

# **Benthic Foraminifera Under Laboratory Culture Experiments: Ecological Implications**

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—————  
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By

**Sujata R. Kurtarkar**

Micropalaeontology Laboratory,  
National Institute of Oceanography,  
Dona Paula 403 004, Goa, India

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**2010**

*Dedicated to my beloved  
Parents and Husband*

## Declaration

As required under the university ordinance OB.9.9 (ii), I hereby state that the present thesis entitled “**Benthic foraminifera under laboratory culture experiments: Ecological Implications**” is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge, the present study is the first comprehensive work of its kind from the area mentioned.

Literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

  
**Sujata R. Kurtarkar.**



Dr. R. Nigam, Ph.D., D.Sc.  
Scientist 'G'  
Project Leader, Paleoclimate

1<sup>st</sup> May, 2010

## Certificate

As required under the university ordinance OB.9.9. (vi), I certify that the thesis entitled "Benthic foraminifera under laboratory culture experiment: Ecological Implications", submitted by Ms. Sujata R. Kurtarkar for the award of the degree of Doctor of Philosophy in Marine Science is based on original studies carried out by her under my supervision. The thesis or any part of thereof has not been previously submitted for any other degree or diploma in any university or institution.

(R. Nigam)

Research Guide

All suggestions & corrections suggested by all the referees have been incorporated.

S. N. Ballal  
20/8/10

# Preface

Climate change has become one of the most important issues of concern today. It has become necessary to forecast imminent climatic changes well in advance. It requires a proper understanding of interrelationship between various factors that affect the climate. Part of such understanding about the interrelationship between various climatic factors is gained by studying the climatic changes during past. But, because of absence of written records of climatic changes during the geologic past, various indirect techniques called proxies have been used for this purpose. Out of several proxies used for paleoclimatic studies, the characteristics of foraminifera are among the most often used techniques. Foraminiferal proxies to infer climatic changes during the past are developed based on study of present day distribution of foraminifera. However, there are a few drawbacks of such studies; most important is the difficulty to precisely define the physico-chemical parameter responsible for particular foraminiferal characteristics. These drawbacks can be overcome by laboratory culturing of foraminifera, wherein the foraminifera can be subjected to a known set of conditions and their characteristic response observed. Therefore, a dedicated laboratory is established at National Institute of Oceanography for laboratory experiments on foraminifera. The present work is first comprehensive study carried out with the objective to understand the response of a few selected benthic foraminiferal species to a known set of physico-chemical conditions in the laboratory. As the change in temperature and monsoonal precipitation are supposed to directly affect the coastal marine environment, emphasis was given to observe the effect of change in salinity, temperature and food concentration, etc. on the foraminifera.

The work is compiled in nine chapters and a brief outline of the layout of the thesis and of the various chapters is given below.

**Chapter I** provides an introduction to the need for laboratory culture studies of benthic foraminifera. In experimental plans, emphasis is given to changes in 'test (hard part)' of foraminifera which is made up of either  $\text{CaCO}_3$  or sand grains cemented together.

**Chapter II** summarizes the previous work done on culture studies of benthic foraminifera throughout the world. Culture studies have been carried out at many labs throughout the world, including the Micropaleontology Laboratory of National Institute of Oceanography, India. The literature review shows that majority of benthic foraminiferal studies were carried out to understand the life-cycle of few foraminiferal species and behavior of soft part (protoplasm) of the organism. Only a few studies were aimed out with the objective to understand the response of foraminifera to various climatic parameters. Therefore, it was decided to perform laboratory culture studies on benthic foraminifera with the following objectives.

- To observe the effect of different concentrations of food on selected benthic foraminifera.
- To observe the growth and reproductive phases of a few benthic foraminiferal species.
- To study the life span of selected benthic foraminifera.
- To observe the response of few benthic foraminiferal species (*Cymbaloporeta plana* (Cushman), *Pararotalia nipponica* (Asano), *Rosalina leei* (Hedley and Wakefield), etc.) to different ecological parameters.
- To conduct isotopic analysis on selected benthic foraminifera.

**Chapter III** includes the details of sampling, which was carried out from the coastal waters off Goa (15° 27' N; 73° 48' E). Sampling area is surrounded by the Mandovi estuary to the right and Zuari estuary to the left. It is about 200 m in length and has rocky cliffs on both sides. List of the materials required for collection of sample for picking live specimens is given. The method of sampling has been elaborated in detail with the help of field photographs. Culturing of diatoms to serve as food for foraminifera is also given.

After successfully maintaining the benthic foraminifera in laboratory, it was decided to study the response of them to different amounts of food, to get an idea about the proper amount of food to be provided to benthic foraminifera. **Chapter IV** documents the experiment conducted on benthic foraminifera *Cymbaloporeta plana* (Cushman) which was subjected to different amount of food (0, 20, 40, 60, 80 and 100 cells/ml) at different temperatures (25°C, 27°C and 30°C). A total of 18 sets,

with 5 specimens in each set were used for the experiment. The experiment was carried out in replicate. *Navicula* sp. was added as food weekly. Based on this experiment it was inferred that the average growth of *C. plana* increases with increased amount of food and 27°C temperature is most suitable for growth and reproduction in this species.

Before starting the work, it was decided to get an idea about the life span of benthic foraminiferal species found in the coastal waters off Goa. It will help plan the experiments and selecting species with shorter life span. Therefore, **Chapter V** summarizes the life span and the growth stages of a few benthic foraminiferal species namely *Cymbaloporeta plana* (Cushman), *Discorbina concinna* (Brady), and *Spiroloculina* sp. All these specimens were subjected to different combinations of temperature and salinity with 100µL of food (~20 cells/ml) in order to observe growth phases, mode of reproduction and life span. Based on this work, it was noted that all the three species reproduce asexually. Juveniles of *C. plana* and *D. concinna* are formed within the parent cell whereas that of *Spiroloculina* sp. reproduces within the cyst built by the pseudopodial network. Life span range of *C. plana* was noted to be from 45-55 days, *D. concinna* from 22-25 days and *Spiroloculina* sp. from 25-30 days. In case of *C. plana* and *D. concinna*, significant relationship ( $R=0.88$  and  $0.97$  respectively) is seen between the number of juveniles and the size of the parent test.

Once the selected benthic foraminiferal species were successfully maintained in laboratory and their favored preferences were known, it was decided to carry out the experiments to understand their response to various physico-chemical parameters. In coastal areas, fresh water influx during monsoon significantly changes the salinity of coastal marine water which in turn affects foraminiferal fauna. **Chapter VI** comprises the results of experiments conducted on benthic foraminifera *Rosalina leei* (Hedley and Wakefield) with salinity as a single parameter keeping rest of all the parameters constant. This experiment was conducted on live specimens isolated from the field material. On the basis of this experiment it is concluded that *R. leei* specimens can tolerate wide range of salinity (25‰ to 80‰). Extremely lower salinities proved to be detrimental to this species.

Precipitations during monsoon season, does not only result in change in salinity of the coastal waters due to increased runoff, but it also lowers the seawater temperature. Additionally, there are seasonal changes in the seawater temperature. Therefore, in **Chapter VII** it was decided to understand the combined effect of both salinity and temperature, on benthic foraminiferal species, *Rosalina leei* (Hedley and Wakefield), *Rosalina* sp. and *Pararotalia nipponica* (Asano). It is conclude that in specimens of *R. leei* the growth rate increases with comparatively lower temperatures and higher salinities but as the temperature increases and salinity decreases the growth rate also decreases.

Specimens of *Rosalina* sp. showed maximum average growth and reproduction at 30°C temperature and 25‰ salinity, whereas, comparatively less growth and reproduction was observed in case of specimens subjected to 25°C temperature.

27°C temperature and 35‰ salinity was the best combination of seawater temperature and salinity for *P. nipponica* specimens as the maximum average growth and reproduction was observed at this combination. Comparatively less growth was observed at higher as well as lower than 27°C temperature and salinity lower than 35‰. Prolonged exposure to lower than 25‰ salinity no matter the temperature, proved detrimental to this species.

The salinity in the coastal waters off Goa becomes as low as 10‰ during monsoon season. But such low salinity conditions are short-lived and do not prevail for long. Therefore, after understanding the effect of low salinity on benthic foraminifera, it was decided to understand the response of benthic foraminifera to short-term salinity changes. **Chapter VIII** deals with the experiment wherein attempt has been made to find the capability of *Rosalina leei* (Hedley and Wakefield) and *Pararotalia nipponica* (Asano) to recover adverse effects of short-term salinity changes. From this experiment it was concluded that extremely lower salinities lead to dissolution of the tests in both the specimens. These specimens are able to recover (with increase in salinity) these short term salinity changes but with morphological abnormalities.

Elemental and isotopic analysis of foraminiferal tests is an important technique used for quantitative determination of past climatic parameters. The chemical composition of the foraminiferal tests varies with different physico-chemical parameters. **Chapter**

**IX** deals with the changes in stable isotopic composition of *Pararotalia nipponica* (Asano) and *Rosalina leei* (Hedley and Wakefield) with reference to temperature and salinity. It was observed that the relationship between  $\delta^{18}\text{O}$  foraminifera and seawater temperature is more consistent for *P. nipponica* than for *R. leei* specimens. As compared to seawater temperature, salinity appears to have little control on  $\delta^{18}\text{O}$  foraminifera, within the studied salinity and temperature range.

The final chapter (**Chapter X**) summarizes major findings of the present work and future scope of this study. This chapter is followed by the list of references quoted in the thesis.

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## Introduction

Foraminifera, unicellular, preferentially marine microorganisms, are one of the most efficient indicators of ambient environment of the geologic past. Changes in abundance, species assemblage, morphology and chemical composition of foraminifera have long been applied to reconstruct the climatic and oceanographic conditions during the earth's geologic past (for Indian region, please see reviews by Nigam and Khare, 1995; Kathal, 1998; Sharma and Srinivasan, 2007; Singh, 2007; Bhalla et al. 2007; Khare et al. 2007; Bandy et al. 1972; Srinivasan, 2007; Sinha, 2007, etc.) as well as to assess the modern changes in the coastal regions due to increasing anthropogenic influence (Murray, 1991; Sen Gupta, 1999; Nigam, 2005). The temporal variation in foraminiferal population and species assemblage are among the most extensively applied foraminiferal proxies for paleoclimatic and paleoceanographic reconstruction (Gooday, 2003). The widespread application of foraminifera for paleoclimatic reconstruction arises from the study of foraminiferal characteristics in modern sediments, showing their high sensitivity to changes in seawater physico-chemical conditions (Boltovskoy & Wright, 1976; Murray, 1991; Gooday & Rathburn, 1999; Gooday, 2003; Saraswat et al. 2005). The foraminifera have been reported to be influenced by a number of ecological parameters, including food, temperature, salinity, pH, dissolved oxygen, etc.

Out of the total foraminifera, the planktic foraminiferal population dominates the deep-sea sediments above the carbonate compensation depth, whereas shallow water regions have high abundance of benthic foraminifera. The planktic foraminifera are almost absent in near shore, shallow water regions. The study of temporal changes in the deepwater planktic foraminiferal population has revealed a number of important paleoclimatic/paleoceanographic changes, including the changes in seawater temperature and thermohaline circulation. Though, the benthic foraminiferal characteristics from the deepwater regions have also been studied to infer paleoclimatic and paleoceanographic changes, it is the shallow and intermediate depth range from where the majority of benthic foraminiferal studies have been carried out. The benthic foraminifera are relatively more reliable and true

representatives of ambient conditions as their chances of transport are relatively less. Additionally, shallow water regions being the site of high sedimentation rate, abundance of benthic foraminifera in these regions offers possibility of high-resolution study of paleoclimatic changes (Nigam, 1993).

The changes in deepwater benthic foraminiferal population and species diversity have mainly been attributed to the change in surface water productivity leading to variation in the organic matter flux to the sea bottom and changes in lower limb of thermohaline circulation (Gooday & Rathburn, 1999, and references therein). It has further been suggested that out of the biotic and abiotic environmental factors, abiotic factors play a dominant role in shaping the benthic foraminiferal assemblage, especially in marginal marine environments (Murray, 1991; Sen Gupta, 1999). Out of abiotic factors, temperature and salinity have been reported as the most important ecological parameters, which govern the distribution, growth, and reproduction of foraminifera along the coastal areas (Boltovskoy & Wright, 1976). According to Bradshaw (1961), temperature may limit the distribution of species geographically and also affect growth, reproduction and other vital functions. In coastal areas the marine water characteristics vary as a result of fresh water influx during monsoon, which in turn affects the foraminiferal assemblages (Murray, 1991; Nigam et al. 1992; Nigam & Khare, 1994; 1999; Murray & Alve, 1999a, 1999b). Again, most of these findings are based on the study of benthic foraminiferal distribution in the surface sediment samples collected from various geographic environmental settings.

The present day knowledge of factors affecting benthic foraminiferal population is largely based on the field studies. A further understanding of the factors affecting the foraminiferal population in general and reproduction in particular can increase the reliability of foraminiferal abundance and species diversity based applications. However, since under natural environmental conditions, a number of ecological parameters simultaneously affect the foraminifera, it is difficult to study the effect of specific change in ecological parameters, on the foraminifera. Therefore, to give more reliability to the field-based proxies, culturing of foraminifera under controlled laboratory conditions is necessary. In laboratory culture studies, the effect of one or a combination of parameters on foraminifera can be studied, by keeping rest of the parameters constant. Therefore, field based observations have continuously been evaluated by laboratory culture studies. Additional information

about the differential response of foraminifera to various physico-chemical conditions is obtained by laboratory culture experiments conducted to understand the response of foraminifera to precisely known parameters (Bradshaw 1955, 1957, 1961; Nigam et al. 2006, 2008). The parameters are well constrained under laboratory studies than that in the field. However, it should be kept in mind that it is very difficult to simulate exact natural conditions in laboratory.

During recent years, a lot of emphasis is given to reconstruct the high resolution records of past climate. Such record is being generated through study of benthic foraminifera from shallow water regimes of high rate of sedimentation. However, the foraminiferal parameters used for such studies were developed fairly on field based circumstantial correlations. There is an urgent need to support these techniques through culture experiments. Realizing the need of the hour, the idea of the present study was conceived.

Therefore, it was decided to study the response of selected shallow water dwelling benthic foraminiferal species to various physico-chemical factors, so that the definite effect of a particular parameter on benthic foraminiferal species is known and can be used to infer paleoclimatic changes. Since, laboratory culture study of benthic foraminifera has been carried out since long at many laboratories abroad (but very few in India) it was decided to review the work done so far with an emphasis on understanding the past climatic and oceanographic changes. It will help in understanding the type of studies that are yet to be carried out. The findings of literature review and the objectives of the present study based on the review of laboratory culture studies of benthic foraminifera done so far are given in the next chapter.

