Ecological and Life History Studies upon a Large Foraminiferan (*Discobotellina biperforata* Collins 1958) from Moreton Bay, Queensland

II. Aquarium Observations

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ECOLOGICAL AND LIFE HISTORY STUDIES UPON A LARGE FORAMINIFERAN (*DISCOBOTELLINA BIPERFORATA* COLLINS 1958) FROM MORETON BAY, QUEENSLAND

II. AQUARIUM OBSERVATIONS

SUMMARY

Discobotellina biperforata moves bodily in aquaria, extruding pseudopodia mostly from its margin.

Survival in aquaria is (1) extended in muddy as against clean aquaria; (2) not prolonged in the presence of mineral sands (e.g. ilmenite) which are normal test constituents; (3) reduced by handling; (4) dependent upon population density; and (5) longest at salinities approaching those of oceanic water.

Specimens feed upon something associated with mud, upon diatoms, and possibly upon blue-green algae. However dense concentrations of the blue-green *Oscillatoria amphibia* reduce survival times. Variations in longevity between samples from nominally identical aquaria are probably due to lack of control over the associated micro-organisms.

A few cases of changes from one morphological form to another were recorded, and also limited growth in certain aquaria.

I. INTRODUCTION

Aquarium observations were undertaken initially to confirm that *Discobotellina biperforata* is a foraminiferan. This was far from obvious, and Collins (1958, p. 343) stated in his original description: "This form is very different from any previously

described genus, and at first raised considerable doubts as to its foraminiferal nature." Similar doubts were expressed by Dr. R. Hedley of the British Museum of Natural History when identifying present material.

Extended survival experiments were undertaken in the hope that growth of

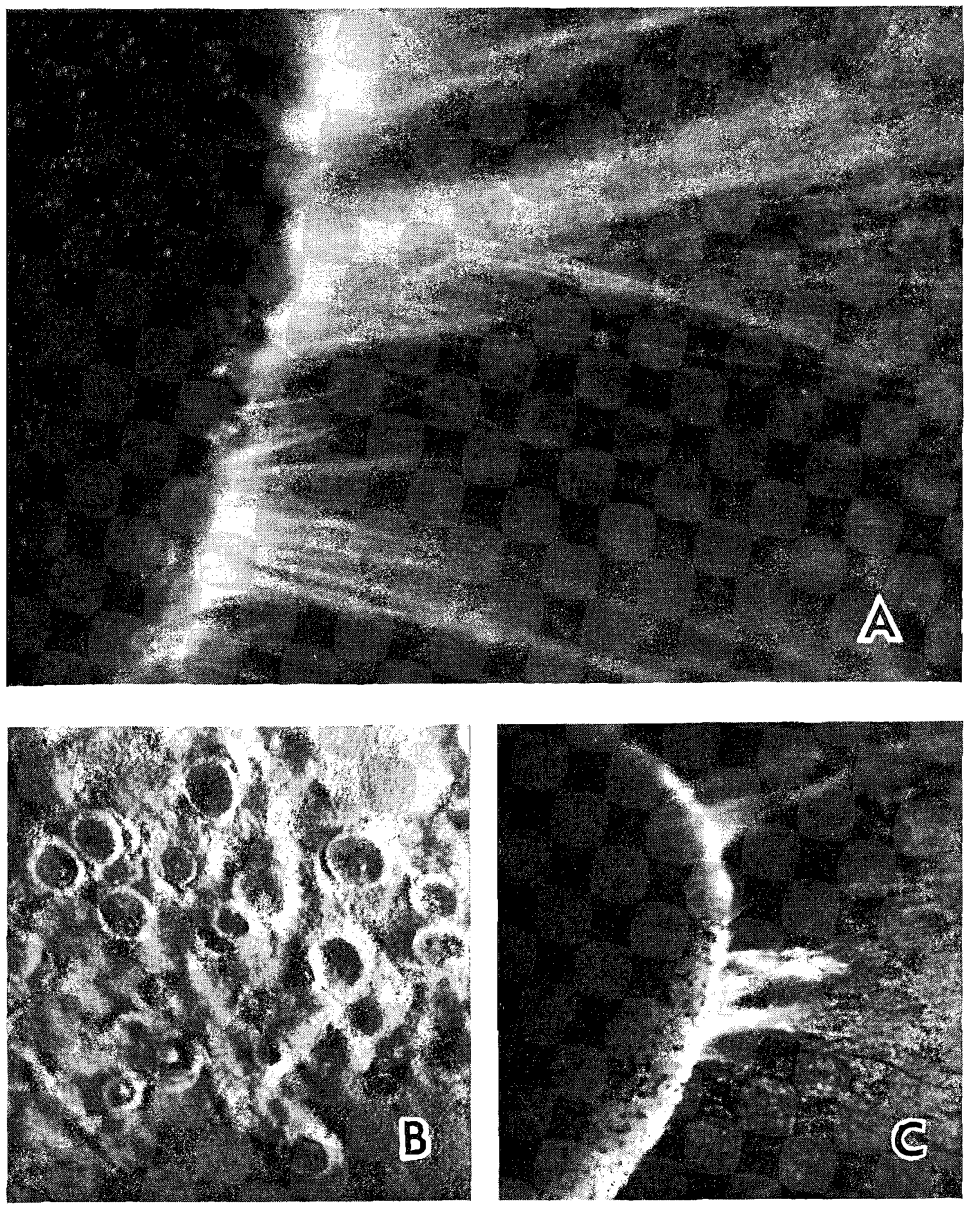

PLATE I

A, B—Pseudopodia of *Discobotellina* through aquarium walls; A, width of view ca. 10 mm; B, width of view ca. 20 mm. C—tracks of movement after 12 hr in sand aquarium; diameter of specimens ca. 20 mm.

specimens would (1) confirm the postulated life history (Stephenson & Rees, 1965); (2) indicate growth rates in nature; (3) in calcareous media provide specimens which after decalcification would be amenable to cytological study. It was also hoped the experiments would give pointers to the critical environmental factors in natural habitats (Bradshaw, 1955, 1961).

No attempts were made to simplify the biotic environment down to the presence of only few organisms (see Lee *et al.*, 1963). The "agnotobiotic" approach of most workers studying Foraminifera in the laboratory was followed.

II. EVIDENCE THAT *DISCOBOTELLINA* IS A LIVING FORAMINIFERAN

Specimens from north of Peel Island, Moreton Bay were kept in three aerated aquaria in dim natural light. The substrata of sand, find sand, and mud* respectively all contained sufficient mud to produce a settled surface layer of fine particles. Twelve hours after introduction specimens had moved up to 6 cm, leaving visible tracks (Pl. I b).

Later some climbed the aquarium walls, through which could be seen, around each specimen, radiating processes composed of hundreds of filaments. These showed faint interference colours, were up to 10 mm in length, slowly extended and retracted, and were typical foraminiferan pseudopodia (see Pl. I a, c). Encrusting film had been removed from the tracks of movement on the glass.

Observation in petri dishes was attempted, but here pseudopodia were not extruded; apparently the surroundings require conditioning by immersion.

