

A NOTE ON THE LABORATORY CULTURE OF BENTHIC FORAMINIFERA COLLECTED FROM NEARSHORE REGION OFF GOA, INDIA

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ABSTRACT

A laboratory culture programme is established to extend credibility to foraminiferal-based techniques being used to generate the high resolution proxy records for paleoclimatic reconstruction. In order to obtain living specimen of foraminiferal species for laboratory culture experiments, a number of samples were analyzed from the nearshore region off Dias beach (Goa), which revealed the presence of 22 species. Among the living benthic foraminifera *Rosalina leei* was found in abundance and was used to establish a laboratory culture programme. Initially, we monitored the effect of different media (like plain sea water and Erdscherbermedium) and food on the growth of foraminiferal test. The results suggest that Erdscherber medium is conducive for general growth and chamber formation. A further observation suggested that food is another governing factor for their growth in sea water. However, in case of Erdscherber medium excess food supply retards the overall growth.

INTRODUCTION

The need to culture foraminifera in the laboratory has long been striking the Indian workers dealing with paleoclimatic studies with the help of foraminiferal proxies (Nigam, 1986; Nigam and Rao, 1987; Nigam *et al.*, 1992). The study of foraminifera in the laboratory helps in solving a variety of problems like taxonomy, development (Lee *et al.*, 1963); growth rate (Kuile and Erez, 1984); test structure and size (Kuile and Erez, 1988); nature of pseudopodia and locomotion (Kitazato, 1988); habit and habitat (Lee *et al.*, 1985); life cycles (Biekart *et al.*, 1985; Grell, 1988; Goldstein and Moodley, 1993), etc.,. A number of attempts have been made on various aspects of foraminifera both planktic and benthic in laboratory culture from different parts of the world. Nevertheless, in India which has a vast coast line (approximately 6,000Km) and prolific presence of *Foraminifera*, no attempt has so far been made to culture foraminifera in laboratory under controlled conditions.

Realizing these facts, we have initiated the laboratory culture programme of the foraminifera. This paper is the first report on this aspect of the laboratory culture of benthic foraminifera collected from Indian region. In order to get living specimen of foraminifera regular (fortnightly) samples were collected from the sub tidal region off Dias beach (Dona Paula), which is a protected beach in north Goa and is situated at the confluence of the Mandovi and Zuari Estuaries (fig. 1) flowing into the Arabian Sea. The tidal amplitude of the study area varies from 2-3 m. It is protected on either sides by rocky cliffs. A few rocks can be seen in the sub-tidal regions during low tide. Such protected areas are suitable to obtain living specimens of foraminifera.

METHODOLOGY

[A] Culturing of diatoms in laboratory as food for foraminifera

The pre-requisite for foraminiferal culture is the availability of food. Therefore, the samples containing the diatoms were collected from the surf water. Samples were brought to the laboratory and one species of diatom, *Navicula subinflata* was cultured as follows.

The sea water containing high number of *Navicula subinflata* was transferred to a 100 ml conical flask with fresh (filtered) sea water and kept near a glass window. Dense growth of the diatoms was obtained after 6 to 7 days. 1-2 ml of this culture was re-inoculated onto fresh (filtered) sea water in two flasks.