## Previous Studies: A review

### 2.1 Introduction

As mentioned in the previous chapter, various foraminiferal characteristics are often been used for paleoclimatic studies. The effect of different ecological parameters on foraminiferal characteristics, however, is not yet clear. Laboratory culture studies can help to understand the effect of different ecological parameters on foraminifera. Laboratory culture studies on benthic foraminifera started soon after the discovery of potential application of benthic foraminiferal characteristics for the paleoclimatic reconstruction. Laboratory culture studies were started because the studies from field have not always provided a definite clue about the factors governing the specific foraminiferal assemblage (Bradshaw, 1955; Boltovskoy et al. 1991 and Cadre et al. 2003). Though large number of laboratory culture studies have been carried out on benthic foraminifera covering different aspects, here findings of only those laboratory culture studies that helped to understand the effect of different ecological parameters on benthic foraminiferal abundance, morphology and chemical composition, as observed in the field have been reviewed, as these are the benthic foraminiferal parameters used for paleoclimatic/paleoceanographic reconstruction. Additionally, those studies have also been included that were carried out on cellular part of the foraminifera, and helped refine the evolutionary history and taxonomic position of the foraminifera, as well as to identify cryptic species. Such studies have significantly improved the application of foraminiferal characteristics in stratigraphic correlation, especially for hydrocarbon exploration studies. Studies that refined the species identification have immensely improved the application of foraminiferal chemical composition (stable isotopic and elemental) for paleoceanographic studies as large differences have been noticed in the stable isotopic and elemental composition of the closely related species belonging to same genus. However, the laboratory culture response should be taken with care as significant differences have been observed in the species behavior under similar conditions (Röttger, 1972). Schnitker (1967) observed that under laboratory culture, specimens of *Triloculina*

*linneiana* attained sexual maturity at one-eighth the size of the parent specimens recovered from the field and were morphologically different from the parents.

Earlier Lister (1895) compiled the studies covering the biological aspects of foraminifera but the results were mainly based on the field observations. Later on, efforts were made to provide a comprehensive review of the factors affecting the abundance and growth of species, as well as its response to various ecological parameters based on the samples collected from the field (Myers, 1943a). Murray (1973) compiled the biological aspects of foraminifera and their potential application for paleoecological studies, but the findings included observations made both in the field and laboratory culture. A comprehensive review of ecological parameters affecting the benthic foraminiferal morphology was provided by Boltovskoy et al. (1991). But, here also, most of the studies included were based on the field observations. Subsequently, the paleoceanographic significance of studies carried out to understand the biological aspects of benthic foraminifera was compiled and discussed by Gooday (1994). In this study, results from laboratory culture of benthic foraminifera were included to some extent; much emphasis was given to the influence of food and oxygen conditions on the benthic foraminiferal communities. Thus, a complete review of the studies carried out to understand the specific effect of a single, or a combination of few ecological parameters on benthic foraminifera under laboratory culture, is not available yet. Therefore, it was decided to review the laboratory culture studies carried out on benthic foraminifera to understand their application for paleoclimatic/paleoceanographic applications. The studies are grouped as per major parameters studied.

## **2.2 Type and Amount of Food**

Food is one of the important factors for all organisms for growth and life activities. Foraminifera mostly feed upon different kinds of organic materials, some small organisms, mainly the diatoms, bacteria, coccolithophores, dinoflagellates, etc., and parts of other plants and animals. It is believed that the type and amount of food could be one of the factors responsible for the changes in the foraminiferal population. Boltovskoy and Wright (1976), noted that the size and morphology of the test may also be influenced by the amount and availability of food. Large number of small and abnormal specimens was noticed in laboratory experiments, due to lack of

food (Murray, 1963). From field studies, observation were made by Showers, (1980) that specimens of *Rosalina globularis* had rounded test in winter when there is lack of food material, while oval shaped test were found during the summer condition when the food materials are better. Myers, (1943b) and Bradshaw, (1955; 1961) observed that when there is abundant food, the growth is continuous and faster but growth retards when the quantity of food decreases. Over abundance of food is also detrimental to foraminifers as reported by Arnold, (1954) and Bradshaw, (1955). Many workers attempted to study the effects of different types and amount of food on various species of benthic foraminifera (Table 2.1).

**Table 2.1: Laboratory culture studies wherein effect of type and amount of food on benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Bradshaw	1955	Different rotalid species have different food preferences; comparatively lower temperature results in reduced growth rate; both higher and lower than normal salinity has adverse effect on the growth of rotalids.
2.	Bradshaw	1961	Higher temperature lead to the increased growth rate and quick reproduction, however the specimens were smaller than the ones grown at lower temperature; effect of temperature and pH on benthic foraminifera was linked with seawater salinity; scarcity of food lead to the decreased growth and reproduction; antibiotics adversely affected the benthic foraminiferal species; only extremely high hydrostatic pressure was fatal; oxygen consumption was species specific and was controlled by the seawater temperature.
3.	Lee et al.	1961	The response of benthic foraminifera to a combined diet of diatom, filamentous algae and bacteria varied from species to species.
4.	Lee & Muller	1973	<i>Allogromia laticollaris</i> , <i>Rosalina leei</i> , and <i>Spiroloculina hyalina</i> are selective feeders and can adjust to the seasonal changes in the food availability.

5.	Lee and Bock	1976	In two species of symbiont bearing soritid foraminifera, feeding is by far the more important process at midday; both species added about 4% of their weight in additional calcium each day; light did not enhance the rate of calcification.
6.	Salami	1976	Studied the feed preference optimum for growth and reproduction (salinity range and temperature range), mode of reproduction, chamber addition, differences in size, number and arrangement of nuclei etc of <i>Trochammina</i> cf. <i>T. quadriloba</i>
7.	Ross	1977	Size of the animal depends on the composition of food available; reproduction is adapted to seasonal changes in food.
8.	Kuile et al.	1987	Attempted to define the role of feeding in the carbon metabolism of the host-symbiont system in larger symbiont bearing foraminifera.
9.	Faber and Lee	1991	Studied the effect of feeding on the growth of foraminifera.
10.	Lee et al.	1991	Response to food was species specific as few species grew more when fed while others showed increased growth when provided with no food; additional nitrate and phosphate does not change the growth rate under certain conditions.
11.	Linke	1992	Two survival strategies in benthic foraminifera based on the ATP content and metabolic rates, namely, the one that maintained uniform rate throughout the year, and those that showed seasonally varying ATP turnover rate.
12.	Goldstein & Corliss	1994	Organic detritus, associated sediments as well as bacterial cells act as food for deep-sea and shallow-water species.
13.	Hemleben & Kitazato	1995	The culture without food survived for longer duration but reproduced less than the ones maintained under continuous food supply.

14.	Nigam et al.	1996	Food and type of media controls the growth in <i>Rosalina leei</i> .
15.	Moodley et al.	2000	<i>Ammonia</i> responded best to the freshly added phytodetritus.
16.	Moodley et al.	2002	Differential response of benthic foraminifera to induced phytodetritus.
17.	Witte et al.	2003	Abyssal foraminiferal response to phytodetritus was delayed and distinct from continental slope foraminifera.
18.	Ernst et al.	2005	Though oxygen availability affected the short term vertical distribution and density of benthic foraminifera, food content was responsible for shaping the long-term benthic foraminiferal assemblages.
19.	Langezaal et al.	2005	<i>Allogromia laticollaris</i> and <i>Ammonia beccarii</i> could distinguish between food (living and dead bacteria) and non-food (inorganic particles) material; inter- and intra specific variation in the uptake rate and final digestion of food.
20.	Nomaki et al.	2005a	Shallow infaunal species ( <i>Uvigerina akitaensis</i> , <i>Bulimina aculeata</i> ) assimilated more carbon as compared to the intermediate ( <i>Textularia kattedgatensis</i> ) and deep infaunal species ( <i>Chilostomella ovoidea</i> ); response varied as per the food and season.
21.	Nomaki et al.	2005b	Vertical migration in response to addition of food; response decreased from shallow infaunal to deep infaunal species; <i>Chilostomella ovoidea</i> does not respond at all.
22.	Nomaki et al.	2006	Recognized three types of food preference, viz. (1) fresh phytodetritus selectively (phytophagous species); (2) fresh phytodetritus selectively but sedimentary organic matter as well when phytodetritus is absent; and (3) sedimentary organic matter at random (deposit feeders).
23.	Kohoa et al.	2008	Reported the response of benthic foraminifera to deposition of phytodetritus, either directly or indirectly due to

			enhances bacterial activity; the response can be measured as an increase in TSS of foraminifera.
24.	Pascal et al.	2008	Effects of abiotic (temperature, salinity and irradiance) and biotic (bacterial and algal abundances) factors were performed to measure uptake rates of bacteria through grazing experiments.

## 2.3 Oxygen Concentration

Besides food, oxygen is suggested as another important parameter that defines the microhabitat of benthic foraminifera (Jorissen, 1999). The relative influence of oxygen concentration on benthic foraminiferal community is debated (Moodley et al. 1998a; Heinz et al. 2001; Geslin et al. 2004). Upon the onset of anoxia, upward migration of deep living foraminifera was observed by Alve and Bernhard (1995) and Duijinstee et al. (2003). However, the response varies from species to species (Gross, 2000). In general, species abundance increases under oxic conditions, and the majority of the species show upward migration when subjected to anoxic conditions (Table 2.2).

**Table 2.2: Laboratory culture studies wherein effect of oxygen concentration on benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Bernhard	1993	No evident statistically significant effect of changing oxygen condition on the survival or ATP pool of foraminifera.
2.	Alve & Brenhard	1995	Studied the vertical migratory response of benthic foraminifera to controlled oxygen concentrations ranging from well-oxygenated to dysaerobic conditions in experimental mesocosm.
3.	Bernhard & Alve	1996	Survival rate of <i>Adercotryma glomeratum</i> , <i>Psammosphueru bowmunnii</i> and <i>Stainforthia fusiformis</i> subjected to anoxic conditions does not vary much from the control specimens; however the ATP

			concentrations were significantly lower. <i>Bulimina marginata</i> behaved differently.
4.	Moodley et al.	1998a	Few foraminifera can survive under anaerobic conditions; soft-shelled foraminifera are less tolerant to anoxia.
5.	Gross	2000	Species-specific effect of change in temperature, oxygen and food quantity on the migrational activity.
6.	Heinz et al.	2001	Benthic foraminiferal abundance increased under increased food supply and oxygen; the within sediment migration was controlled by the availability of oxygen.
7.	Heinz et al.	2002	Time series experiment to investigate the response of cultures deep sea benthic foraminifera to simulated phytodetritus pulses under stable oxygen concentrations.
8.	Duijnsteet et al.	2003	Anoxic conditions lead to the comparatively shallower dwelling depth for most of the species.
9.	Geslin et al.	2004	<i>Globobulimina affinis</i> , <i>Hoeglundina elegans</i> , <i>Pyrgo murrhina</i> , <i>Uvigerina peregrina</i> , <i>Uvigerina mediterranea</i> can all live in the oxic sediment layer, whereas <i>G. affinis</i> can also live under anoxic conditions; oxygen concentration regulates the microhabitat.
10.	Pucci et al.	2009	Experimental results show that all dominant foraminiferal taxa from the sixteen short sediment cores from a 35 m deep site in the Adriatic Sea survive strongly hypoxic conditions.
11.	Nomaki et al.	2009	In situ feeding experiment using <sup>13</sup> C-labelled unicellular algae, showed microbial degradation of <sup>13</sup> C labeled algal material and the production of bacterial biomass within 2 days. The biomass produced was gradually turned over by respiration or predation within 6 days.

## 2.4 Light and Symbionts

The food intake of certain algal symbiont bearing benthic foraminiferal species is affected by the changes in the light intensity. Thus, in laboratory culture experiments carried out to understand the effect of light intensity on the benthic foraminifera, much emphasis is given to the symbiont bearing benthic foraminiferal species. The very high light intensity as well as continuous darkness results in decreased growth and even morphologically distinct specimens (Table 2.3).

**Table 2.3: Laboratory culture studies wherein effect of light and symbionts on benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Röttger	1972	Low intensity and darkness lead to cessation of growth activity; growth pattern changed after the specimens were subjected to normal light again.
2.	Röttger & Berger	1972	Very high light intensity lead to decreased growth rate and tests grown under such conditions were morphologically distinct.
3.	Röttger & Spindler	1976	Studied the optimum condition for growth of <i>Heterostegina depressa</i> including the light intensity and the symbiotic algae; described the embryonic and nepionic developmental stages of the living individuals.
4.	Lopez	1979	The food intake varies as per the density of chloroplast; the chloroplast abundance varies as per changing light-dark conditions.
5.	Lee	1979	Nutrition and physiology of foraminifera from littoral, sub littoral to temperate zones is discussed.
6.	Lee et al.	1979	The symbiosis is responsible for the comparatively larger size of the symbiont bearing benthic foraminifera.
7.	Lee et al.	1980	<i>Amphisorus hemprichii</i> and <i>Amphistegina lobifera</i> were photoinhibited above 200 klx illumination, while photoinhibition in

			<i>Amphistegina lessonii</i> and <i>Heterostegina depressa</i> occurred at lower than 10 klx light intensity.
8.	Hallock	1981	Effect of light on growth rates of <i>Amphistegina lessonii</i> and <i>Amphistegina lobifera</i> are studied in the laboratory as well as the field conditions.
9.	McEnergy & Lee	1981	Three species of larger foraminifera <i>Amphistegina lobifera</i> , <i>Amphisorus hemprichii</i> and <i>Heterostegina depressa</i> were studied for their endosymbiotic associations and also fine structure analysis.
10.	Kuile & Erez	1984	Growth rate decreases under dark conditions in symbiont bearing foraminifera; shell thickening occurs under turbulent conditions.
11.	Hallock et al.	1986	Influence of environment, especially availability of light and water motion on the test shape of <i>Amphistegina</i> .
12.	Lee et al.	1991	Symbiont bearing species could not survive prolonged darkness.
13.	Williams & Hallock	2004	The growth rate of <i>Amphistegina</i> increased in blue light while was not affected by ultra-violet light towards the lower end.

## 2.5 Seawater Temperature

Seawater temperature is one of the most important ecological parameter for all marine organisms. Bradshaw (1955) noted that specimens reproducing at lower temperatures had larger diameter and more number of chambers compared to the specimens reproducing at higher temperatures. He also noted that unless and until there are favorable environmental conditions, foraminifera will not reproduce though it has reached maturity. Further experiments (Bradshaw, 1961) concluded that temperature may limit species distribution as well as prevent certain vital activities such as growth and reproduction. In view of this many laboratory culture studies have focused on understanding the response of benthic foraminifera to changing seawater temperatures (Table 2.4). The studies show that each species has a narrow

range of optimum temperature for both growth as well as reproduction. Furthermore, growth rate changes if the species are subjected to temperatures other than the optimum.

**Table 2.4: Laboratory culture studies wherein effect of seawater temperature on benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Myers	1935b	The optimum temperature for <i>Patellina corrugata</i> is very near to the upper temperature tolerance limit; lower temperature leads to decreased rate of reproduction.
2.	Arnold	1954	<i>Discorinopsis aguayi</i> and <i>Discorinopsis vadesens</i> can survive extremes of temperature only if subjected for a short period.
3.	Bradshaw	1957	Temperature and salinity below and above tolerance limits, lead to cessation of growth in <i>Streblus beccarii</i> (Linné); within the temperature tolerance limit, growth increases with temperature; lower temperature and extreme salinity leads to delayed reproduction.
4.	Bradshaw	1961	Higher temperature lead to the increased growth rate and quick reproduction, however the specimens were smaller than the ones grown at lower temperature; effect of temperature and pH on benthic foraminifera was linked with seawater salinity; oxygen consumption was species specific and was controlled by the seawater temperature.
5.	Röttger	1972	Lower temperature resulted in reduced rate of chamber formation but does not affect the size of chambers or shape of the test.
6.	Gross	2000	Species-specific effect of change in temperature, oxygen and food quantity on the migrational activity.