When specimens lay horizontally on mud, this was disturbed to a distance of 1 cm by radiating pseudopodia. On removal from aquaria some loosely adherent grains fell from the under surface, and evidently pseudopodia were present here too. Sometimes particles with a light elastic attachment were observed on the upper surface. This and the removal of settled mud particles from the upper surface further indicate pseudopodial presence.

In sand and fine sand, movement usually ceased after a few days and specimens built under themselves mounds 1–2 mm high. This hillock formation is due to particles brought inwards by pseudopodia. In soft mud, hillock formation presumably minimizes any tendency to sink below the surface.

Criteria were established progressively for determining whether or not specimens were alive:

(i) movement of specimens as a whole;

(ii) clearing from upper surface of mud particles settled from turbid waters;

(iii) boundary between rim of animal and surrounding substratum indefinite. Outer sand grains are retained semi-elastically and have a characteristic feel to the fingertips. Early confusion was caused by loose grains on dead specimens. This is due to erosion, and such specimens can be recognized by peripheral irregularities and the absence of elastically attached particles;

(iv) test not blackened. Except in complete absence of mud, the test becomes black and fragile the day after death. Sometimes partially black specimens were found, but with remaining portions showing some signs of life; within a day these specimens died. If left undisturbed, blackened specimens disintegrated into a mass of normally coloured sand grains.

III. SURVIVAL EXPERIMENTS

Throughout, the general forms of survival curves and the times in days for 50 per cent casualties are given.

*These terms are defined on p. 243.

Preliminary Experiment

Observations in section II above were extended to a survival experiment. Survival curves (see Fig. 1) showed a rapid initial decline, followed by a plateau, then by a

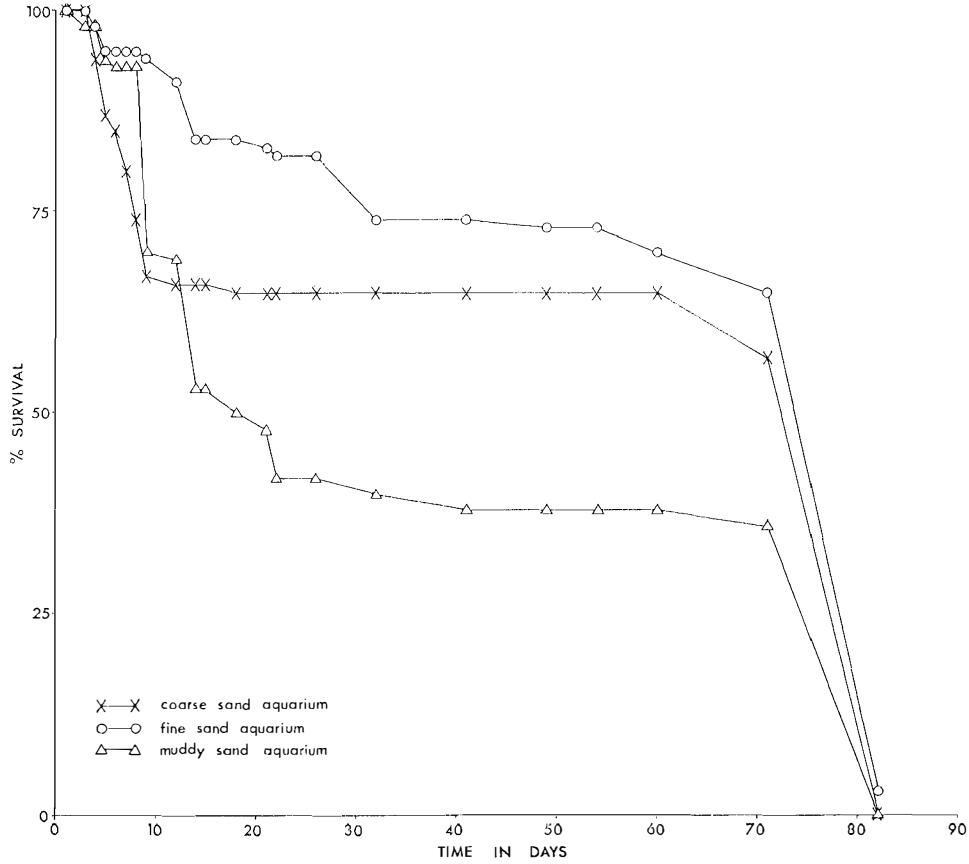

FIG. 1.—Survival curves, Preliminary Experiment.

catastrophic decline. This last coincided with hot weather, the maximum weekly water temperature of 31.5°C being 2.5°C above that of the preceding week. Fifty per cent survival times (see Table 1) were just over 70 days in sand and fine sand aquaria, and 18 days in a mud aquarium.

TABLE 1

FIFTY PER CENT SURVIVAL TIMES (IN DAYS) WITH DIFFERENT SEDIMENTS

Experiment Number	Coarse Sand	Sand	Fine Sand	Muddy Sand	Mud	Coral Sand	Mixed Mineral	Ilmenite	Fine Sand and Rutile
Preliminary	—	72	74	18	—	—	—	—	—
I	10	3	2	15	28	15	13	14	15
II	20	20	19	34	46	29	29	21	22
III	30	34	28	50 56	65 90	33 63	39	29	30

General method

In the remaining experiments, general conditions were:

Aquarium size

Surface 42 x 24 cm, depth 20 cm; substratum depth ca.4 cm except where otherwise stated.; water depth ca.10 cm.

Aeration

By oil-free compressor through porous stone cubes. A compromise was struck between vigorous and feeble aeration, because with the former salinities increased rapidly.

Temperature

Aquaria were kept in a constant temperature room at 23°C, where water temperatures were accurate to ca. ± 0.1°C.

Salinity

Measured by temperature compensated conductivity cell (Hamon, 1956) calibrated at intervals with results accurate to ± 0.1 ‰. In Experiments I–IV salinities were adjusted weekly. The extreme range of 32–35 ‰ occurred during vigorous aeration in dry weather; the normal range was 33–34 ‰. In Experiments V and VI measurements and adjustments were made twice weekly, and salinity increases between adjustments never exceeded 0.3 ‰.

Throughout, evaporation was reduced by (a) covering aquaria with corrugated glass plates to facilitate drip-back of condensed water; and (b) placing a large vessel full of tap water adjacent to the inlet fan of the constant temperature room.

Illumination

Because facilities were not available for diurnal changes, a choice had to be made between constant light and constant darkness. In Experiments I–IV constant light by overhead incandescent lighting was used to encourage photosynthetic organisms and provide nutriment. This gave a $2\frac{1}{2}$-fold variation in illumination between extreme positionings of aquaria. From Experiment V (inclusive) onwards the room was kept dark apart from minimal observation periods.

Substrata

In Experiments I–III various substrata were used, differing in particle sizes and mineral compositions. From Experiment IV onwards only mud (from St. Lucia) was used.

(1) *Particle size.* Marine sands containing fragmented shell were obtained from Wellington Point (Moreton Bay), oven dried, graded by sieve, and kept in aquaria for 12 months before use. Only three grades were obtainable in this manner:

(i) "Coarse sand", particle diameters 1.02–0.53 mm.
(ii) "Sand", diameters 0.53–0.21 mm.
(iii) "Fine sand", diameters 0.21–0.10 mm.