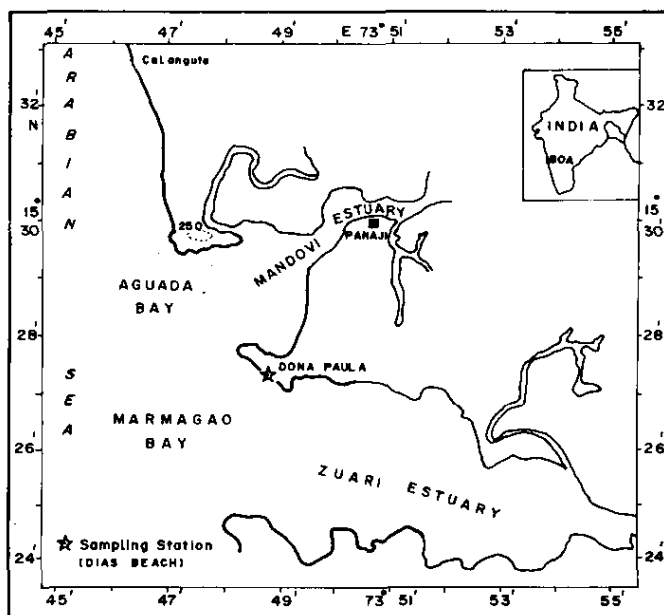


Fig. 1. Location of the study area.

[B] PREPARATION OF CULTURE MEDIA

(I) *Filtered Sea Water*: Natural sea water is simple to use as a culture medium and yields good results in the culture of foraminifera. Sea water was collected from the sampling site and filtered through ashless Whatman Filter paper No. 42.

(II) *Erdscheriber medium* : According to Foyn (1934) "Erdscheriber" has given highly satisfactory results in the cultures. The medium was prepared according to the formula given by Scheriber (1927) as following:

Earth Extract		50.00	cc
NaNO ₃		0.1	gm
Na ₂ HPO ₄ 12	H ₂ O	0.02	gm
"Aged" seawater		1000.00	cc

The earth extract was prepared by autoclaving one kilogram of humus rich garden soil in a litre of glass distilled water for one hour and allowing it to settle for several days until a clear or straw colored fluid can be decanted from the sediment. Sterile technique is generally desirable and the extract should be stored under refrigeration to prevent bacterial growth. After the addition of the extract and salts to the sea water the medium was ready for use.

[C] FORAMINIFERAL STUDIES

(I) *Field Collection*: Periodic (fortnightly) samples were collected from the sub tidal regions of Dias beach including bottom sediments and seaweeds. Foraminifera attached to sea weeds respond better in the laboratory culture (Arnold, 1954). After collection, the sample (Seaweeds + sea water) was poured in a plastic bucket, with additional seawater. The algae were gently shaken by hand for about 10 min. and allowed to stand for 15 min., so that the foraminifera can settle to the bottom. The vegetation and water were replaced by fresh sea water. Filtration was done through different sieves of 850 microns and 63 microns respectively. The material which passed through 850 microns was gathered over a 63 microns sieve, rinsed 2 to 3 times and whatever remained was subsequently kept in fresh sea water, which was filtered using Ashless Whatman Filter paper No. 42.

(II) *Laboratory Procedures* : Living specimen of benthic foraminifera were separated from the field samples under binocular Microscope, having 20X eye pieces. Recognition of living specimen was possible by their thin wall test and dark grey to brownish colored protoplasm (Boltovskoy and Wright, 1976). Such living specimen, after repeated rinsing in filtered sea water,

were kept individually in wells of the autoclaved Costar culture dish and filled with sea water. Such containers have lot of surface area with respect to volume. The advantage of using small culture dish is to be able to study directly under the Inverted Microscope. In this way the foraminiferal specimen do not have to be disturbed. While viewing these culture dish under the microscope one must be careful that the microscope light does not overheat the water and the cover should be removed from time to time to allow aeration.

The sea water of the dishes was changed alternatively and food (diatoms) was provided. Before feeding the diatoms to foraminifera, the culture flasks were shaken well, because diatoms attach to the glass. With the help of a Pasteur pipette, a small quantity was taken out for feeding the foraminifera. A single drop was poured weekly in each individual well of the culture dish of foraminifera. The size of the foraminifera was measured using a binocular microscope equipped with an ocular micrometer.

(III) *Experimental set up to study the effect of the Medium and Food*: It was proposed that different media like plain seawater, artificial sea water, F₁ and F₂ media and Erdscheriber media could be used for culture (Lee *et al.*, 1963). Nevertheless the species cultured in artificial sea water survived but did not grow or reproduce (Boltovskoy and Wright, 1976).