7.	Nigam & Caron	2000	The pairing, probably a requisite for sexual reproduction in <i>Rosalina leei</i> , was affected by the seawater temperature.
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## 2.6 Seawater Salinity

Salinity is also an important ecological parameter which governs the survival growth and reproduction in foraminifera, especially in coastal areas. Salinity decreases during monsoon season in coastal areas due to a lot of fresh water influx. This change in salinity affects the marine organism. De Rijk, (1995) reported that salinity is one of the important ecological parameter which influences the foraminiferal population in marginal marine areas. Bradshaw, (1955) reported that cultures maintained at 26.8‰ and 30.2‰ resulted in reduced growth. Salinity less than 13‰ and greater than 40‰ leads to delay or absence of reproduction (Bradshaw, 1961; 1957). The studies carried out to understand the effect of salinity are helpful in assessing the changing monsoon intensity based on the changes in benthic foraminiferal abundance, diversity and morphology (Table 2.5). It is observed that the growth decreases considerably with the lowering of salinity and even dissolution was noted in a few species at significantly low salinity (Nigam et al. 2006).

**Table 2.5: Laboratory culture studies wherein effect of seawater salinity on benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Bradshaw	1955	Both higher and lower than normal salinity has adverse effect on the growth of rotalids.
2.	Bradshaw	1957	Temperature and salinity below and above tolerance limits, lead to cessation of growth in <i>Streblus beccarii</i> (Linné); lower temperature and extreme salinity leads to delayed reproduction.
3.	Bradshaw	1961	Effect of temperature and pH on benthic foraminifera was linked with seawater salinity
4.	Freudenthal	1963	Developed a tidal system for laboratory studies on eulittoral foraminifera and

	et al.		found that the higher salinity is correlated with early reproduction.
5.	Murray	1963	Performed various ecologic experiments on foraminifera.
6.	Sliter	1965	Laboratory experiments on the lifecycle and ecologic controls of <i>Rosalina globularis</i> d'Orbigny.
7.	Stouff et al.	1999a	Increased number of abnormal <i>Ammonia beccarii</i> and <i>Ammonia tepida</i> specimens under hypersaline conditions; abnormalities similar to those reported from similar environments in field.
8.	Stouff et al.	1999b	Though abnormal specimens were also present under normal conditions, hypersaline conditions lead to the increased abundance of abnormal specimens.
9.	Nigam et al.	2006	<i>Pararotalia nipponica</i> (Asano) shows reduced growth at lower salinities and tests start dissolving at very low salinity.

## 2.7 Seawater pH

Range of pH in open sea varies from ~7.5 to 8.5, whereas in tide pools, bays and estuaries the pH may exceed 8.5 or at times fall below 7.0 (ZoBell, 1946). Bradshaw, (1961) reported that response of benthic foraminifera are species specific to changes of seawater pH. At lower as well as normal pH, dissolution occurs in calcium carbonate test of foraminifera. Dissolution in the foraminiferal test proceeds from the last chamber to the initial chamber (Cadre et al. 2003). Thus efforts have been made to understand the role of seawater pH on the benthic foraminifera (Table 2.6).

**Table 2.6: Laboratory culture studies wherein effect of seawater pH on benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Bradshaw	1961	Effect of temperature and pH on benthic foraminifera was linked with seawater salinity

2.	Angell	1967	<i>Rosalina floridana</i> can recover the dissolution of the tests incurred while subjected to seawater with acidic pH; however no evidence of any special mechanism to regenerate the test.
3.	McEnery & Lee	1970	Incorporation of radionuclides of Ca, Sr, P and S was proportional to the growth rate of <i>Rosalina leei</i> and <i>Spiroloculina hyalina</i> ; both the species have the capability to regenerate the test.
4.	Muller	1975	Assessed temperature, salinity and pH limits for <i>Allogromia laticollaris</i> , <i>Rosalina leei</i> and <i>Spiroloculina hyalina</i> ; type and amount of food affect the food intake.
5.	Cadre et al.	2003	Temporary acidification of the environment can cause morphological abnormalities in the <i>Ammonia beccarii</i> foraminiferal tests during recalcification.
6.	Kuroyanagi et al.	2009	Growth rate, measured by shell diameter, shell weight and the number of chambers added, generally decreased with lowering pH after 10 weeks of culture in asexually produced individually of <i>Marginopora Kudakajimensis</i> .

## 2.8 Reproduction and test morphology

Life cycle in foraminifera is characterized by alternation of sexual and asexual generations. The microspheric form is the asexual one with large test and small proloculus, whereas the megalospheric form is the sexual form with small test and large proloculus. Such morphological change in foraminifers due to alternation of generation has led to the recognition of new species. Morphological changes might possibly be attributed to climatic changes also. Laboratory culture studies helped to outline the effect of mode of reproduction on morphology of benthic foraminifera (Table 2.7). A few studies have also showed a link between mode of reproduction and coiling direction (Myers, 1936).

**Table 2.7: Laboratory culture studies wherein effect of reproduction on morphology of benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Myers	1936	Close relationship between mode of reproduction and coiling direction in <i>Spirillina vivipara</i> .
2.	Myers	1940	Megalospheric specimens preferentially coiled sinistrally while the microspheric ones coiled dextrally in <i>Discorbis patelliformi</i> .
3.	Lee et al.	1963	Close link between mode of reproduction and coiling direction with gamont specimens being preferentially dextrally coiled while the agamonts being sinistral.
4.	Schnitker	1967	At sexual maturity under laboratory culture, offsprings of <i>Triloculina linneiana</i> attained only the one-eighth the size of the parent and were morphologically distinctly different than the parent.
5.	Schnitker	1974	Physiological acclimatization takes place during and possibly after reproduction over a span of generation; clones culture lead to morphological variations.
6.	Röttger	1978	Unusual multiple fission in the gamont of the larger foraminifera <i>Heterostegina depressa</i> ; observed regeneration following multiple fission during which a small residue of protoplasm remained within the vacated test.
7.	Goldstein	1988	Reported the alternation of generations in the life cycle of <i>Saccamina alba</i> Hedley.
8.	Grell	1988	Reported the extreme heteromorphy in the alternative generations of monothalamous foraminifera <i>Heterotheca lobata</i> .
9.	Goldstein & Moodley	1993	Life cycle of <i>Ammonia becarii forma tepida</i> includes both sexual and asexual phases and is probably best characterized as a facultative alternation of generations.
10.	Alve &	2010	Propagules of certain species are sufficiently resilient to survive transport,

	Goldstein		remain dormant for two years, and then start growing and reproducing once conditions permit.
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## 2.9 Other factors affecting the abundance and morphology

There are many other factors or processes other than those discussed above affecting the abundance and morphology of benthic foraminifera. Studies carried out to understand the factors, other than those reported above, affecting the abundance and morphology have been tabulated in Table 2.8.

**Table 2.8: Laboratory culture studies wherein effect of other parameters on morphology and abundance of benthic foraminifera was studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Myers	1942	About 1000 <i>Elphidium crispum</i> tests per square foot are contributed annually to the sediments; larger tests evade degradation during ingestion by macrofauna.
2.	Frankel	1974	<i>Trochammina ochracea</i> attaches to the cavities in the surfaces and may be missed during counting.
3.	Schwab	1977	Described the biology (size, colouration, behaviour and the ability to rapidly change shape) of a new foraminifera belonging to genus <i>Boderia</i> and family Lagynidae.
4.	Bowser & Bloodgood	1984	Phase contrast light microscopy and membrane surface marker studies on two protozoan systems including that of <i>Allogromia laticollaris</i> and <i>Ammonia</i> sp. indicated that surface motility does not occur by a surf-riding/surf boarding mechanism.
5.	Buzas et al.	1989	Conducted experiments on predation, substrate preference and colonization of benthic foraminifera at the Shelf break off the Ft. Pierce inlet, Florida.
6.	Boltovskoy	1991	Conducted laboratory experiments to study the destruction of foraminiferal tests.

7.	Fujita	2004	Field colonization experiment using artificial substrate was conducted to examine the small scale distribution of algal symbiont bearing larger foraminifera on reef rubble.
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## 2.10 Factors affecting chemical composition

By culturing benthic foraminifera under controlled laboratory conditions, information about the effect of different physico-chemical parameters and vital factors on the foraminiferal chemical composition has been obtained. These studies have thus, helped to refine the applications of benthic foraminiferal chemical composition to paleoclimatic/paleoceanographic studies.

### 2.10 A. Factors affecting $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$

In laboratory culture studies, it is possible to maintain constant physico-chemical parameters throughout the experiment; therefore, studies have been carried out on benthic foraminifera to understand the factors controlling the stable isotopic composition. Laboratory culture studies showed that the carbonate ion composition of seawater, growth rate, and developmental stage, light levels etc. affect the stable oxygen and carbon isotopic composition of the benthic foraminifera. The studies dealing with factors affecting stable isotopic composition of benthic foraminifera have been compiled in Table 2.9 A.

**Table 2.9 A: Laboratory culture studies wherein factors affecting stable isotopic composition of benthic foraminifera were studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Erez	1978	Carbon isotopic composition of the foraminifera becomes more depleted under increased rate of photosynthesis by attached symbiont algae.
2.	Hallock	1978	Studied the rate of carbon fixation by <i>Amphistegina lessonii</i> (in the light as well as in dark) and noted that carbon fixation in light was consistently higher than that in the dark.

3.	Williams et al.	1981	Identified significant disequilibrium in carbon and oxygen isotopic fractionation in <i>Heterostegina depressa</i> and attributed it to the vital effects that varied with changing light intensity and age.
4.	Zimmerman et al.	1983	<i>Heterostegina depressa</i> tests, secreted at increased light levels are depleted in heavier isotopes of oxygen and carbon, probably under the influence of symbiont photosynthetic activity.
5.	Kuile & Erez	1988	Established the existence of an internal inorganic carbon pool in the perforate foraminifer <i>Amphistegina lobifera</i> using $^{14}\text{C}$ tracer method.
6.	Chandler et al.	1994	Utility of sediment microcosm cultures for the study of ontogenetic and sediment microhabitat effects on isotopic composition of benthic foraminifera.
7.	Chandler et al.	1996	Determined the sediment microhabitat effects on carbon isotopic signatures of microcosm-cultured benthic foraminifera <i>Ammonia beccarii</i> .
8.	Wilson-Finelli et al.	1998	While <i>Cibicides pachyderma</i> incorporated the changing carbon isotopic signal of the medium, <i>Uvigerina peregrina</i> $^{18}\text{O}$ was relatively enriched, in contrast to the field studies.
9.	Rollion-Bard et al.	2008	Reported that the vital effect for oxygen isotopic composition in foraminifera is mainly due to the presence of primary calcite, which is lighter than the secondary calcite.

## 2.10 B. Factors affecting elemental composition

Elemental composition of foraminiferal test is another proxy used for paleoceanographic and paleoclimatic interpretations. Foraminiferal test is composed of extremely pure calcite which amounts to 99% by weight of  $\text{CaCO}_3$  and the remaining 1% constitutes of the trace elements such as Mg, Sr, Ba and Cd (Lea, 1999). During the test precipitation trace elements are directly incorporated into the

test from the seawater. Therefore studies on elemental composition of foraminiferal test will be able to reflect seawater composition as well as the physical and biological condition present during the precipitation (Lea, 1999). Laboratory culture studies have helped evaluate elemental composition of several benthic foraminiferal species for paleoclimatic studies. Additionally, a few calibration equations to estimate seawater temperature from elemental composition have also been developed from laboratory culturing of benthic foraminiferal species. Below is the list of researchers who have studied factors affecting elemental composition of benthic foraminifera (Table 2.9B).

**Table 2.9 B: Laboratory culture studies wherein factors affecting elemental composition of benthic foraminifera were studied.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Angell	1979	Studied the crystal growth during chamber development in <i>Rosalina floridana</i> ; also studied the calcium and carbonate uptake during the same developmental periods using tracer method.
2.	Duguay	1983	Calcium incorporation and photosynthetic carbon fixation was examined in three species of benthic foraminifera that harbor symbiotic microalgae.
3.	Delaney et al.	1985	Temperature dependence for the minor elemental composition of foraminiferal shells was investigated in the laboratory and by analysis of several planktic and one benthic foraminiferal species from sediment trap and sediment core samples.
4.	Kuile & Erez	1987	Uptake of inorganic carbon and internal carbon cycling in symbiont bearing benthonic foraminifera.
5.	Buzas	1989	No effect of mineralogically different sediments on the colonization potential of foraminifera.
6.	Toyofuku et al.	2000	Concluded that monospecific Mg/Ca can be used to infer paleotemperature, based

			on culture of <i>Planoglabratella opercularis</i> (d'Orbigny) and <i>Quinqueloculina yabei</i> Asano under controlled conditions; salinity does not have significant effect on the foraminiferal Mg/Ca.
7.	Havach et al.	2001	Ba/Ca and Cd/Ca partitioning coefficients of <i>Bulimina marginata</i> , <i>Cibicidoides pachyderma</i> and <i>Uvigerina peregrina</i> were within the range observed from the field.
8.	Maréchal-Abram et al.	2004	Reported cadmium partitioning coefficient value close to one for <i>Ammonia beccarii</i> under one set of conditions, and opined that cadmium does not segregate in the foraminiferal carbonate from the surrounding water; deviation in other sets was attributed to presence of food material.
9.	Hintz et al.	2006a	Large difference in the Mg, Ba, Cd, Sr versus Ca partitioning coefficients of <i>Rosalina vilardeboana</i> and <i>Bulimina aculeata</i> .
10.	Hintz et al.	2006b	Sr/Ca partitioning coefficient of <i>Bulimina aculeata</i> was not affected by the ontogenetic development, whereas large variation was observed in Mg/Ca partitioning between different individuals, as well as at different developmental stages in the same individual.
11.	de Nooijer et al.	2007	Copper partitioning coefficient was constant in <i>Ammonia tepida</i> and symbiont-bearing <i>Heterostegina depressa</i> over a large range of seawater Cu/Ca and was not affected by the seawater salinity, temperature or presence of symbionts.
12.	Dissard et al.	2010	Magnesium incorporation increases with increasing temperature (DMg increases by 4.1%, 4.6% and 5.5% per °C (temperature range 10-15 °C for salinities of 20, 33 and 40‰, respectively), but to a lesser extent than previously described for other low-mg calcite foraminifera.

## 2.11 Studies that helped to identify cryptic species

As discussed above, alteration in physico-chemical parameters tend to change the morphology of species of the same genus. These changes make species level identification of foraminifera difficult, which is the basic requirement for almost all foraminiferal characteristics based proxies for paleoclimatic/paleoceanographic reconstruction. Molecular systematic analysis can now be used to differentiate between morphotypes of benthic foraminifera. The genetic makeup of each individual is characteristic. It thus helps in identification of species or genus, based on the percentage of differences in the genetic make-up of the individuals. The extraction and enzymatic characterization of foraminifera DNA lead the way for application of molecular systematic analysis for identification of foraminiferal species and for tracing the evolutionary history of foraminifera (Langer et al. 1993; Wray et al. 1993). These studies have far reaching implications for species identification in foraminifera (Table 2.10).