In each case finely particulate matter was present due to disintegration after sieving and the presence of micro-organisms.

Two naturally occurring sediments were used for finer grades, i.e. "muddy sand" from Wynnum and "mud" from St. Lucia. These again had been kept in marine aquaria for 12 months before use. Results of particle analyses (see Appendix for method) are given in Table 2.

(2) *Mineral composition.* The above were predominantly siliceous, with a little calcareous material. In the remainder various minerals were used:

(a) *Coral sand:* pure $CaCO_3$, fairly coarse grain (see Table 1). A little mud was added, and the whole "conditioned" in aquaria for one week.

TABLE 2
RESULTS OF PARTICLE ANALYSES ON SEDIMENTS USED IN SURVIVAL EXPERIMENTS I–III

PARTICLE SIZE IN mm	PERCENTAGE DRY WEIGHT (TO NEAREST 0.1)					
	MUDDY SAND	MUD	CORAL SAND	MINERAL SAND	ILMENITE	FINE SAND + RUTILE
>1.96	0.1	0.2	1.2	0	0	0
1.96–1.02	1.5	0.9	1.1	0	0.1	0.7
1.02–0.53 (coarse sand)	15.1	29.9	57.4	0.5	0	10.9
0.53–0.21 (sand)	76.4	19.4	39.4	74.4	67.7	84.4
0.21–0.10 (fine sand)	6.0	19.6	0.6	23.6	29.6	3.3
0.10–0.05	0.7	14.2	0.1	1.3	2.1	0.2
<0.05	0.1	13.1	0	0	0	0

(b) *Mixed mineral sand:* mixed concentrate of ilmenite, rutile etc. (for particle size see Table 2). Sediment depth was 1 cm (only).
(c) *Ilmenite:* mixed with a little mud, and as (b) above.
(d) *Rutile:* 4 cm of fine sand with a thin surface film of rutile and a little mud.

Source of specimens

Fine sandy mud in a triangle bounded by M3 beacon, Tangalooma, and Dring Banks (Moreton Bay).

Experiment I. Effects of substrata of different particle sizes and mineral compositions

Imperforate specimens (diameter 17 mm) collected 23/ii/1962 were used, with population density of 25 per aquarium.

Fifty per cent survival times (Table 1) were spectacularly less than in the Preliminary Experiment, probably due to:
(a) New aquaria, involving risks of contact with small areas of red-lead putty, and with reduced "conditioning" of surfaces.
(b) In some cases, insufficiency of food, including the absence of obvious microbenthos upon glass walls. Coarse sand, sand, and fine sand aquaria were the cleanest, and gave shortest survival times.

Experiment II. Possible causes of failure in Experiment I

The same substrata and general conditions were used. Specimens were collected on 14/iv/1962. Modifications were:
(a) Seawater was changed frequently to remove possible contaminants.
(b) Aquaria were previously kept in bright indirect daylight for three weeks to encourage plant development.
(c) Handling during collection and in aquaria was kept to a minimum.
(d) Specimens were kept in "quarantine" aquaria for four days, and those dying were regarded as damaged during collection.
(e) Twenty specimens per aquarium were used.

A typical survival curve (Fig. 2) shows that early deaths were reduced, presumably because of quarantine. The rapid final decline remained.

Survival times (see Table 1) were roughly double those of Experiment I, although still less than in the Preliminary Experiment. Survivals in the coarse sand, sand, and fine sand were effectively identical and considerably below those in muddy sand, which in turn were lower than mud. Coral sand and the "mineral" sands gave equivalent survival times to those of corresponding grades of siliceous sands.

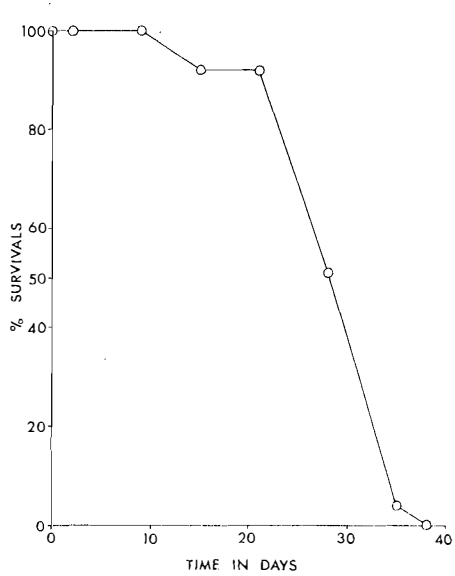

FIG. 2.—Survival curve, mud aquarium, Experiment I.

In aquaria with "abnormal" sediments, the introduced mud which originally formed a surface film became progressively concentrated round the specimens, suggesting that mud is a food source. To minimize starvation, approximately 7 ml of mud were introduced weekly into the aquaria.

Experiment III. Repetition of Experiment 2, with mud added to all aquaria

Specimens collected on 1/vi/1962 were kept four days in quarantine; 25 specimens per aquarium were used.

Mud was added to all cleaner environments, i.e. "abnormal" substrata, coarse sand, sand, and fine sand. Two coral sand aquaria were established, one receiving 7 ml of mud weekly, the other double this amount. Muddy sand and mud aquaria were duplicated.

Survivals were prolonged, being longest in muddy aquaria, followed by coral sand with double mud addition, and then by muddy sand (see Table 1). Survivals with cleaner coral sand and "abnormal" substrata were comparable to those with equivalent grades of "normal" minerals.

Nominally identical pairs of aquaria gave different survival times, apparently due to uncontrolled variables. Dense growths of the blue-green alga *Oscillatoria amphibia* C. Ag. ex Gom. were observed in aquaria with unexpectedly short survivals. A film of diatoms dominated by *Neidium affine* (Ehrenberg) Pfitzer and *Amphora proteus* Gregory covered the walls of aquaria giving longest survival times.

Survival curves (Fig. 3) again show the rapid final decline. If mud provides food, it seems unlikely that starvation is responsible. Possibly a minor nutrient becomes exhausted, and to test this in Experiment IV newly collected and re-used mud were compared.

Another possibility is spread of disease; if so it would be more obvious in overcrowded aquaria. Various population densities were used in Experiment IV. Alternatively growth of a harmful organism other than a specific pathogen (e.g. *Oscillatoria*) may have been involved. To test this, in Experiment IV light and dark conditions were compared.