In view of this, we have initially selected two media namely plain sea water and Erdscheriber. Twelve specimens, were kept in each medium. However, out of these, half the number (6) was provided food (diatoms) weekly, whereas the remaining half was fed only at the beginning of the experiments and then left starving during the experimental period (Table 1). The media was changed at alternate days following the same procedure described earlier.

RESULTS AND DISCUSSION

The Culturing of benthic foraminifera was carried out in two phases. In phase 1 we used living organisms collected from the field as parent to be cultured under laboratory conditions using filtered sea water as media. This attempt was made to obtain the offsprings to be utilized for various experiments under phase 2. However, in phase 1 we changed half of the medium on alternate days and supplied food (diatoms) weekly. While changing the medium, care was taken to maintain the salinity by adjusting the loss due to evaporation through addition of distilled water. The results of both phase culture are discussed separately.

Table 1. Benthic foraminiferal species recorded from Dias beach (attached to sea weeds).

Name of the species
<i>Ammonia tepida</i>
<i>A. sobrina</i>
<i>Asterorotalia dentata</i>
<i>Bulimina exilis</i>
<i>Florilus scaphum</i>
<i>Globigerinoide ruber</i>
<i>Miliolina australis</i>
<i>M. labiosa</i>
<i>Nonion elongatum</i>
<i>Pararotalia calcar</i>
<i>Quinqueloculina ludwigi</i>
<i>Q. kerimbatica</i>
<i>Q. seminulum</i>
<i>Q. lamarckiana</i>
<i>Q. laevigata</i>
<i>Quinqueloculina sp.</i>
<i>Rosalina leei</i>
<i>Rotalidium annectens</i>
<i>Triloculina trigonula</i>
<i>Triloculina sp.</i>
<i>Spiroloculina communis</i>
<i>S. indica</i>

FORAMINIFERAL CULTURE

Phase 1

A total of 22 benthic foraminiferal species attached to seaweeds were recorded from the study area (Table 2). However, only *Ammonia sobrina*, *Spiroloculina communis*, *Pararotalia calcar* and *Rosalina leei* species were found alive. Within the living foraminiferal populations *Rosalina leei* was found in abundance and hence was considered for first phase culturing (PH, figs. a-f). Twelve specimen were initially put under culturing and

Table-2 Total number of specimen and responded specimen of *R. leei* in media experiment

Sr. No.	Name of the Experiment	No. of specimen in Expt.	No. of specimen responded
1.	Sea Water (food was provided only at the beginning)	6	5
2.	Erdscheriber (food was provided only at the beginning)	6	4
3.	Sea Water (food was provided weekly)	6	6
4.	Erdscheriber (food Was provided weekly)	6	5

detailed observations were made at regular intervals. The results of this study suggested that-

- (1) All the specimen initially responded to culture.
- (2) Food has conducive effect
- (3) The formation of initial chambers took less time as compared to latter chambers.

Fig. 1 shows the growth of successive chambers. However, some of the parents reproduced at varying intervals (between data of collection of samples and delivery). On an average, 30-40 offsprings were produced by an individual. Being tiny in size they appeared as small black dots under the microscopic field (Pl.I, figs. g-h). We waited for 4-5 days and after they got stabilized in the wells, the individual offsprings were transferred to various wells to start different experiments as phase 2 of the present study. Before starting any experiments initial size of each offspring was measured.