**Table 2.10: Laboratory culture studies that helped to identify cryptic species of benthic foraminifera.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Arnold	1952	Desiccation experiments showed that a part of tubuliferous ring may get preserved after fossilization and help in identification from fossil assemblage.
2.	Arnold	1953	Observed live specimens of <i>Allogromia laticolaris</i> in the laboratory and inferred morphological differences in gamonts and agamonts; helped in clubbing of previously different species under single species.
3.	Nyholm	1958	Observed that previously considered separate taxa were in fact different ontogenetic stages of <i>Cibicides lobatulus</i> .
4.	Biekart et al.	1985	Existence of two biologically different types of megalospheric <i>Heterostegina depressa</i> in laboratory cultures as well as Hawaiian sediments.

5.	Röttger et al.	1986	Proposed that the earlier considered schizont of <i>Heterostegina depressa</i> , in fact belonged to a new species.
6.	Röttger	1987	Describes and illustrates a new species of <i>Heterostegina</i> ( <i>H. apogama</i> , n.sp.) based on the reproductive as well as morphological characteristics.
7.	Röttger et al.	1990b	Observed the formation of megalospheric schizonts by a microspheric agamont for the first time, which verifies part of the hypothesis of biologic trimorphism.
8.	Röttger et al.	1990a	The gamonts and schizonts of <i>Calcarina gaudichaudii</i> vary in their test size, size of the proloculus and number of chambers.
9.	Pawlowski & Lee	1992	Showed that <i>Rotaliella elatiana</i> has a classical heterophasic lifecycle, with a regular alternation of diploid agamontic phase and a haploid gamontic phase.
10.	Wray et al.	1993	Discussed the extraction and enzymatic characterization of foraminiferal DNA.
11.	Pawlowski et al.	1994	Identified two species of <i>Glabratella</i> from eight morphotypes collected from the field, by using molecular systematic analysis.
12.	Pawlowski et al.	1995	Identified three species of <i>Ammonia</i> based on the partial sequences of large subunit ribosomal DNA from six morphotypes of <i>Ammonia</i> ; all the six morphotypes had distinct LSU rDNA.
13.	Holzmann et al.	1996	Sequence variations in the large-subunit ribosomal RNA gene of <i>Ammonia</i> (foraminifera, Protozoa) and their evolutionary implications.
14.	Fahrni et al.	1997	<i>Miliammina fusca</i> is porcellaneous and not agglutinated as suggested before.
15.	Holzmann & Pawlowski	1997	Identified two species of <i>Ammonia</i> based on the molecular, morphological and ecological evidence.

16.	Holzmann et al.	1998	Identified two <i>Ammonia</i> sp based on the differences in the partial sequencing of large subunit ribosomal DNA, but the species could not be identified morphologically.
17.	Holzmann	2000	Deciphered the subspecies of <i>Ammonia</i> through molecular systematics.
18.	Tsuchiya et al.	2000	Ribosomal DNA sequence studies show that the single specimens of <i>Glaboratellidae</i> are genetically less diverse as compared to <i>Ammonia</i> .
19.	Pawlowski et al.	2002	Identified 49 Allogromids species belonging to 28 genera/families, from the 27 morphotypes, collected from Explorers Cove, McMurdo Sound, Antarctica, based on 135 partial small-subunit ribosomal DNA sequences.
20.	Saraswat et al.	2003	Proposed that three species of <i>Pararotalia</i> ( <i>P. nipponica</i> , <i>P. ozawai</i> , <i>P. taiwanica</i> ) are probably one, based on base pair length of 12 S mitochondrial gene.
21.	Tsuchiya et al.	2003	Identified two different species of <i>Planoglabratella opercularis</i> by sequencing internal transcribed spacers of ribosomal DNA.
22.	Grimm et al.	2007	Reported highly divergent genotypes among <i>Chilostomella</i> collected from different geographical locations, based on sequencing of 3' region of the small subunit ribosomal RNA (SSU rDNA), 5.8 subunit and the internal transcribed spacers.

## 2.12 Other studies that refined application of benthic foraminifera for past climatic/oceanographic reconstruction

Other than the studies discussed above there are a few other factors which influence the foraminiferal abundance and morphology (Table 2.11).

**Table 2.11: Laboratory culture studies that helped to refine application of benthic foraminifera for past climatic/oceanographic reconstruction.**

Sr. No.	Author & Reference	Year of Publication	Study Details
1.	Frankel	1974	<i>Trochammina ochracea</i> attaches to the cavities in the surfaces and may be missed during counting.
2.	Kuile & Erez	1991	Presence of internal carbon pool for calcification and partial contribution of carbon to this pool from the organic matter respiration.
3.	Kitazato	1995	<i>In situ</i> recolonization experiments to understand the possible substrate preferences of deep sea benthic foraminifera; faunal density was much lower and mainly confined to the natural sediment deposited over the artificial substrate.
4.	Linke et al.	1995	The response of deep sea benthic foraminifera to a simulated sedimentation was assessed in a strip-board microorganism by using TEM (Transmission Electron Microscopy) Organic Carbon, Adenosine Nucleotide, ETS assay and line observation.
5.	Moodley et al.	1998b	Sulphidic conditions resulted in significant reduction of foraminiferal density; none of the species reproduced under sulphidic conditions.

### 2.13 Objectives of the Study

After reviewing the benthic foraminiferal culture studies it is noticed that the proper understanding of parameters affecting the morphology of benthic foraminifera is still lacking. Exact mechanism that leads to deformities in the benthic foraminiferal tests is yet to be explored. A collective effort is needed to understand the specific effect of different ecological parameters, so that the benthic foraminiferal response can be used efficiently in the field. Similarly the factors affecting the foraminiferal isotopic composition, especially the carbon isotopic composition are still not known fully.

Although usefulness of benthic foraminifera under laboratory culture experiments is well understood, such studies in India are yet to take concrete shape. Therefore, based on the literature review, it was decided to perform laboratory culture studies on benthic foraminifera with the following objectives.

- To observe the effect of different concentration of food on selected benthic foraminifera.
- To observe the growth and reproductive phases of selected benthic foraminiferal species.
- To study the life span of a few benthic foraminifera.
- To observe the response of selected benthic foraminiferal species (*Cymbaloporeta plana*, *Pararotalia nipponica*, *Rosalina leei* etc.) to different ecological parameters.
- To conduct isotopic analysis on selected benthic foraminifera.

Before carrying out experiments on selected benthic foraminiferal species, it was necessary to understand its food preferences and life-span. Therefore, laboratory culture studies were carried out to understand the food preferences and life spans of a few benthic foraminiferal species and are discussed in subsequent chapters.

## Sample Collection and Laboratory Processing

### 3.1 Introduction

The study of different aspects of live foraminifera in laboratory culture provides an efficient tool to test and validate various foraminiferal proxies being used for (paleo) climatic and (paleo) environmental reconstruction. As stated earlier, since benthic foraminifera live in both shallow as well as deep water sediments, the samples for separating live benthic foraminifera for the present study were collected from shallow water regions. The material for isolating live foraminifera was collected during the lowest low tide. In the present study the material for separating live specimens of benthic foraminifera was collected from the area off central west coast of India, near the National Institute of Oceanography, Dona Paula, Goa (Fig. 3.1).



Figure 3.1: Aerial view of sampling area

## 3.2 Sampling Location

Sampling was carried out from the coastal waters off Goa, mainly the Dias Beach (15° 27' N; 73° 48' E), located near the Dona Paula bay (Fig. 3.2). It is surrounded by the Mandovi estuary to the right and Zuari estuary to the left. It is about 200 m in length and has rocky cliffs on both the sides (Fig. 3.1). Three prominent seasons, namely the pre-monsoon (February to May), the southwest monsoon (June to September) and the post monsoon (October to January), are noted at the sampling site. The monthly or bimonthly sampling was carried out during lowest low tide. The study area is dotted with lateritic rocks, the submerged ones of which provide the substratum for the sea-grass to grow. The sampling station is at walking distance from the laboratory, which made it easier to collect the sample as well as the sea water required for laboratory culturing.

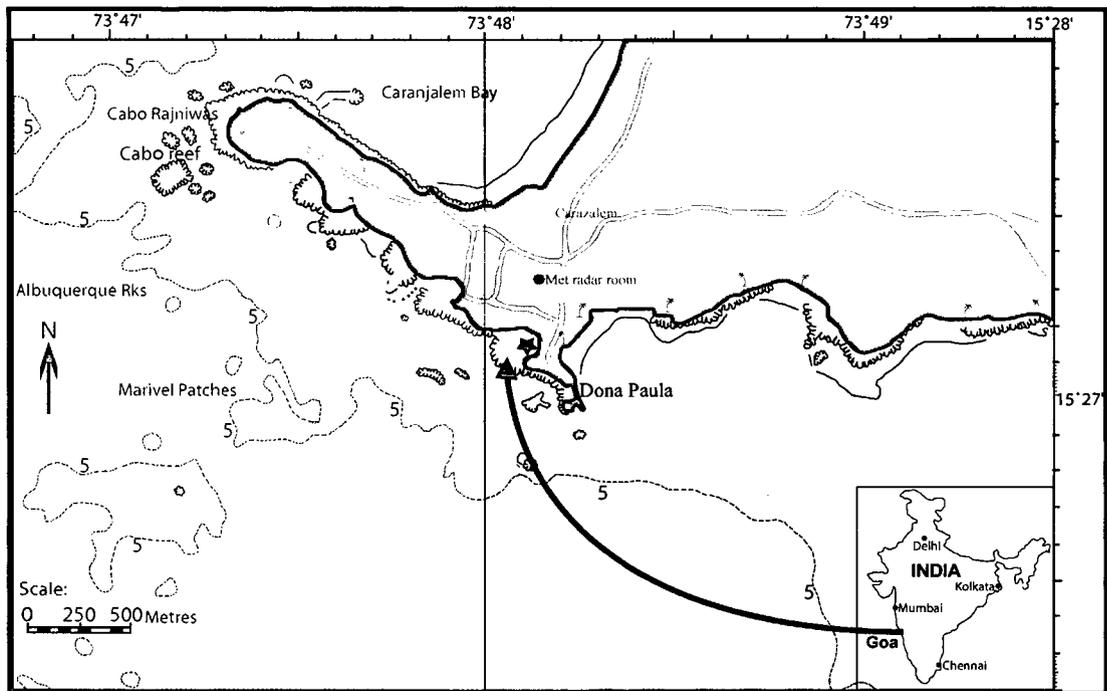


Figure 3.2: Location of sampling station.

### 3.2 A. Checklist of Materials Required for Sampling

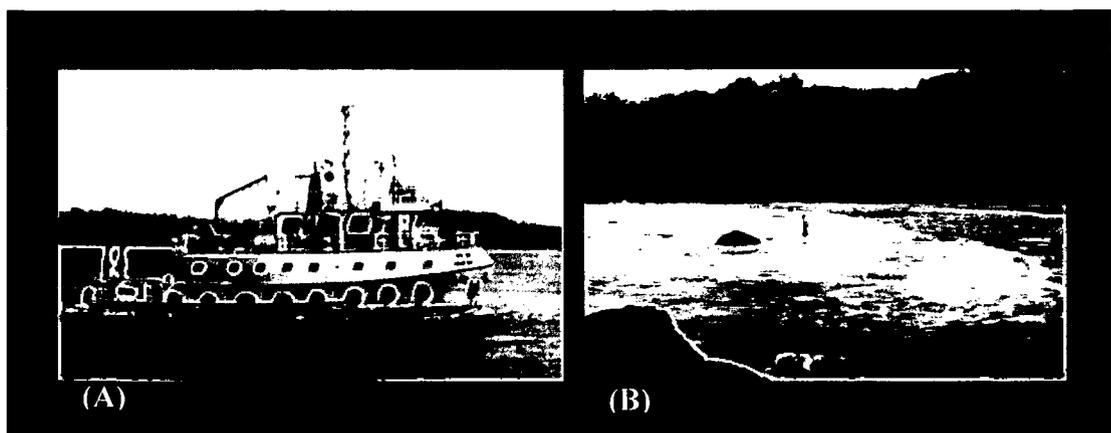
Since the pre-processing of the material collected to pick live benthic foraminiferal specimens was carried out in the field, a set of material (Table 3.1) was taken during field trips.

**Table 3.1: Checklist of material required for sampling live foraminiferal specimens**

1. Wet sieves 63 $\mu\text{m}$ and 1000 $\mu\text{m}$	2. DO meter	3. Scraper
4. Plastic Buckets	5. Plastic Mug	6. Sieve/Funnel Stand
7. Jerry Canes (10 l and 20 l)	8. Spray Bottle	9. Disposable Hand-Gloves
10. Plastic Funnel (8" diameter)	11. Wash Bottle	12. Hand Corer
13. Polythene Bags	14. DO bottle	15. Beakers (1000 ml)
16. Muslin Cloth of 63 mesh size	17. Salinometer	18. Glass bottle for pH

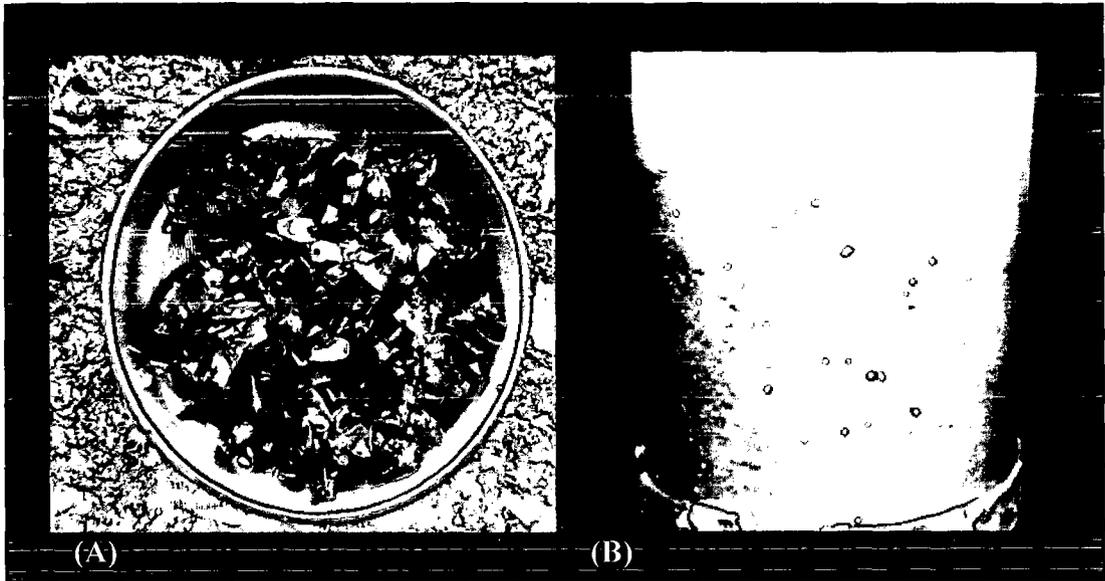
### 3.3 Methodology

The detailed methodology followed to collect material for picking live benthic foraminifera and to maintain them in laboratory is provided in this section. Benthic foraminifera live in almost all types of marine environments. They live as free as well as attached to the substratum, which may be submerged rocks, sea grass, other larger organisms etc. Material for live picking is collected through diving in shallow areas or by collecting surface sediments on-board research vessels (Fig. 3.3).



**Figure 3.3: Collection of sample on board (A) and by diving (B).**

The collected material, which include seaweed/sea-grass and sediments are then brought to the shore (Fig. 3.4). The seawater from the place from where material for picking live foraminifera was taken, was also collected at the same time. The seawater brought to the shore is then filtered through the muslin cloth and this filtered seawater is used for further processing of the material.