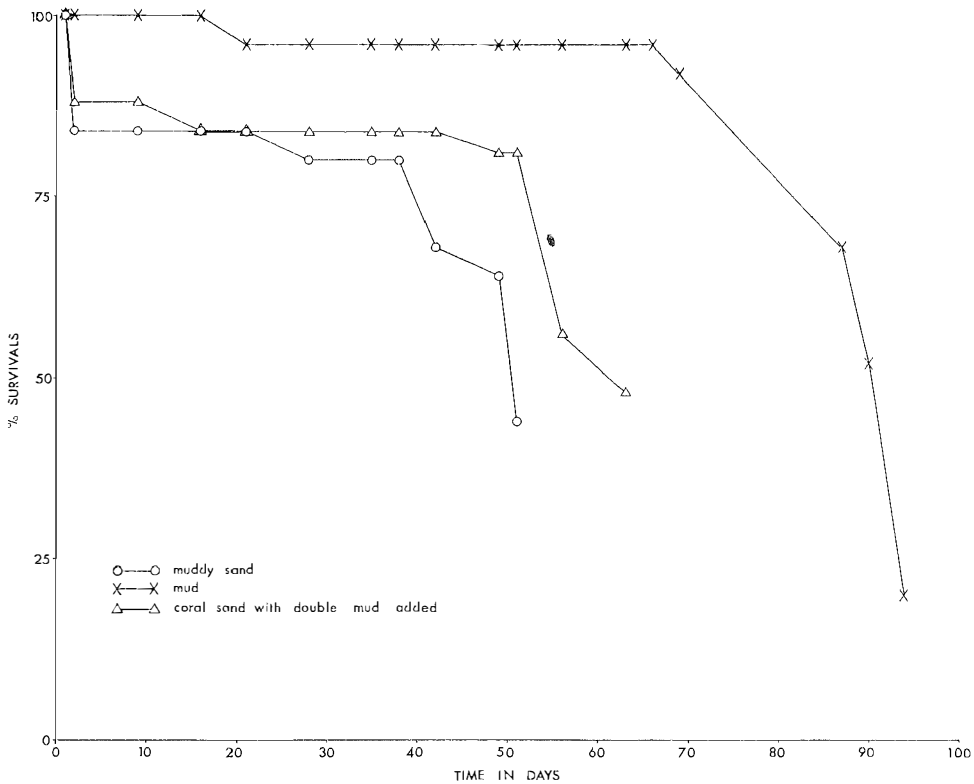

FIG. 3.—Survival curves, Experiment III.

Experiment IV. Densities of specimens, light versus dark conditions, and old versus new mud

Imperforate specimens of ca. 15 mm diameter (collected 29/x/1962) were selected after two days' quarantine. Aquaria contained St. Lucia mud previously kept in the constant conditions of the aquarium room for three months.

Numbers of specimens in aquaria were:

In light—100 (duplicated), 50 (triplicated), 20 (triplicated), 10 (quintuplicated), 5 (quintuplicated).

In dark (i.e. in closed cupboard within illuminated room)—20 (triplicated).

Of the three illuminated aquaria each with 20 specimens, two contained "old" mud unchanged from Experiments I–III, while the third contained "new" mud.

Fifty per cent survival times are given as a scatter diagram (Fig. 4) which shows:

(i) Variability between supposedly identical aquaria; with survivals of 37 and 104 days in the two densest aquaria. (The former developed dense growths of *Oscillatoria*.)

(ii) With low densities (5 and 10 specimens), mean survival time was 118 days, as against 77 days with high densities (20–100 specimens).

(iii) No obvious difference between light and dark conditions.

(iv) Shorter survivals in "old" than in "new" mud.

Early deaths were again reduced, but in most cases the final decline remained. It was delayed but not eliminated by (a) reducing densities (Fig. 5); (b) using new instead of old mud (Fig. 6); and (c) darkness instead of light (Fig. 7).

Although specimens were not measured (to reduce handling), it was apparent that, apart from one specimen, no obvious growth occurred in four months. The

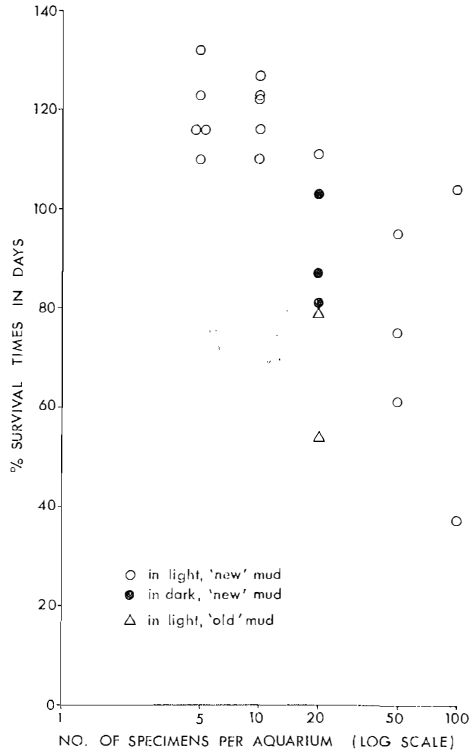

FIG. 4.—Fifty per cent survival times, Experiment IV.

exception was a specimen bearing a small bud which grew into a crescent, and was detached during gentle handling. After three months of aquarium life, ten imperforates produced uniperforates with central holes.

Experiment V. Effects of disturbance

In previous experiments, handling was avoided during initial stages when specimens were obviously alive, but later as movement declined it was necessary to examine specimens. Increased handling during experiments could have been responsible for final rapid declines.

In Experiment V effects of handling were investigated. Two obvious factors could be involved, contact with specimens and stirring sediment. Three sets of aquaria were maintained in triplicate:

(a) *Controls*—minimal handling and sediment disturbance. Inevitably these increased in the later stages.

(b) *Turbid*—as above, except that at each observation mud from an unoccupied aquarium corner was stirred up.

(c) *Turbid with handling*—at each examination specimens removed, handled and returned, and turbidities similar to those of (b) above were obtained.

Specimens were collected on 23/iv/1963, and 20 placed in each aquarium. "Old" mud from Experiment IV was used, the experiment carried out in darkness, and salinities adjusted at bi-weekly observations, to lie within the range 33·5–34·0‰. Fifty per cent survival times in days were: Controls 100, 110, 114—Mean 108; Turbid 62, 100,130—Mean 97; Turbid with handling 65, 66, 108—Mean 77.