Phase 2

The offsprings of *R. leei* obtained in phase 1 culture were kept for two experiments to test the relative effects of culture media. The results of each experiment were

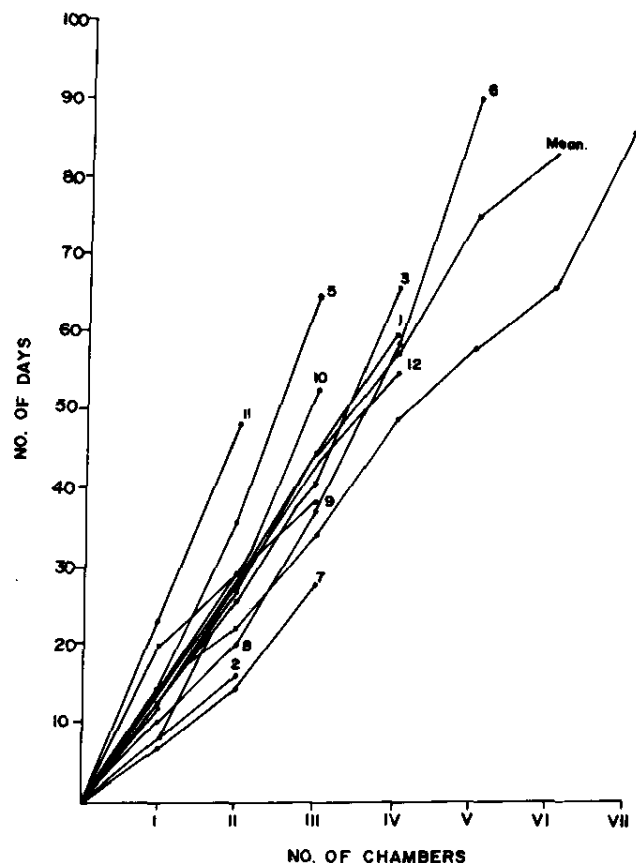


Fig. 2. Growth of successive chambers of different specimens of *R. leei* with reference to number of days.

determined in terms of number of chambers, test size, (which is expressed in terms of growth/day). The details of the experiments and the results are given below.

EFFECT OF MEDIA

Based on the regular observations, made on twelve specimen (six in each medium), which were fed only at the beginning of the experiments, the following inferences are made on test size and chamber formation.

Test size: The growth experiment shows that Erdscheriber medium has a positive effect on growth (fig. 3a) as the size of the specimen kept in Erdscheriber medium increased by 168 μm , with average growth of 5.58 $\mu\text{m}/\text{day}$. Whereas, specimen kept in plain sea water were increasing in size (Table 3a) at a slow rate of 3.5 $\mu\text{m}/\text{day}$.

Chamber: Erdscheriber medium was conducive (11.12 days/chamber) for chamber formation (fig. 3b) than plain sea water (13.53 days/chamber).

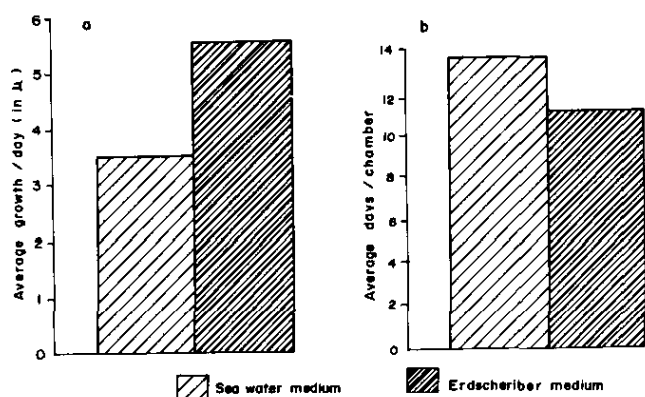


Fig. 3a. Effect of plain sea water and Erdscheriber media on the size of *R. leei* specimens, which were provided food only at the beginning of the experiment.

Fig. 3b. Effect of plain sea water and Erdscheriber media on the average growth of *R. leei* specimens, which were provided food only at the beginning of the experiment.