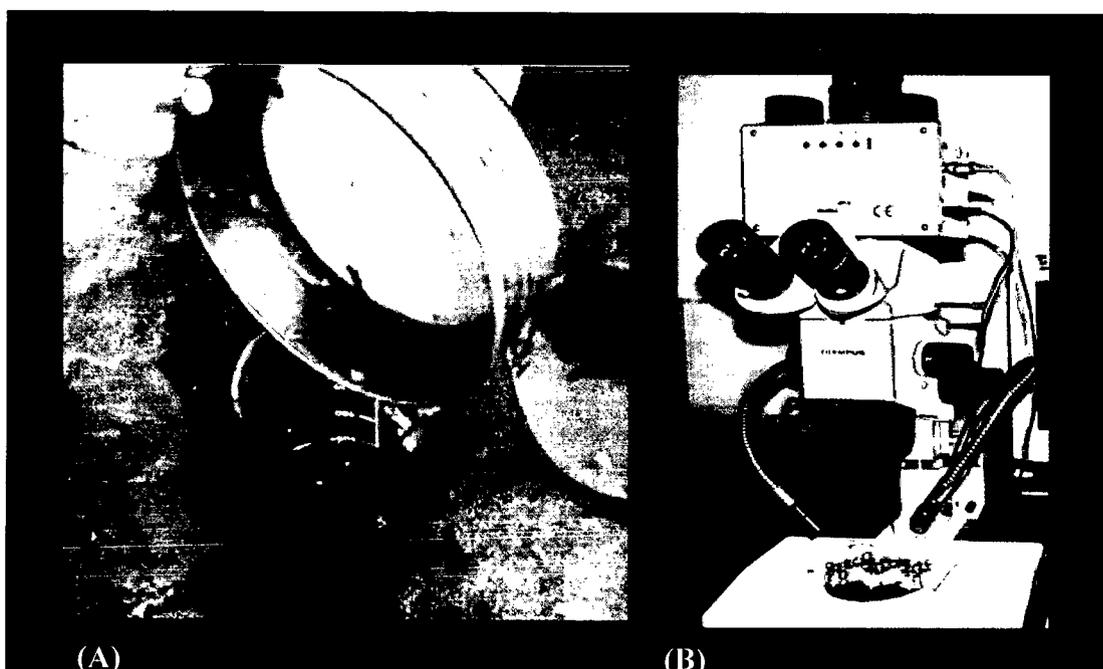


**Figure 3.4: Seagrass (A) and the sediment sample (B).**



**Figure 3.5: Vigorous shaking (A) and sieving of sample (B).**

The sea-grass or the seaweeds were then transferred into plastic buckets containing filtered sea water. The sea-grass/seaweed was then shaken vigorously to detach the foraminifera attached to it. This material was then sieved through 1000 $\mu$ m, (to remove large unwanted material) and 63 $\mu$ m sieves in order to concentrate the foraminiferal tests (Fig. 3.5). The >63 $\mu$ m and <1000 $\mu$ m material was then transferred to glass beakers and little seawater was added to it. The sieved material was brought to the laboratory and scanned for live benthic foraminifera, under the reflected light stereo-zoom microscope (Fig. 3.6). The prospective live specimens were isolated and kept under observation in 'Nunclan' multiwell culture dishes. The live nature of the specimens was confirmed when response such as movement, collection of food material or extended pseudopodia were noticed under the inverted microscope (Fig. 3.7).



**Figure 3.6: Transferring of the +63 $\mu$  material in beaker (A) and the Stereozoom microscope used for isolating foraminifera from the material (B).**

Once confirmed to be live, foraminiferal specimens became subject to different experiments. First few specimens were kept under observation to check their various growth stages till they reproduce. After the foraminifera reproduced, the juveniles were subjected to various experiments. Food for the foraminifera, in the form of diatoms, was cultured separately and foraminifera were fed accordingly.

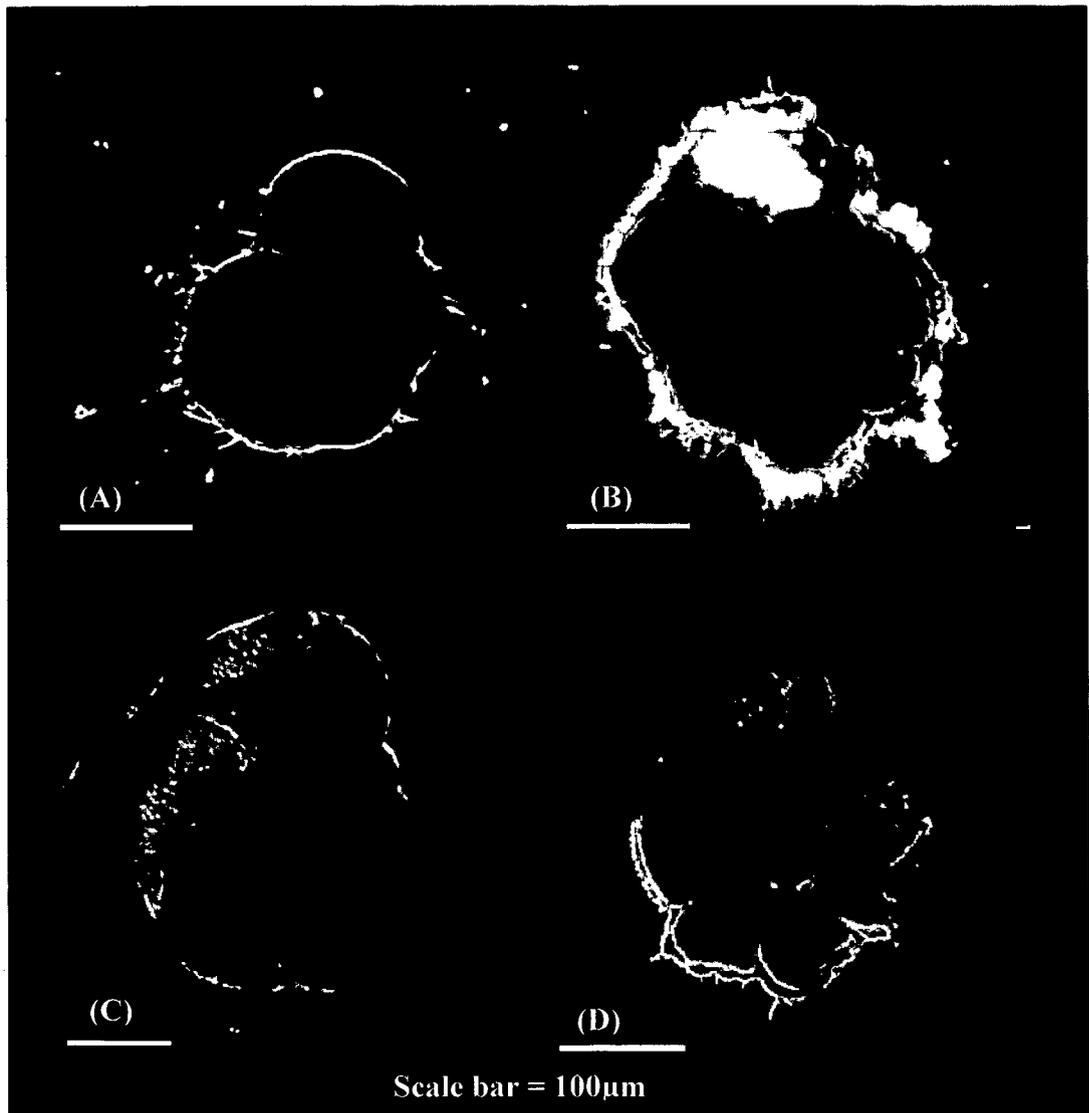


**Figure 3.7: Live image of specimen scanned under the Inverted Microscope.**

### **3.4 Species Abundance: General observations**

The abundance of live foraminifera in the collected material varied from place to place and from time to time. Specimens of *Pararotalia nipponica* (Asano) as well as *Rosalina leei* (Hedley and Wakefield), were observed to be abundant in protected location of the sampling site. At the open ocean location, however *P. nipponica* specimens were present in good number but the specimens of *R. leei* were less abundant. The protected location is surrounded by small rocks and thus is devoid from direct wave action. This signifies that *P. nipponica* being more robust can sustain the influence of more rough conditions than the *R. leei* specimens which have a thin test (Plate 3.1). During monsoon season there was a huge general decrease in the abundance of both the species. Specimens of *R. leei* were almost absent, though a few specimens of *P. nipponica* were present. This decreased abundance during monsoon season can be attributed to salinity changes. The salinity varied considerably throughout the year. During premonsoon season, the salinity varied between 35‰ and 36‰ while in monsoon season the salinity decreased upto 10‰, whereas during the post monsoon season, the salinity fluctuated between 33‰ and

37‰. The decreased salinity was probably responsible for the decreased abundance of live foraminiferal tests during monsoon season.



**Plate 3.1: Live specimens of *Rosalina leei* (Hedley and Wakefield) (A and C) and *Pararotalia nipponica* (Asano) (B and D). (A) and (B) photographs are taken with light, whereas (C) and (D) photographs are taken without light (where different specimens were used).**

Except above mentioned species, live specimens of many other species were also found in the study area (Plate 3.2 A&B). These live specimens did not reproduce in laboratory. The lack of reproduction in such specimens may be due to longer life spans or a narrow range of physico-chemical conditions required for reproduction, which could not be inferred during the course of experiments.

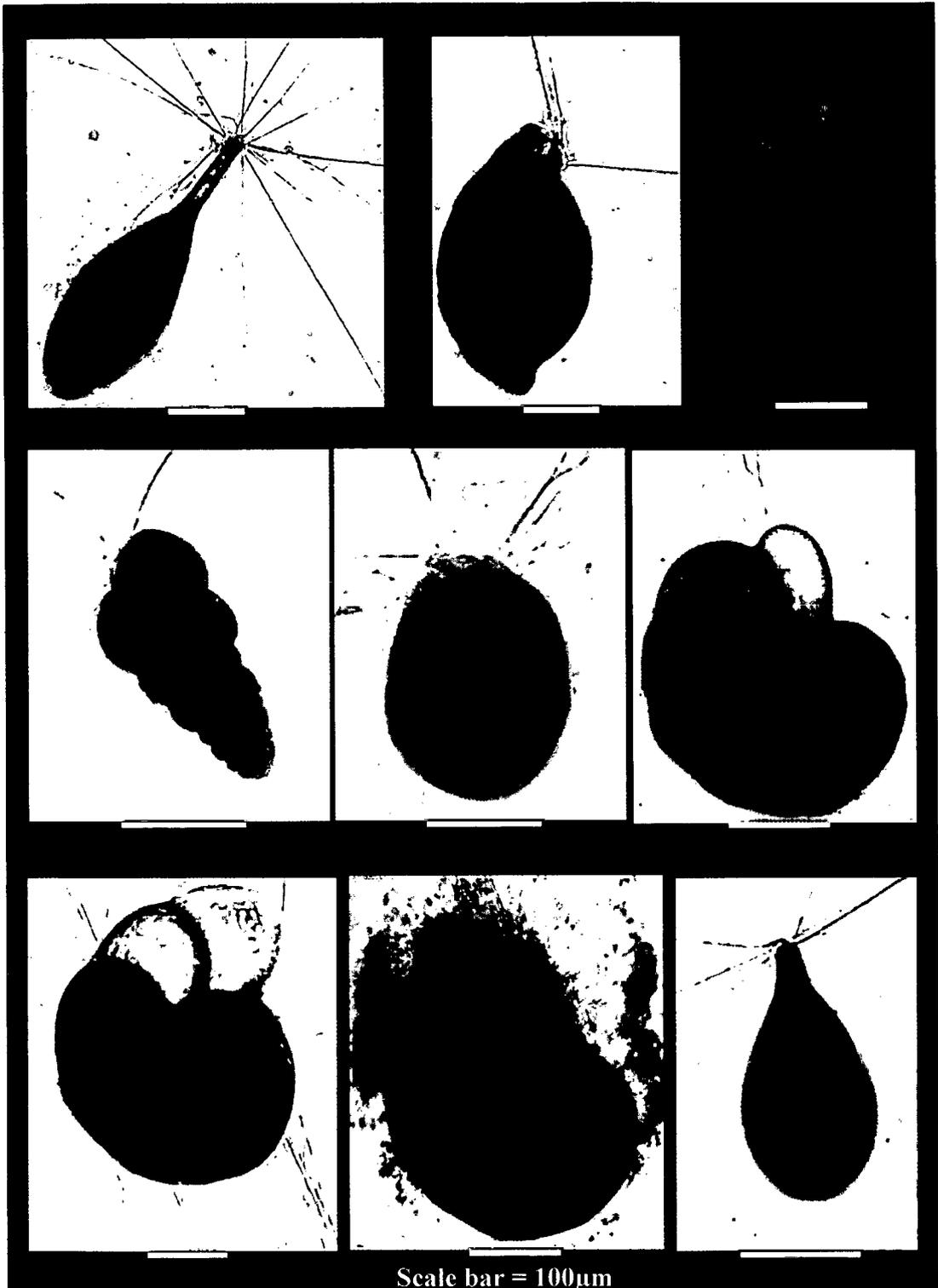
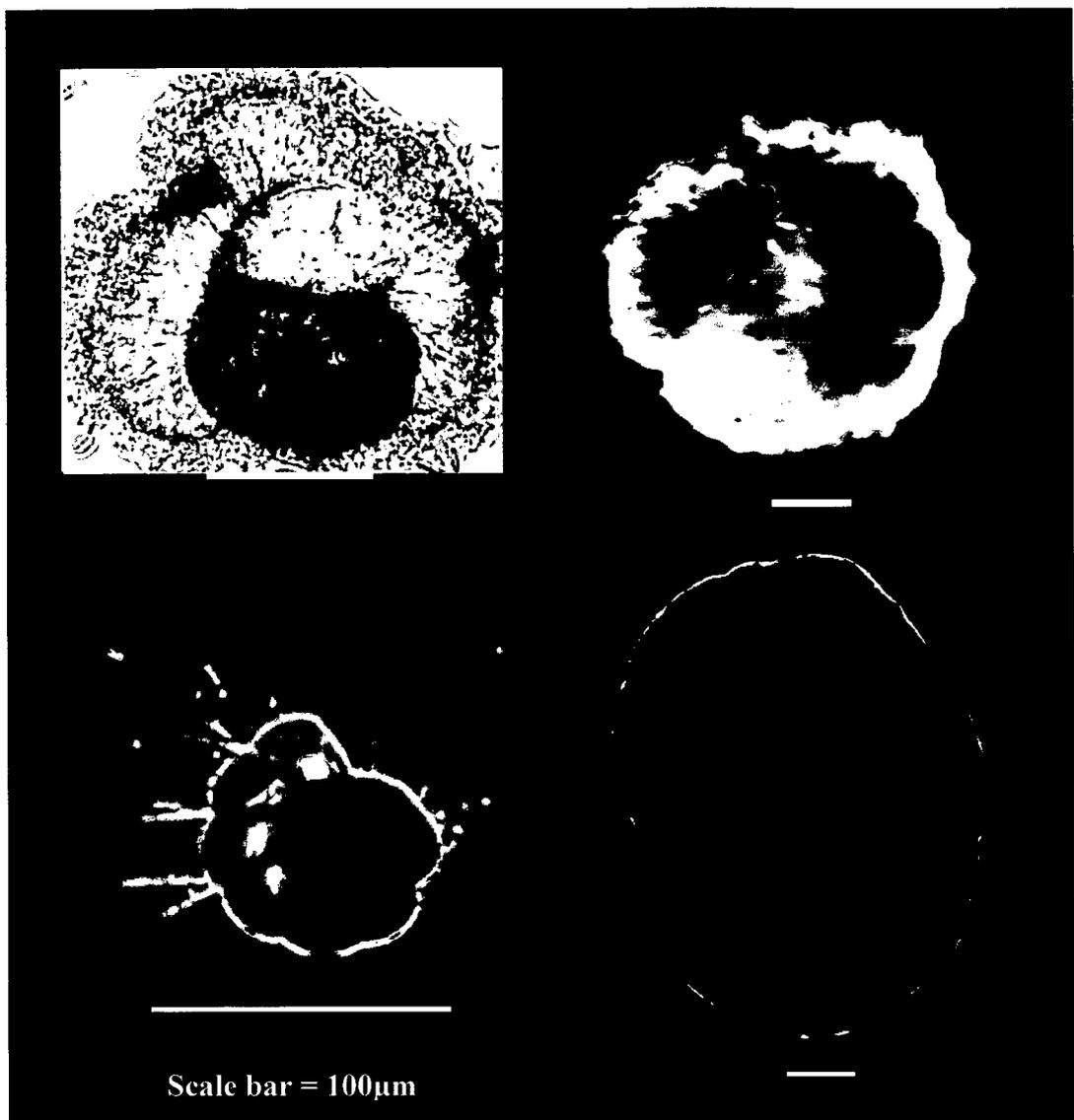


Plate 3.2 A: Live Specimens of *Lagina* sp., *Brazilina* sp., *Spiroloculina* sp., *Elphidium* sp., etc. found in the study area.