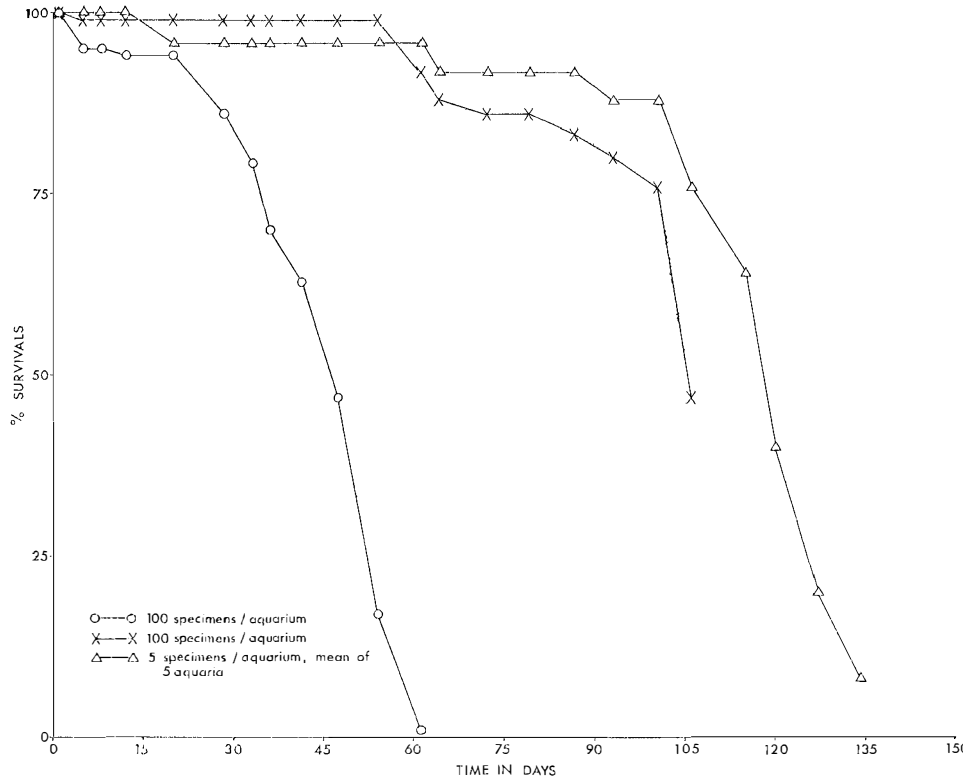

FIG. 5.—Survival curves, different population densities, Experiment IV. The two curves for 100 specimens per aquarium indicate the great variation in this experiment. (See also Fig. 4).

These data show that disturbance of specimens reduces survival times. The effects of turbidity are more doubtful; possibly they act harmfully by partially burying specimens and also beneficially by supplying food.

The survival curves (Fig. 8) confirm that the rapid decline may be due to handling.

Experiment VI. Effects of (constant) salinities

In previous experiments salinities were normally maintained in the range 33–34‰, with extremes of 32–35‰. The chosen mode of 33.5‰ approximated to the salinity of the Peel Island grounds, where early specimens were obtained, but later material came from the Tangalooma grounds where salinities are ca. 35‰ (C.S.I.R.O. Aust., 1953).

Sources of specimens, general conditions, etc. were as in Experiment V. Salinity at the collecting site was ca. 35‰, and specimens were quarantined in water of this salinity overnight, then transferred directly to one of six different salinities (in triplicated aquaria). These were maintained to ± 0.5‰ by biweekly adjustments.

Fifty per cent survivals were:

Salinity ‰	*Survival Times (Days)*	*Mean Survival Times*
37.5	<1, <1, <1	<1
35.5	130, 118, 126	125
33.7	100, 110, 114	108
31.5	19, 40, 57	39
29.5	10, 15, 19	15
25.5	<1, <1, <1	<1

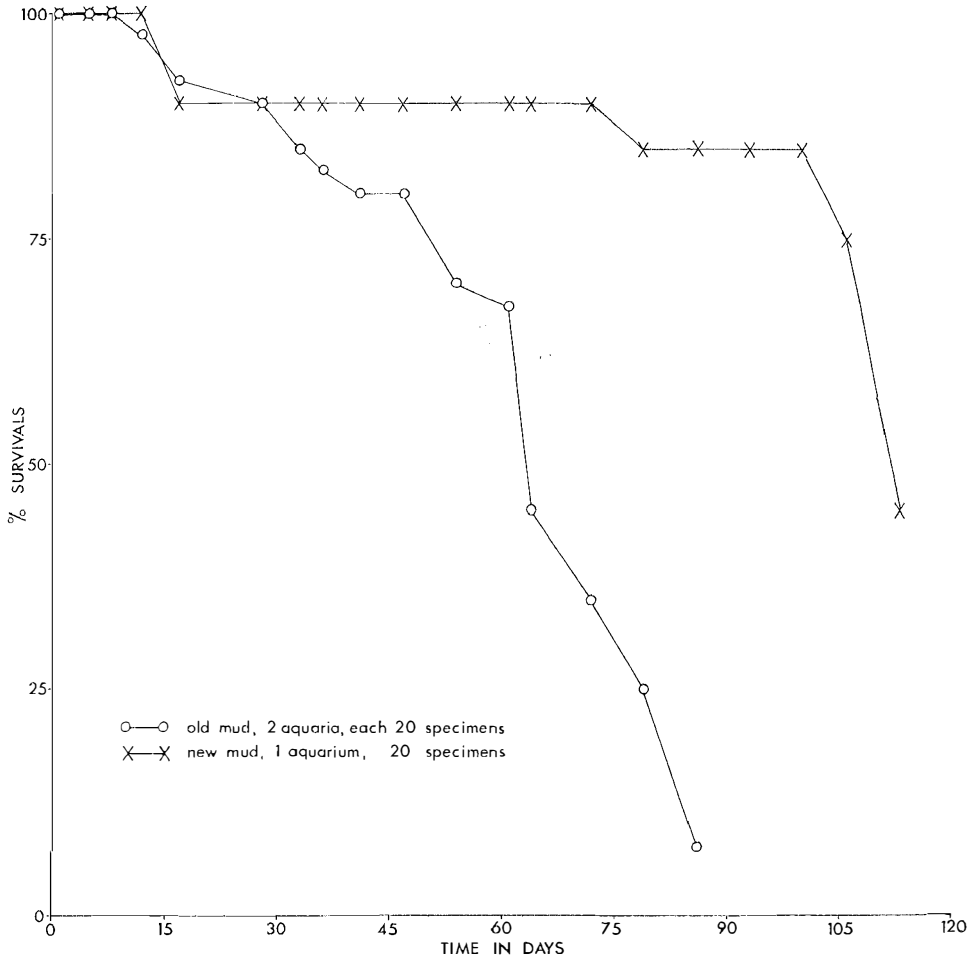

FIG. 6.--Survival curves, "new" and "old" mud, Experiment IV.

Optimum survival is in water of oceanic salinity, with a sharp decline in higher and more gradual decline in lower salinities. Experiments I--IV were conducted at salinities approximating to 33.7‰. Their shorter survivals may be due either to less frequent salinity adjustments, or to presence of light. Most survival curves were approximately linear. The rapid initial decline in 29.5‰ may be a shock effect due to the instantaneous salinity change.

Only in the present experiments was there any noticeable growth of specimens. An annulus ca. 2 mm thick was added to specimens kept four months in 35.5‰ salinities.

Experiment VII. Effects of changing salinities, to evaluate "shock effects" of rapidly reduced salinities

Media were diluted from 34.5‰ to 29.5‰ at three rates: (a) two equal stages over 7 days; (b) seven approximately equal stages over 17 days; and (c) twelve approximately equal stages over 29 days. Salinities were returned to normal by evaporation, which increased values ca. 0.3‰ per day.