Similarly regular observations on remaining twelve (six in each medium) specimens, which were fed weekly, suggested the following facts:

Test size: As compared to sea water, the growth in terms of the average size of the specimen (fig. 4a) in Erdscheriber medium after 30 days increased by 140 μm , with average growth of 4.66 $\mu\text{m}/\text{day}$. It was noticed only 4 $\mu\text{m}/\text{day}$ with an average increase in size of 120 μm in sea water (Table 4a).

Chamber: The formation of chamber took less time (average 11.76 days/chamber) in Erdscheriber medium (fig. 4b). On the other hand, average time taken by the specimen in plain sea water to form one complete chamber was 12.48 days (Table 4b).

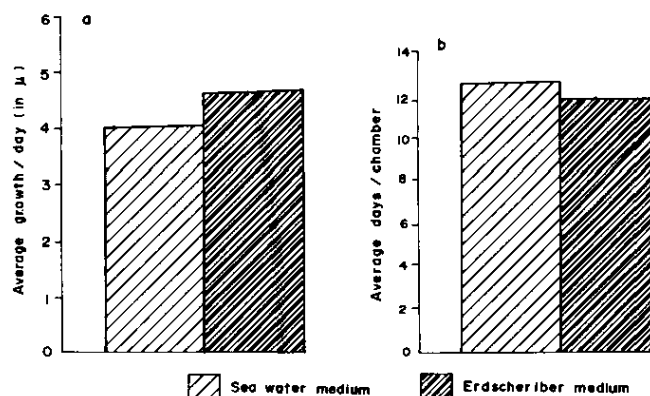


Fig. 4a. Effect of plain sea water and Erdscheriber media on the size of *R. leei* specimens, which were provided food weekly.

Fig. 4b. Effect of plain sea water and Erdscheriber media on the average growth of *R. leei* specimens, which provided food weekly.

EFFECT OF FOOD

The specimen which were fed weekly showed better growth (4 $\mu\text{m}/\text{day}$) in plain sea water, with average days taken to form one chamber being 12.48. On the contrary, those fed only at the beginning of the experiment took 13.53 days to form one chamber (figs. 5a-b; Tables 3a-b; 4a-b).

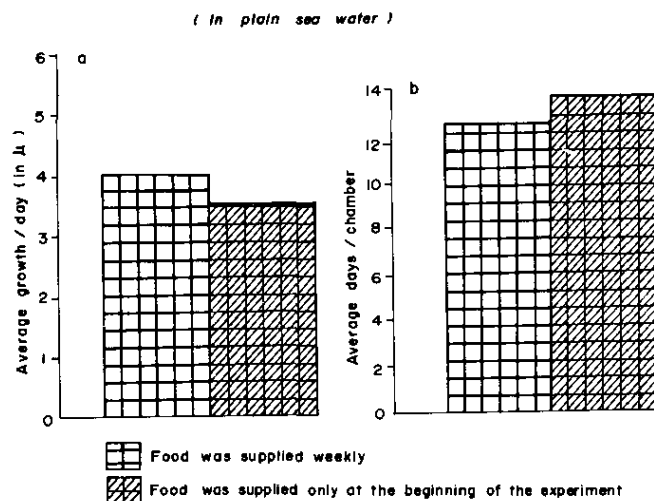


Fig. 5a. Effect of food on the size of *R. leei* specimens, kept in plain sea water.

Fig. 5b. Effect of food on the average growth of *R. leei* specimens, kept in plain sea water.

In Erdscheriber, it was noted that as far as growth was concerned *R. leei* with weekly food supply, did not show better response (average growth rate 4.66 $\mu\text{m}/\text{day}$). Instead, the specimen which were fed only once, showed faster growth (5.58 $\mu\text{m}/\text{day}$) with average time of 11.12 days to form a complete chamber (fig. 6 a-b; Tables 3a-b; 4a-b).