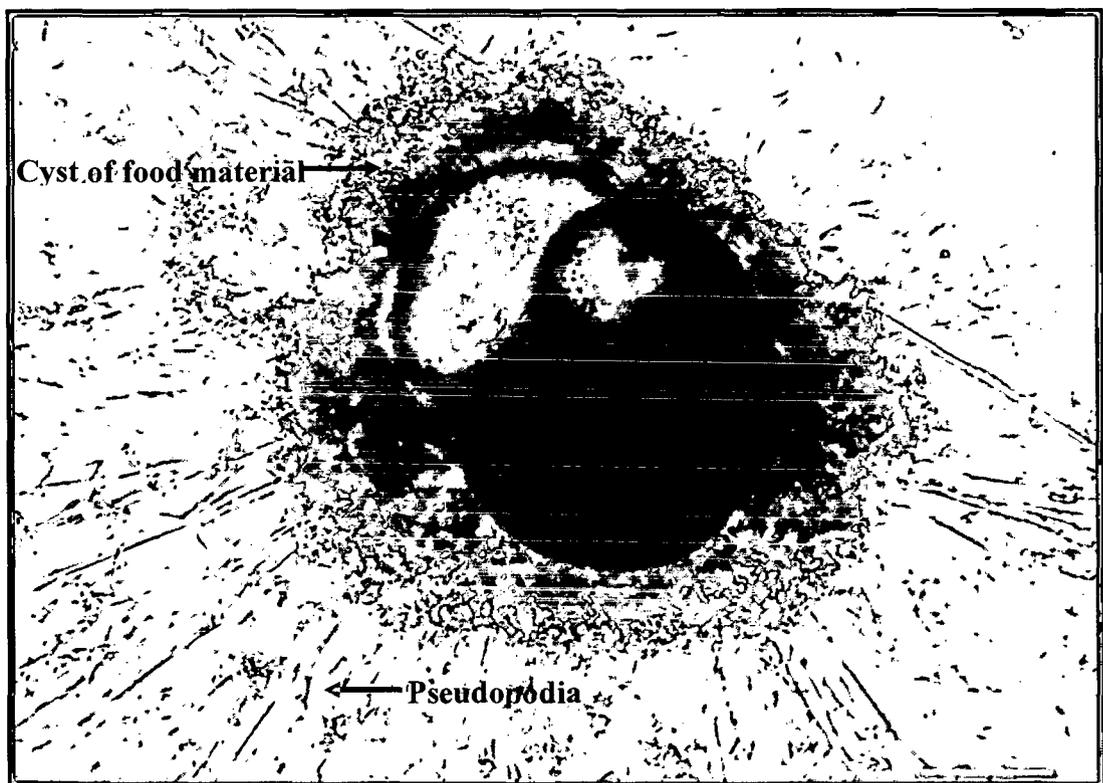


**Plate 3.2 B: Live Specimens of *Rosalina* sp., *Cavarotalia* sp., etc. found in the study area.**

### **3.5 Pseudopodia Activity: Sign of Being Alive**

The confirmation of benthic foraminiferal specimens being alive is done by observing any activity of the soft part of the specimens. The soft part of foraminifera comprise of single eukaryotic cell. This single cell, like other eukaryotic cells consists of cytoplasm, which can be differentiated into outer ectoplasm and inner endoplasm. Dispersed in cytoplasm are the cellular organelles including nucleus, mitochondria, golgi apparatus, endoplasmic reticulum etc. This single cell along with its organelles performs all the vital functions necessary for life. One of the characteristic features of foraminiferal cell is its ability to extend its ectoplasm into

long slender, thread like structures called pseudopodia (Plate 3.3). These pseudopodia exhibit a filamentous streaming motion. The organisms spread their pseudopodia as far as possible when in need of food. At times the length of pseudopodia was found to be as long as  $\sim 1520 \mu\text{m}$  (Plate 3.4). When foraminifer is disturbed the pseudopodia retract but are re-emitted from the test after a short interval. The pseudopodia perform various functions including sense of touch, collection of food, locomotion, respiration and test or chamber formation. To collect food, the pseudopodia are extended out up to the food particle where they branch out and entangle the food particle. Once the food particle gets entangled, the pseudopodia are retracted so as to bring the food particle near to the aperture from where the food particle is ingested in the test. The pseudopodia also take part in reproduction. When the specimen is about to reproduce it forms a cyst of food material around the parent cell so that when the juveniles are formed they have enough food to form new chambers before being released in the open (Plate 3.5). Live specimens of *Operculina* sp. deep sea benthic foraminifera which was isolated from the sediment samples collected on research vessel (Plate 3.6).



**Plate 3.3: Fine thread like structures protruding out of the organism the pseudopodia and cyst of food material formed around the specimen. Scale bar =  $100\mu\text{m}$**

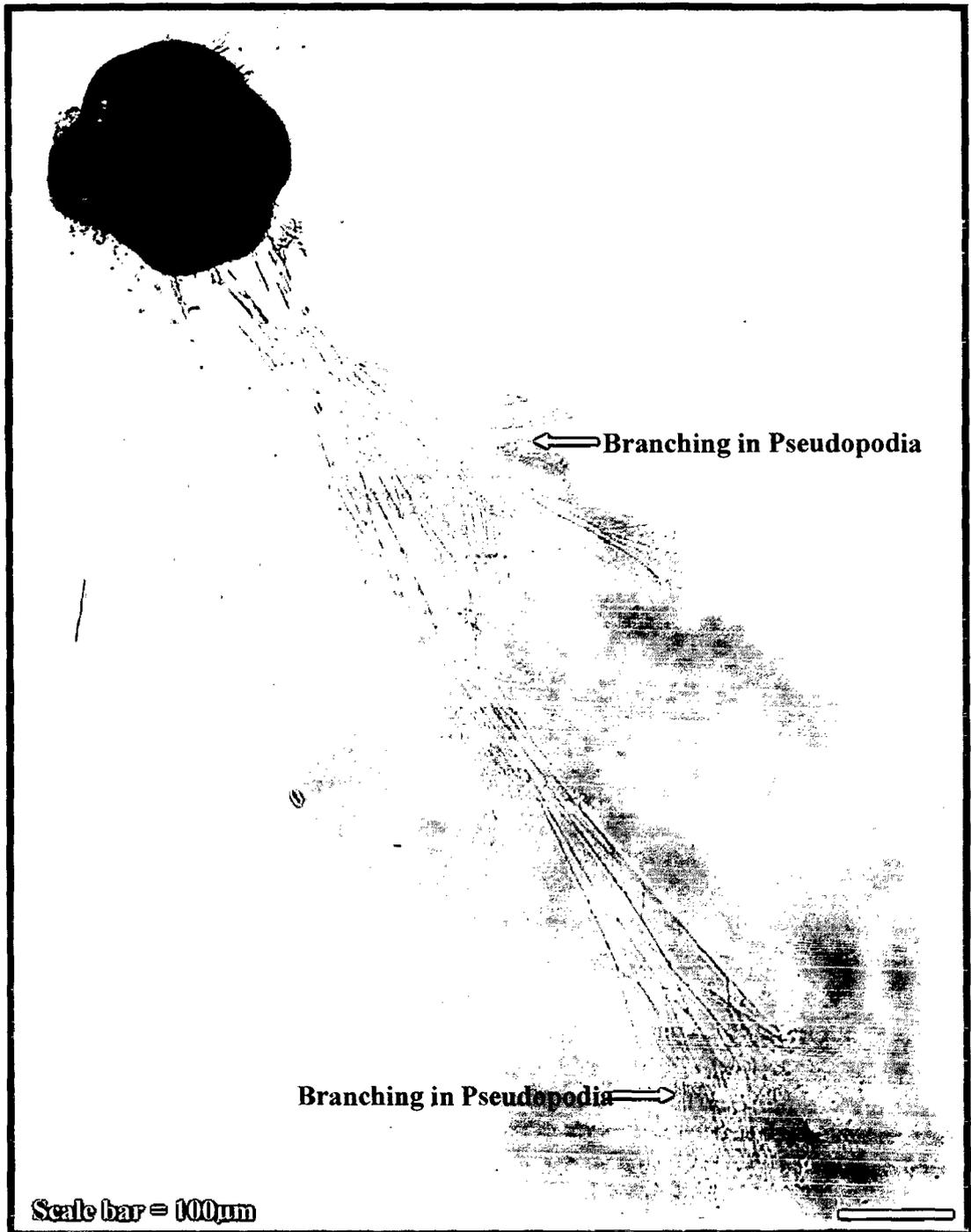
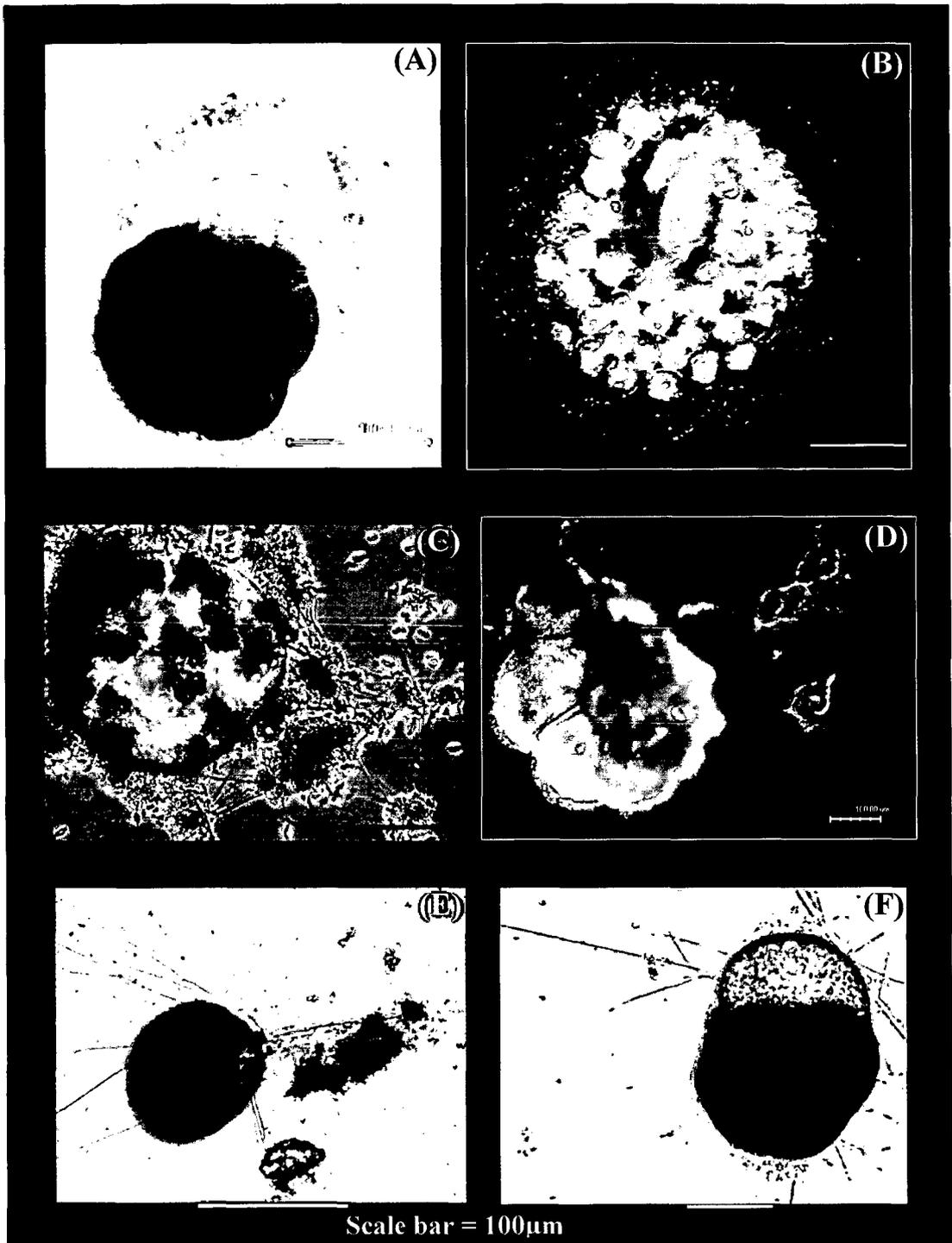


Plate 3.4: Pseudopodia protruding out of the organism. Length of the pseudopodia varies and may be as long as several hundred micron meters.