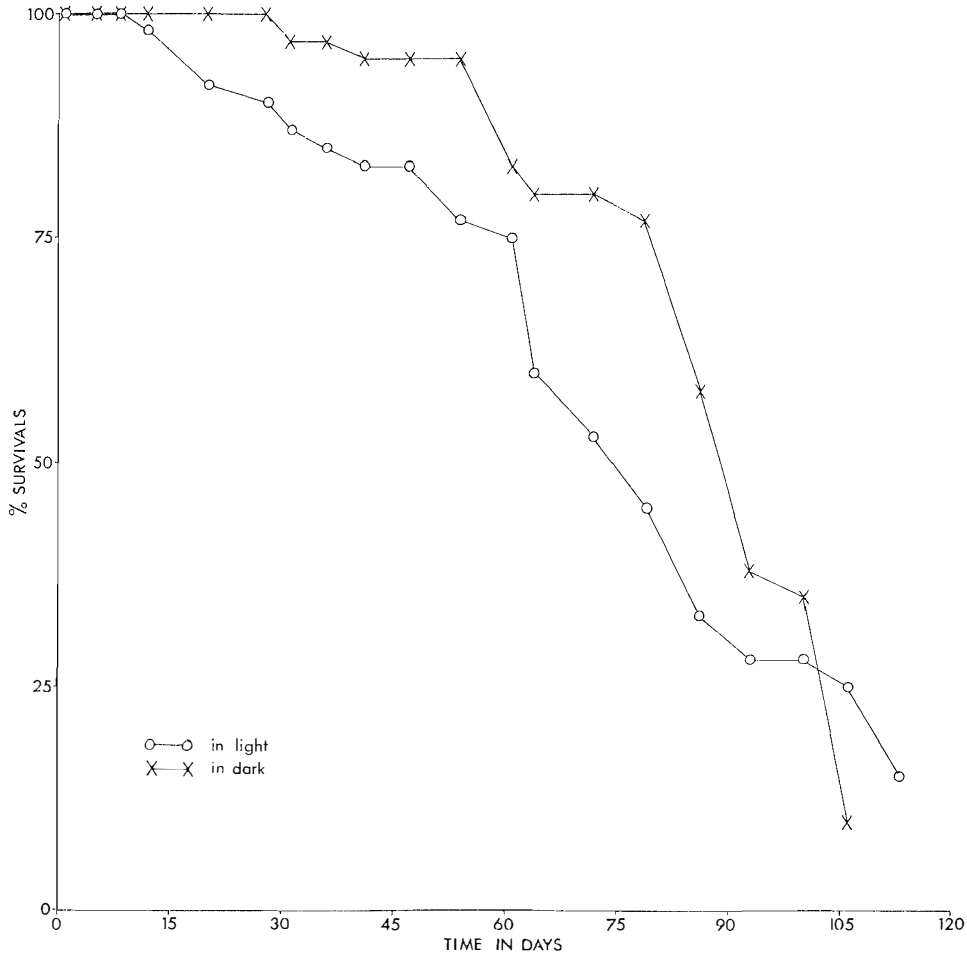

FIG. 7.—Survival curves, light and dark conditions, Experiment IV.

Fifty per cent survivals in days in duplicated aquaria each with 20 specimens were: rapid change 35, 48; moderate change 22, 21; slow change 24, 30. These equivocal results suggest that the shock effect of rapid change is approximately counterbalanced by longer exposures to reduced salinities in slower changing aquaria.

Experiment VIII. Attempted summation of optimal conditions for prolonged survival

This involved low population densities (10 / aquarium); "new" mud; transfer at two-month intervals to aquaria with replaced mud; apart from these transfers, no handling; dark conditions throughout; addition of small amounts of $CaCO_3$.

In the first attempt 96 per cent survival was obtained after 26 days, when aerator failure terminated the experiment. In the second attempt 50 per cent survival was approximately ten days, with deaths attributed to development of rust-red patches on the mud surface (due to bacterial action).

Because daily observation of aquaria was impossible, and because of the evident risk of mechanical or biological "catastrophes", further experiments were not attempted.

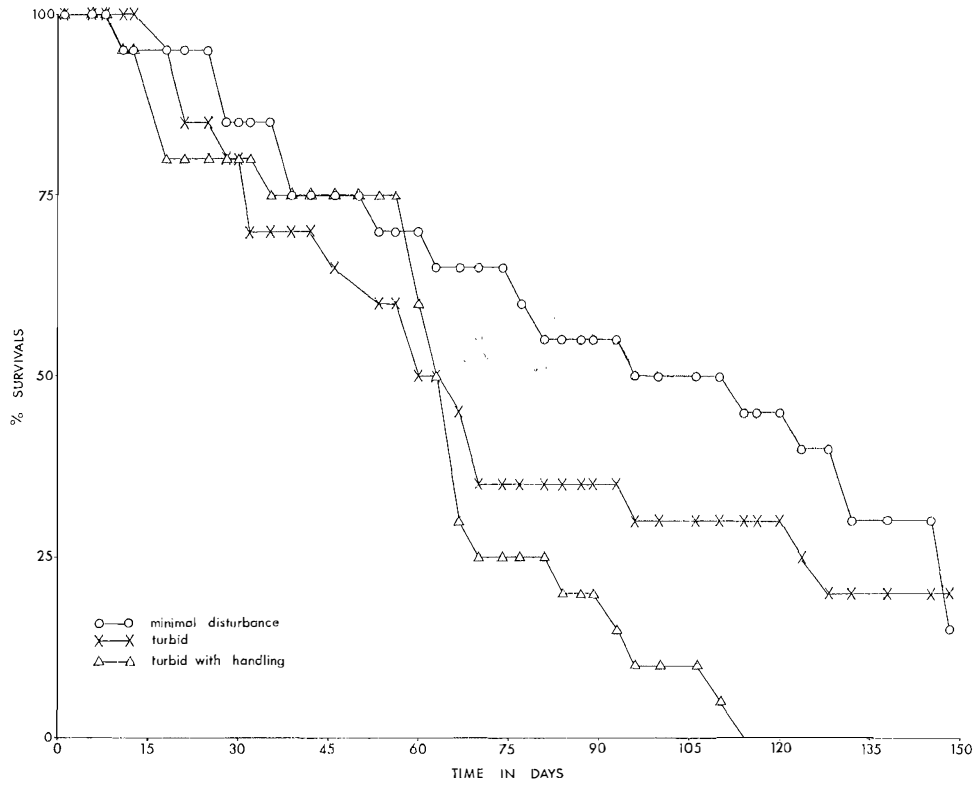

FIG. 8.—Survival curves, different degrees of disturbance and turbidity, Experiment V.

IV. DISCUSSION

Discobotellina biperforata is a very large disc-shaped arenaceous foraminiferan, capable of moving up to 6 cm in 12 hr. This is similar to the distance moved by the much smaller *Elphidium crispum* (L.), which was 12 cm in 12 hr (Myers, 1943). Occasionally *Discobotellina* climbed the glass faces of aquaria where dense clusters of pseudopodia were visible radiating up to 10 mm from the rim. These lengths are roughly comparable to those observed in the much smaller *Peneroplis pertusus* (Forskål) by Winter (1907).

Sand or mud particles settling on the upper surface from turbid water are removed by the animal, indicating that some pseudopodia are present on this surface. (Other observations suggest they are also on the lower surface.) In nature this "cleaning mechanism" would have obvious survival value. Myers (1943) noted that due to their shape and relatively low specific gravity Foraminifera sink more slowly than quartz grains in their surroundings when sediments are disturbed. These considerations would not apply to the large arenaceous *Discobotellina*, so here the "cleaning mechanism" may have particular importance.

