930). They were less than half the size of marine representatives. Gunter (1947*a*, *b*) noted that in following a salinity gradient from a river to the sea, the variety of animals and their size increased with increasing salinity. Sinclair (1947) criticized this statement and pointed out similar changes along temperature gradients, but in fact he was discussing a separate matter. Evidence in favour of Gunter's statement is seen in Expts. 11, 12, 13 and 14. The rate of feeding in *E. crispum* was lowered as the salinity fell and therefore growth fell. However, Bradshaw (1957, p. 1145) observed that, 'When reproduction is delayed by unfavourable temperatures and salinities, and other factors, food, etc., remain favorable, growth may continue as long as the conditions are not excessively detrimental. The specimens may thus finally reproduce at a larger size and with a greater number of chambers than would be true under more favorable circumstances'.

The evidence from Bradshaw's experiments (1961) shows that this statement clearly holds true for adverse temperatures; that is, forms living at lowered temperatures tend to grow large. The relationship is less obvious with salinity although perhaps still true for *small* changes. The field evidence noted above supports this concept. It is of interest that Lankford (1959) observed that in the Mississippi Delta area, the largest living populations occurred where sedimentation was most rapid and the individuals were smaller; again, this demonstrates that forms living close to their optimum are smaller than those that are not.

It is generally true that animals living in estuaries are smaller than their marine counterparts. Certainly, the overall size of estuarine foraminiferids is very much less than that of marine forms. However, estuarine foraminiferids are usually distinct from marine forms although they may be found mixed together in inshore waters. An animal living in estuarine conditions,

which are more rigorous than marine conditions, can be considered to do so either because it is well adapted to the environment, or because it cannot compete with its marine counterparts and must therefore exist where they choose not to. The first possibility seems most likely. If we accept this, it follows that optimum conditions must prevail for part, at least, of the life of these forms. The factor which is most likely to be responsible for their small size is the availability of calcium. Sea water of salinity 35‰ contains 400 p.p.m. calcium whereas in comparison the hardest of river waters contains an almost negligible amount. As brackish water usually results by the dilution of sea water with river water, it follows that subsaline waters contain less calcium than sea water. The author found that this affected both agglutinated and calcareous foraminiferids at Christchurch Harbour (in preparation) and led to their smaller size.

This is not intended as a comprehensive analysis of the factors controlling size, but it does reveal three trends. First, a shortage of food will lead to small size. Secondly, field and experimental evidence shows that forms living close to their optimum will reach maturity and reproduce at a smaller size than those living under adversely lowered temperatures and slightly lowered salinities. Thirdly, in estuarine conditions the shortage of calcium will be responsible for small size, although all the animals will live at their optimum for part of the time.

SUMMARY

Fifteen experiments on the foraminiferid *Elphidium crispum* (L.) are described. They deal with feeding, movement, the substratum, and salinity, and their bearing on ecology.

The daily production of feeding cysts was used as a measure of the metabolic rate and healthiness of the foraminiferids. *Phaeodactylum tricornutum*, both living and dead, was used as food; a distinct preference was shown for living food. However, kaolin and graphite having a similar size to the *Phaeodactylum* were also accepted as 'food'. It is suggested that *E. crispum* selects its food on the basis of size. The colour of the protoplasm is shown to be closely related to the pigments of the food.

Movement in the horizontal plane is normally random. However, this species prefers a clean, hard substratum to one of clay; once a specimen has escaped from a clay substratum, it is loathe to return to it and therefore movement in this instance must be directed and not random.

The rate of feeding is shown to be closely related to the salinity of the water, the feeding rate decreasing with the salinity. The amount of calcium present in sea water or in subsaline waters does not appear to affect the rate of feeding. However, lowered temperatures help *E. crispum* to survive for longer periods in subsaline water. At temperatures of 8° and 16° C., cultures survived subsaline water of 20‰ salinity for 38 days, but at a salinity of 15‰, only the culture kept at 8° C survived (for 15 days).

A shortage of food was considered to be the cause of the formation of small chambers which produced notches in the outline of the tests of juvenile specimens. A general review of the palaeoecological import of these results is given together with a brief discussion on size.

I should like to express my gratitude to the Commissioners for the Exhibition of 1851 for the Senior Studentship which made this work possible, to Dr F. S. Russell for allowing me the use of the facilities at the Plymouth Laboratory, to Dr M. Parke who so kindly provided me with *Phaeodactylum*, and to Dr R. H. Hedley for his valuable and constructive criticism of the final manuscript.

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