**Plate 3.5: Chamber formation with a cyst of food particles around the specimen (A), reproduction with a cyst around the specimens (B), release of Juveniles from parent cells (C and D) and gathering of food with the help of pseudopodia (E and F).**

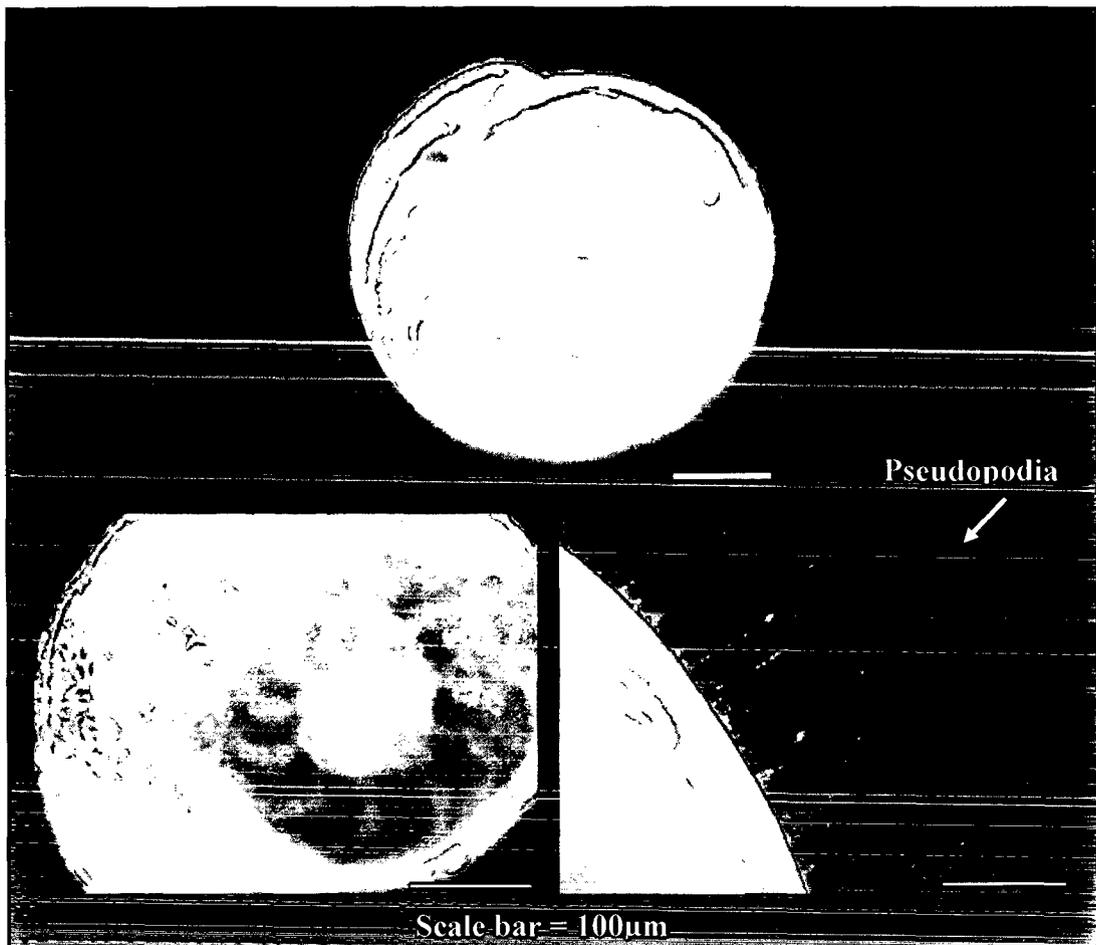


Plate 3.6: Live *Operculina* sp with different views.

### 3.6 Field trips and collection of physico-chemical data

Field trips were carried out to collect sediment as well as seaweed samples for live specimens, every month except during very rough conditions that prevailed during monsoons. During monsoon season, the waves were very high and the sea was rough which made it difficult to venture into the sea. Seaweeds were also not found during the monsoon season. Therefore pre-monsoon and post-monsoon sampling was mainly carried out. A preliminary investigation of the samples, show seasonal change in faunal distribution. This seasonal change may be attributed to several abiotic as well as biotic parameters that affect diversity and distribution of foraminifera. Therefore it was decided to record a few of the important abiotic factors in the field, at regular intervals. As part of it, salinity, temperature, pH and dissolved oxygen, which are among the important ecological parameters affecting the growth and

reproduction of these marine microorganisms were routinely measured in the field. In order to measure these physicochemical parameters, water samples were collected every alternate day. Salinity was measured with the help of Salinometer. The seawater pH was measured with LABINDIA  $\mu$ p controlled pH analyser and the dissolved oxygen and temperature was measured with Cyber Scan DO 300 Dissolved Oxygen/ $^{\circ}$ C/ $^{\circ}$ F data meter. The measured physico-chemical parameters were plotted against the Julian days.

### 3.6 A. Seawater Salinity

During the period from post monsoon season till premonsoon season, salinity changes are comparatively small (34‰ to 36‰). Subsequently, with the onset of monsoon, which is from 530 Julian days, the salinity decreased significantly.

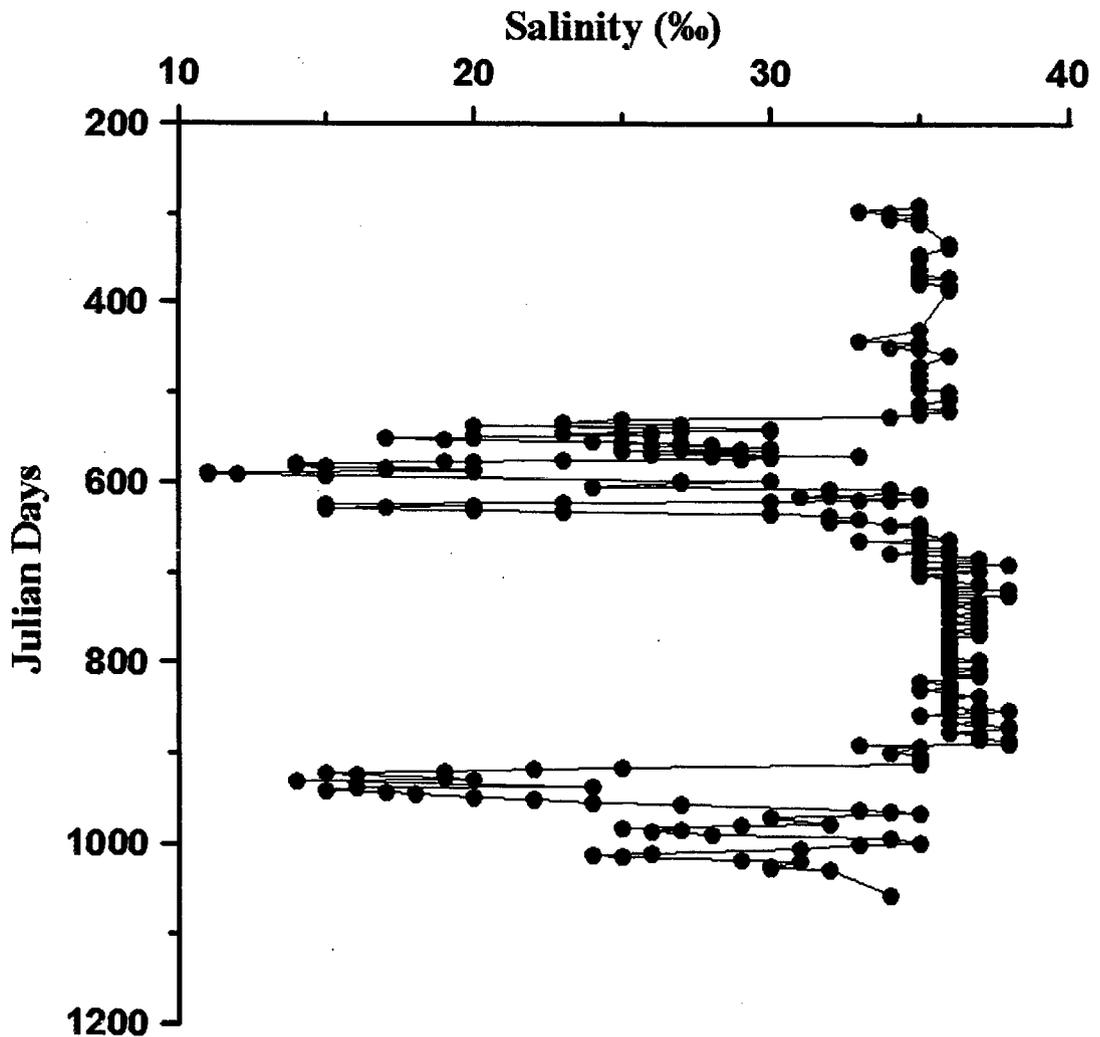


Figure 3.8: Salinity measured at the sampling site plotted against Julian days.

This was because the monsoon had just commenced and the precipitation was moderate. The salinity decreased up to 11‰ from 589 to 590 Julian days (Fig. 3.8). This was due to heavy monsoon which brought lot of freshwater from the land via various rivers and estuaries. Later, the salinity increased again from 606 Julian days up to 621 Julian days, which can be attributed to moderate rainfall or no rains during breaks in summer monsoon season. As the rain showers became moderate, salinity increased slightly but again when there was continuous rainfall, the salinity decreased up to 15‰ during 624 to 628 Julian days. From 640 to 911 Julian days the salinity fluctuated between 35‰ and 37‰ which are attributed to the negligible precipitation during post monsoon as well as the pre-monsoon season. Again after 916 to 950 Julian days the salinity decreases from 35‰ to 14‰ which signifies the onset of monsoon. Later again the salinity started increasing signifying the post monsoon season.

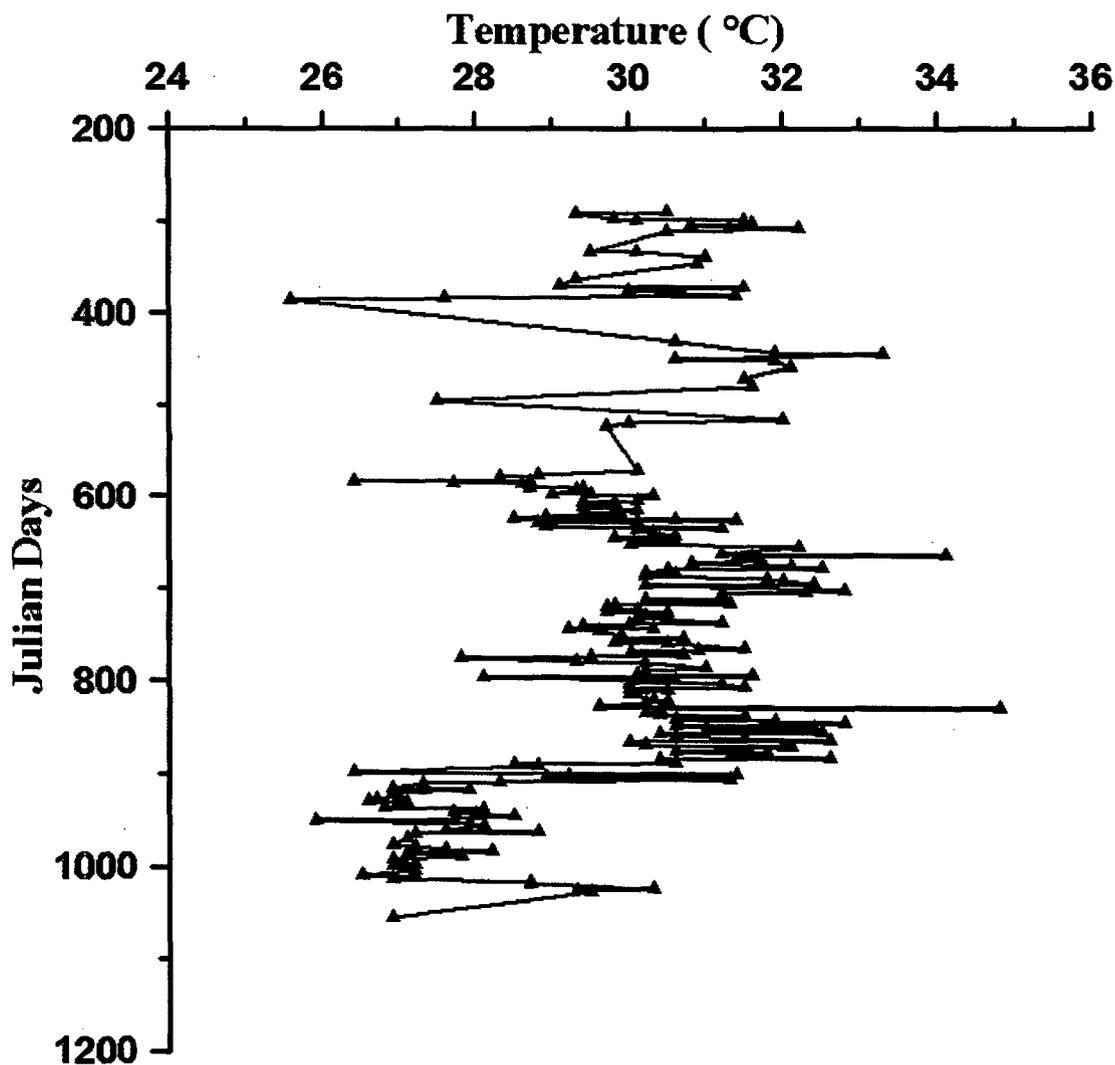
### **3.6 B. Seawater Temperature**

The seawater temperature was also regularly measured (Fig. 3.9). Between 290 to 381 Julian days, temperature varied from 29°C to 32°C. Decrease in temperature from 31°C to 25°C was noted from 383 to 386 Julian days, which is attributed to the winter cooling. Later again increased seawater temperatures were noted between 430 to 523 Julian days. Measurement could not be carried out during 524 to 571 Julian days. But from 572 to 652 Julian days the temperature fluctuated between 28°C to 31°C. Later from 654 to 887 Julian days the temperature started increasing. Again slight decrease in temperature was measured during 890 to 1022 Julian days where in the temperature varied from 29°C to 26°C. The seawater temperature showed influence of seasonal wind speed, freshwater influx and insulation changes.

### **3.6 C. Seawater pH**

The seawater pH varied from 7.7 to 8.3 between 290 to 522 Julian days. Boltovskoy and Wright (1976) mentioned that, the normal seawater has pH of 8.1 but varies from 7.8 - 8.3. From 523 to 592 Julian days onwards, a drop in the pH was noted, which

may be because of heavy rains resulting in the fresh water influx from the land into the sea, thus leading to decreased alkalinity. Between 597 to 652 Julian days the pH increased and remained more alkaline (7.9-8.2). But, from 662 to 721 Julian days the pH values again decreased from 7.9 to 7.6, which are attributed to the receding monsoons. The pH values again increased from 7.9 to 8.3, between 722 to 822 Julian days, which is pre-monsoon phase (Fig. 3.10).