In aquaria containing fine sand, the animals build hillocks beneath themselves, but these are not evident in mud aquaria. Observations upon aquaria with only a thin layer of mud above the glass bottom show that the specimens bring particles to their immediate vicinity, as they evidently do during hillock formation. Possibly hillocks are not obvious in mud aquaria because they sink as rapidly as they are formed. Hence hillock formation presumably prevents sinking into soft substrata, and no doubt the radiating halo of pseudopodia also helps. Probably hillock formation

makes the animals particularly susceptible to surface collecting devices such as trawls and dredges, from which they can be obtained in immense numbers (Stephenson & Rees, 1965).

Numerous authors have successfully cultured marine benthic Foraminifera *in vitro*, and have followed the details of their life cycles. Culture conditions have tended either towards extreme simplification in attempts to evaluate food requirements (Bradshaw, 1957; Lee *et al.*, 1959, 1960, 1961, 1962, 1963); or towards moderate complexity with encouragement of desirable cohabitants and discouragement of undesirables (Myers, 1935, 1937; Jepps, 1942; Arnold, 1954; Bradshaw, 1955); or towards extreme complexity with inclusion of macrofauna, macroflora, and an artificial tidal system (Freudenthal *et al.*, 1963). The present experiments were similarly aimed at prolongation of survival in the hope of confirming postulated details of the life cycle (Stephenson & Rees, 1965). It was appreciated at the outset that extreme simplification of culture conditions presented formidable difficulties; it would have involved elimination of just those small particles which the animals would require for growth, or alternatively their sterilization. Previous attempts at culturing arenaceous species in simple conditions have not been successful (Lee *et al.*, 1960), but in complex conditions they have thrived (Freudenthal *et al.*, 1963). Early in the present experiments it was evident that without prolonged "conditioning" of the environment by preimmersion in seawater survivals were reduced, and hence that a moderately complex system offered the best chances of prolonged survival. From this stage onwards variants around the presumed natural environment were employed.

The approach was empirical throughout, and the results obtained confirmed, in many cases, those similarly obtained by previous workers. Thus Myers (1935, 1937) noted high mortalities just after collection and selected species for culturing after collections had been acclimatized for some days to laboratory conditions. In the present work, within the one species, there were numerous casualties in the first few days after collection, and, by selecting specimens after a quarantine period, subsequent survivals were increased. It was suspected that specimens dying during quarantine might have been damaged mechanically during collection, and further that variations between survival times in early series of experiments might have been due to different severities of handling. Mechanical damage via handling would also explain the form of most of the survival curves, in which, after a long period with few deaths, there is a sudden onset of casualties. In these experiments handling of specimens to determine whether or not they were alive was unnecessary and avoided early in the experiments. Later, when the animals were dubiously alive, it became necessary and accelerated the death rate (Experiment V).

Tests of specimens from Southeast Queensland typically contain appreciable amounts of mineral sands, particularly ilmenite (see Stephenson & Rees, 1965). Because of this, and because their abundance in Southeast Queensland coincides with an abundance of mineral sand (Connah & Newman, 1960), it was thought that these minerals might be of special importance. In their presence one might have expected preferential additions to the test, increased survival times, or increased growth rates, but the survival experiments showed no such relationships.

Survival times in different grades of sediments from mud to coarse sand were variable, but in general the muddier aquaria gave longest survivals. This was unexpected because in nature the species is collected in greatest numbers from fine sand. Addition of mud to otherwise clean aquaria prolonged survivals, and it was concluded that mud, or something associated with it, provides the main food. No doubt in nature plankton and suspended mud particles are used as food in addition to benthic material, as occurs in *Elphidium* (= *Polystomella*) (see Myers, 1943). Without supernatant food (as in settled aquaria) there would be increased dependence on bottom deposits for nutriment. In a single experiment (Experiment V) certain aquaria contained settled mud, and others were made turbid at regular intervals. Increased turbidity gave slightly increased survivals. However disturbance of mud could well

produce opposite effects; by increasing food supply it could prolong survival, and by tending to bury the specimens it could initiate death by asphyxiation. This does occur in foraminiferans generally (Myers, 1943), and even on the mud surface of aquaria dead specimens become the typical black of (chemically) reduced sand. If left undisturbed they disintegrate completely. This explains why such a dense surface population of apparently robust forms fails to produce abundant subfossils. In field collections only a few black or partially black specimens were obtained.

Information upon the food of the species was obtained by observations upon specimens climbing the glass walls of aquaria. They left behind an "abrasion track" and the fouling film upon the walls was evidently removed as food. Thin and newly formed films were composed mainly of two diatoms *Neidium affine* (Ehrenberg) Pfitzer and *Amphora proteus* Gregory. There is abundant evidence in the literature that diatoms, particularly pennate forms, are a main food of benthic foraminiferans (Winter, 1907; Myers, 1935, 1937; Jepps, 1942; Myers, 1943; Arnold,1954; Bradshaw, 1955; Nyholm, 1955; Hedley, 1958; Lee *et al.*, 1959, 1961, 1962, 1963). Older films included in addition ciliates (particularly a species of *Vorticella*), green and colourless flagellates, blue-green algae, and nematodes. Evidently the bulk of this material was used as food, particularly the sessile elements. This compares with previous listings of foraminiferal food sources: diatoms, flagellates, algal gametes, filamentous algae, small pieces of seaweed, protozoa, minute eggs, cysts, decaying vegetation; organic debris, bacteria, and copepods (Myers, 1943; Arnold, 1954; Bradshaw, 1957; Hedley, 1958). In the oldest films the diatoms were grown over and largely replaced by blue-green algae. *Discobotellina* appeared only occasionally upon such films, and when dense populations of blue-greens appeared in certain aquaria they were associated with early deaths. Arnold (1954) has already noted the dangers to foraminiferan cultures of an overgrowth of filamentous algae with resultant algal choking.

In most experiments nominally identical aquaria gave different survival times, and it was suspected that the main uncontrolled variable was the nature and density of the microbenthos, both animal and plant. Use of "well-seasoned" aquaria and of dim continuous illumination were attempts to encourage suitable conditions, and particularly suitably dense growths of diatoms (Myers, 1935, 1937; Bradshaw, 1955; Lee *et al.*, 1963). Illumination sometimes gave the opposite result, and, to avoid the resultant variations in survival times, other factors were evaluated in dark aquaria which gave comparable average survival times (see Lee *et al.*, 1959). Prolonged survival in darkness indicates that *Discobotellina* can obtain adequate nutriment in complete absence of diatoms.

Population density was investigated in a single extensive experiment (IV) which showed, as expected, longer survivals at lower densities. These low densities approximated to those in nature.

The optimum salinity for survival approximated to that of oceanic water (35·5‰), with only slightly reduced survivals in dilute media, but greatly reduced ones in higher salinities. Not unexpectedly different species of foraminiferans show different salinity tolerances. Thus normal growth and reproduction occurred in *Streblus beccarii* var. *tepida* in salinities of 20–40‰, and growth alone occurred in the even wider range of 7–67‰ (Bradshaw, 1957). A species of *Allogromia* can reproduce in salinities of 20–45‰ (Lee *et al.*, 1962).