(In *Erdscheriber medium*)

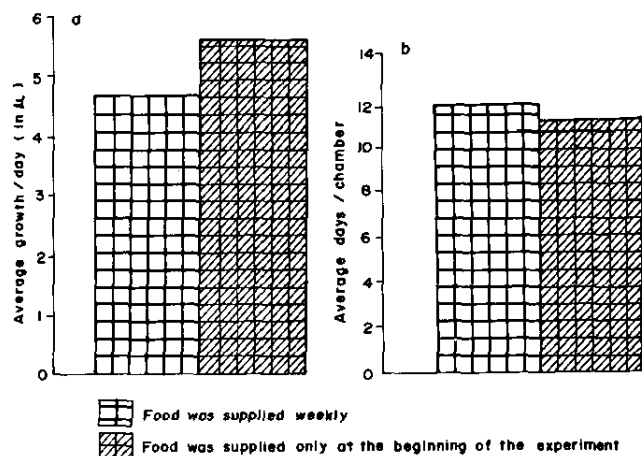


Fig. 6a. Effect of food on the size of *R. leei* specimens, Kept in Erdscheriber medium.

Fig. 6b. Effect of food on the average growth of *R. leei* specimens, kept in Erdscheriber medium.

Table 3A. Average size variations of *R. leei* in different media when food was supplied only at the beginning of the experiment

Size (in μ)	Seawater Medium	Erdscheriber Medium
Avg. initial size	295	205
Avg. size after 30 days	400	372.5
Growth/day.	3.5	5.58

Table 3B. Average time variations in the chamber formation of *R. leei* in different media when food was supplied only at the beginning of the experiment Values in the parentheses are ranges of the time taken by different specimen of *R. leei*.

Chambers	Seawater Medium	Erdscheriber Medium
I Chamber	13.66 (11-16)	12.25 (10-15)
II Chamber	13.4 (13-18)	10.00 (8-13)
Avg. days/Chamber	13.53	11.12

Table 4A. Average size variations of *R. leei* in different media when food was supplied weekly.

Size (in μ)	Seawater Medium	Erdscheriber Medium
Avg. initial size	290	303.3
Avg. size after 30 days	410	443.3
Growth/day.	4.0	4.66

Table 4B. Average time variations in the chamber formation of *R. leei* in different media when food was supplied weekly. Values in the parentheses are ranges of the time taken by different specimen of *R. leei*.

Chambers	Seawater Medium	Erdscheriber Medium
J Chamber	11.66 (7-20)	11.2 (8-15)
II Chamber	13.8 (10-17)	12.33 (11-14)
III Chamber	12.0 (11-13)	
Avg. days/Chamber	12.48	11.76

Such distinct anomaly in the response towards food (diatoms) may be explained in the light of the study of Bradshaw (1955), who postulated that the abundance of nutrient material (due to over growth of *Navicula subinflata*, which grows faster in Erdscheriber medium) causes adverse effect on the growth, perhaps due to overfeeding. In this connection, he further noticed that the initial population growth was retarded when the foraminifera was fed twice a day.

CONCLUSION

Erdscheriber medium is conducive for general growth of foraminiferal specimen. Food is another governing factor for growth but excess of food supply retards the growth rate. These results, though preliminary, are promising.

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EXPLANATION OF PLATE

Plate I

- a. process of collection of food (diatoms), photo through T.V. Monitor (X 500).
- b. Living foraminifera with its pseudopodial network, photo through T.V. Monitor (X 500).
- c. Magnification of foraminiferal pseudopodia, photo through T.V. Monitor (X800).
- d. *Rosalina leei* with newly formed chamber (X475)
- e. *Rosalina leei* with newly formed, four abnormal chambers, photo through Inverted Microscope (X450)
- f. Living *R. leei* where one chamber has newly formed (X 450).
- g. Parent *R. leei* about to deliver, has gathered excess of food (X 300).
- h. Tiny offsprings of *R. leei* along with ruptured parent (X 300).
- I. Magnified offsprings of *R. leei* along with ruptured parent (X 750).