High temperatures killed *Discobotellina*, with mass deaths at 31·5°C. *Streblus beccarii* var. *tepida* ceases growth above 30–35°C and dies rapidly at 35°C (Bradshaw, 1957). Throughout the later experiments temperatures were maintained at 23°C as approximating to the mean annual temperature of the natural environment (see Myers, 1935, 1937).

In most experiments no detectable growth was observed, in disappointing contrast to work upon species of *Allogromia* (Lee *et al.*, 1962), *Bolivina* (Lee *et al.*, 1959), *Discorbis* (Myers, 1935), *Elphidium* (= *Polystomella*) (Jepps, 1942; Myers, 1943), *Rosalina* (Lee *et al.*, 1963), and *Streblus* (Bradshaw, 1957).

In a single experiment an annulus about 2 mm thick was added to *Discobotellina* after four months, suggesting that conditions were not too abnormal, and that natural growth is slow. Myers (1935) has noted that small species develop and mature more rapidly than large ones; presumably *Discobotellina* with its extreme size grows very slowly. Assuming that a diameter increase of 1 mm per month is a normal growth rate, sexual reproduction probably occurs in specimens about 12–18 months old, as indicated by the size (15–20 mm diameter) of the uniperforate (presumably post-reproductive) forms (Stephenson & Rees, 1965). On this basis the largest specimens collected would be about three years old. In spite of slow growth, the aquarium observations have confirmed the origin of crescents from buds, and the origin of symmetrical uniperforates from imperforates as postulated earlier (Stephenson & Rees, 1965).

Possibly the greatest value of the aquarium observations has been in providing data of ecological significance in relation to such matters as salinity tolerance, effects of mechanical disturbance, and food requirements. These will be related to field data in a later paper.

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REFERENCES

ARNOLD, Z. M. (1954). Culture methods in the study of living Foraminifera. *J. Paleont.* **28**(4): 404–16.

BRADSHAW, J. S. (1955). Preliminary laboratory experiments on ecology of foraminiferal populations. *Micropaleontology* **1**(4): 351–58.

BRADSHAW, J. S. (1957). Laboratory studies on the rate of growth of the foraminifer, '*Streblus beccarii* (Linné) var. *tepida* (Cushman)'. *J. Paleont.* **31**(6): 1138–47.

BRADSHAW, J. S. (1961). Laboratory experiments on the ecology of Foraminifera. *Contr. Cushman Fdn foramin. Res.***12**(3): 87–106.

COLLINS, A. C. (1958). Foraminifera. *Sci. Rep. Gr. Barrier Reef Exped.* **6**(6): 335–437; pls. I–V.

CONNAH, T. H. & NEWMAN, P. W. (1960). Beach sands and heavy mineral concentrations. In HILL, DOROTHY & DENMEAD, A. K. (eds.). The Geology of Queensland. *J. geol. Soc. Aust.* **7**: 409–12.

C.S.I.R.O. (AUST.) (1953). Onshore hydrological investigations in Eastern and Southwestern Australia, 1951. *Oceanogrl Stn List Invest. C.S.I.R.O. Aust.* **14**: 1–64.

FREUDENTHAL, H. D., LEE, J. J., & PIERCE, S. (1963). Growth and physiology of Foraminifera in the laboratory: Part 2—A tidal system for laboratory studies on eulittoral Foraminifera. *Micropalaentology* **9**(4): 443–48.

HAMON, B. V. (1956). A portable temperature-chlorinity bridge for estuarine investigations and seawater analysis. *J. Sci. Inst.* **33**: 329–33.

HEDLEY, R. (1958). A contribution to the biology and cytology of *Haliphysema* (Foraminifera). *Proc. zool. Soc. Lond.* **130**(4): 569–76.

JEPPS, MARGARET W. (1942). Studies on *Polystomella* Lamarck (Foraminifera). *J. mar. biol. Ass. U.K.* **25**: 607–66.

LEE, J. J., FREUDENTHAL, H. D., MULLER, W. A., KOSSOY, V., PIERCE, S., & GROSSMAN, R. (1963). Growth and physiology of Foraminifera in the laboratory: Part 3—Initial studies of *Rosalina floridana* (Cushman). *Micropalaentology* **9**(4): 449–66.

LEE, J. J., MCLAUGHLIN, J. J. A., & RAYNOR, J. (1959). Preliminary studies on the growth of Foraminifera in the laboratory. *J. Protozool.* **6** (Suppl.): 12. [Abstract.]

LEE, J. J., PIERCE, S., FREUDENTHAL, H. D., TENTCHOFF, MARILYN, & MULLER, W. A. (1962). Studies on *in vitro* growth of Foraminifera. III. *J. Protozool.* **9** (Suppl.): 16–17. [Abstract.]

LEE, J. J., PIERCE, S., MCLAUGHLIN, J. J. A., & ELLIS, B. F. (1960). Preliminary studies on *in vitro* growth of Foraminifera. *J. Protozool.* **7** (Suppl.): 13. [Abstract.]

LEE, J. J., PIERCE, S., TENTCHOFF, MARILYN, & MCLAUGHLIN, J. J. A. (1961). Growth and physiology of Foraminifera in the laboratory. Part I—Collection and maintenance. *Micropaleontology* **7**(4): 461–66.

MYERS, E. H. (1935). Culture methods for the marine Foraminifera of the littoral zone. *Trans. Am. microsc. Soc.* **54**: 264–67.
MYERS, E. H. (1937). Culture methods for marine Foraminifera of the littoral zone. In GALTSOFF, P. S. (ed.). *Culture methods for invertebrate animals*, pp. 93–6. Ithaca.
MYERS, E. H. (1943). Life activities of Foraminifera in relation to marine ecology. *Proc. Am. phil. Soc.* **86**: 439–58.
NYHOLM, K. G. (1955). Observations on the monothalamous *Hippocrepinella alba* Heron-Allen and Earland. *Zool. Bidr. Uppsala* **30**: 475–84.
STEPHENSON, W. & REES, MAY (1965). Ecological and life history studies upon a large foraminiferan (*Discobotellina biperforata* Collins 1958) from Moreton Bay, Queensland. I. The life cycle and nature of the test. *Pap. Dep. Zool. Univ. Qd* **2** (10): 205–224.
WINTER, F. W. (1907). Zur Kenntniss der Thalamophoren. I. Untersuchung über *Peneroplis pertusus* (Forskål). *Arch. Protistenk.* **10**: 1–113.

APPENDIX

Particle analysis method

Excess of sediment was dried to constant weight at ca. 105°C, cooled, and pulverized. From this, 100 g were placed in the top of a nest of sieves and shaken mechanically for ca. 5 min. Fractions retained by each sieve were weighed.

The main errors are likely to occur during pulverizing and sifting techniques. Under-pulverization leaves aggregates of particles and over-pulverization breaks up more fragile material, e.g. shell fragments. Even with prolonged sieving, finely particulate material, e.g. dried bacteria, remains attached to larger particles.