**Figure 3.9: Temperature measured at the sampling site plotted against Julian days.**

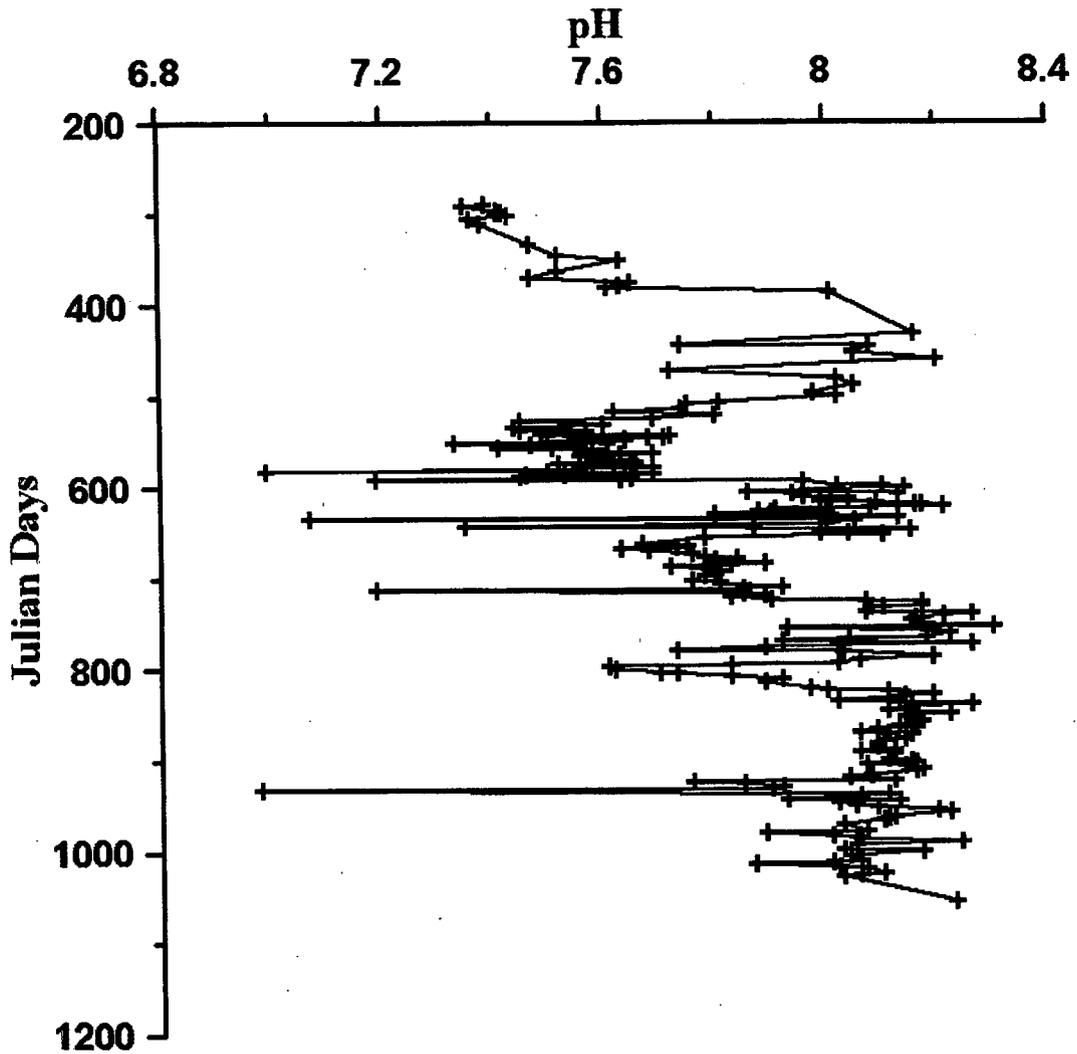
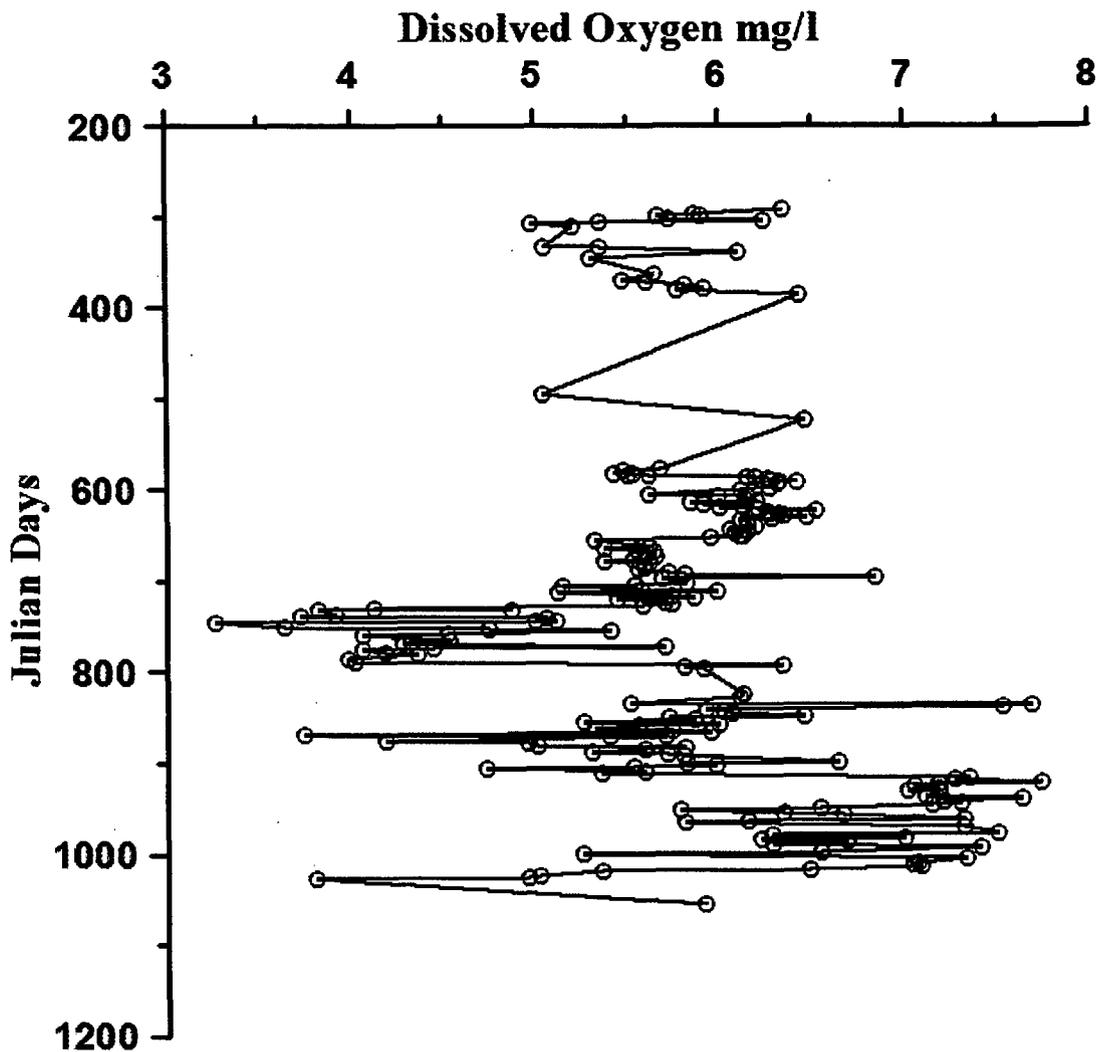


Figure 3.10: pH measured at the sampling site plotted against Julian days.

### 3.6 D. Seawater Dissolved Oxygen (DO)

Initially the measurements for dissolved oxygen were taken from 292 to 386 Julian days. But later, unfortunately there was some problem with the DO meter and therefore, the dissolved oxygen (DO (mg/l)) could not be measured from 387 to 576 Julian days. The DO content increases slightly from 584 to 652 Julian days and later decreased between 662 to 726 Julian days. From 729 to 788 Julian days, the concentration of DO further decreased and the values varied from 6.5 to 4.5mg/l. Increased DO was noted between 792 to 1013 Julian days (Fig. 3.11). The DO content showed an influence of wind speed and fresh water influx.

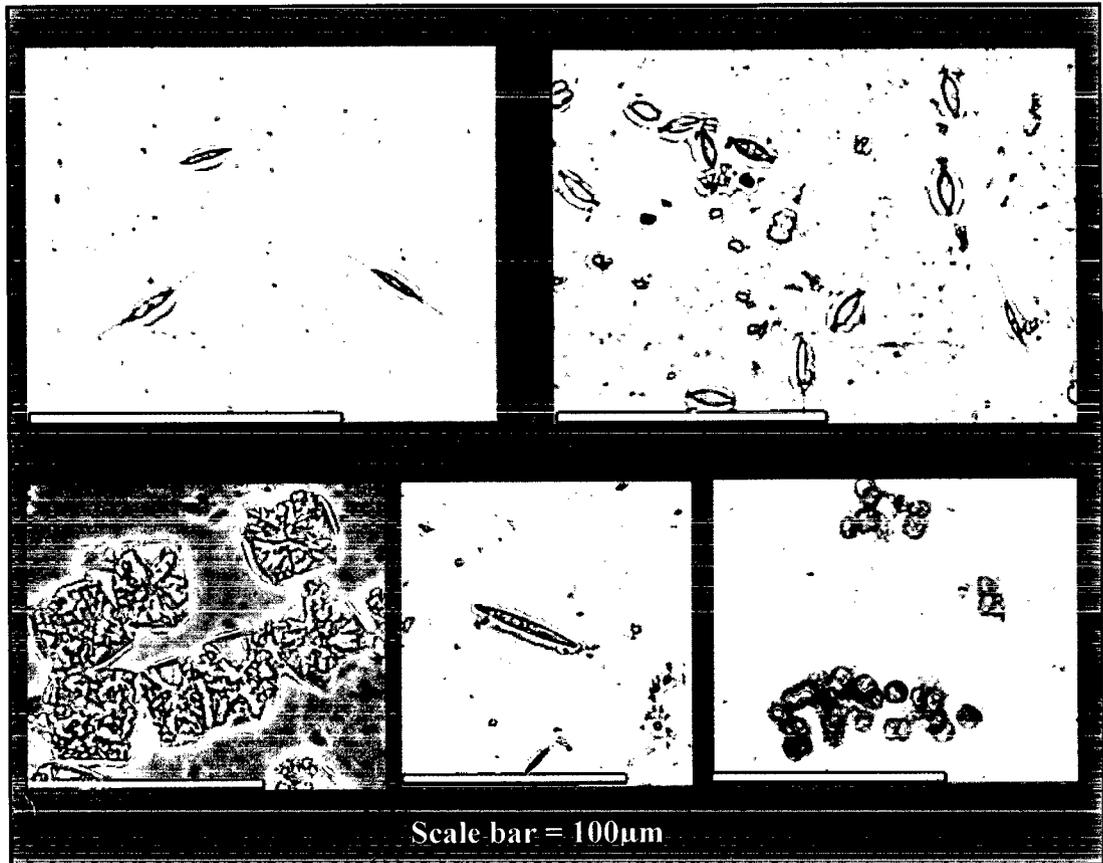


**Figure 3.11: Dissolved Oxygen measured at the sampling site plotted against Julian days.**

### **3.7 Diatom Culture (Food)**

As mentioned previously, foraminifera feed upon a variety of organisms. Previous laboratory culture studies have shown that benthic foraminifera feed upon mostly diatoms. Diatoms are a major component of plankton, free-floating microorganisms of marine or freshwater environments. They are unicellular algae generally belonging to family Bacillariophyceae. The cell walls of these organisms are made of silica. They are also among the most important aquatic microorganisms today. They are extremely abundant both in the plankton and in sediments in marine and freshwater

ecosystems, and because they are photosynthetic they are an important food source for marine organisms. It is estimated that 20% to 25% of all organic carbon fixation on the planet (transformation of carbon dioxide and water into sugars, using light energy) is carried out by diatoms. This is possible because they contain chlorophyll. Diatoms are a major source of atmospheric oxygen.



**Plate 3.7: Some species of diatoms found in the study area.**

Diatoms were collected from the phytoplankton bloom and brought to the laboratory for culturing. Only one species of diatom, *Navicula* sp. was cultured for the present work to be used as food for foraminifera though many different species of diatoms were found in the study area (Plate 3.7). The seawater samples brought from the field that contained diatoms were poured into a 1000ml flask containing filtered seawater. This flask was closed by using cotton plug in order to avoid contamination and was left near the window. Diatoms being photosynthetic prepare their own food and also multiply well in presence of sunlight.

The flask was checked regularly and within a few days it was noticed that the diatoms had reproduced to a huge number. In order to keep the diatoms alive to feed foraminifera, it was necessary to do sub-culturing of diatoms. Again two more flasks were taken with fresh seawater and a small quantity of diatom culture from the old flask was poured in both the new flasks and kept near the window for further growth. The cultured diatoms were used as food for the foraminifera. Before feeding the foraminifera, the flask was shaken well and with the help of a dropper only one drop was put into each well of the culture dish containing foraminifera.

The specimens thus collected and maintained in laboratory were used for various experiments, described in next chapters.

## Effect of food on growth and reproduction

### 4.1 Introduction

Foraminifera feed on phytoplanktons, coccolithophores, dinoflagellates, algae, spores, algal gametes, infusoria, other sarcodina, radiolarian, etc. (Winter, 1907; Sandon, 1932; Myers, 1935a, 1937, 1943c; Jepps, 1942; Arnold, 1954, Bradshaw, 1955, 1957; Nyholm, 1955; Hedley, 1958; Lee et al. 1961; Murray, 1963; Lee et al. 1966; Lee & Pierce, 1963; Boltovskoy, 1969; Lengesfeld, 1969; Muller & Lee, 1969; Lipps & Valentine, 1970; Christiansen, 1971; Rottger, 1972; Ross, 1972; Kathal, 1998). While a few species of foraminifera are very selective towards the kind of food they eat, others feed on a variety of organic matter. A few species like to feed on the living organisms, whereas others feed on the dead remains of organisms. Some of them like only a particular species of diatom whereas others prefer a mixed culture of diatoms. The food preferences of several species have been studied. Rhumbler (1911) reported that *Globorotalia menardii* feeds on diatoms and small radiolarian whereas *Globigerina* sp. eats crustacean fragments. Similarly, Lee, (1980) reported that most littoral foraminifera feed on pinnate diatoms, small chlorophytes and bacteria. The food preference of species belonging to same genus may vary. Grell, (1954) reported that *Rotaliella heterocaryotic* did quite well when supplied with heat-killed *Dunaliella*, but at the same time *R. roscoffensis* did not.

Based on the experimental studies on *Elphidium crispum*, Murray (1963) observed that it selects its food on the basis of size of the available food. *Ammonia beccarii*, *Quinqueloculina* sp., *Elphidium poeyanum* and *Rosalina leei* were found to feed on pinnate diatoms and chlorophytes (Murray, 1991), whereas deep sea foraminifera survived, while provided with freeze-dried *Chlorella* sp. as food (Gross, 2000). The field and laboratory studies showed that although the deep-sea foraminifera are adapted to limited or no supply of food material for long, means starvation, freshly deposited food material is quickly used (Linke, 1992; Graf and Linke, 1992). The food intake has also been linked with foraminiferal activity.

Langer and Gehring (1993) observed that when foraminifera have taken enough food, its pseudopodial activity decreases significantly.

Foraminifera collect its food with the help of pseudopodia and the food material is ingested through the aperture. Thus the type of food material ingested also depends on the size of the aperture. If the aperture is small, the foraminifera will try to take in the small organisms and if the aperture is big even large food material can be ingested. When the apertures are small mostly the gametes of algae and minute diatom frustules are preferred as food (Lister, 1895; Foyn, 1936).

In coastal area though temperature and salinity are the important parameters which govern the growth and reproduction, food is also equally important for these minute organisms to flourish. Influence of nutrition on chamber formations of few species was described by Myers (1943c). He observed that at high latitudes specimens receive less amount of food in winter and therefore the size of the chamber is smaller compared to the chambers formed during the summer, when plenty of food is available. In areas where the food material was abundant, specimens of benthic foraminifera *Elphidium crispum* were larger and elongated, but in areas of deficiency of food, these specimens were more rounded and globular (Boltovskoy and Wright, 1976). These field based observation were further confirmed by laboratory observations wherein it was noticed that the size of the specimen decreased and abnormal specimens were formed when there was less amount of food (Myers, 1936; Murray, 1963).

Significant seasonal differences in the foraminiferal population and assemblage (Gooday, 2003; Gooday and Lambshead, 1989), lead to the belief that changes in the type and amount of food may be one of the factors responsible for the changes in the foraminiferal population. Therefore, it was imperative to understand the effect of changes in the phytodetritus on the benthic foraminiferal population. The study can help to understand the changes in the species assemblages and the abundance of certain species under changing oxygen and food conditions in the field. Based on foraminiferal culture studies it was observed that changes in the foraminiferal diversity and abundance were brought by the amount of food and the response was species specific (Heinz et al. 2001). The differential food preferences result in change in abundance of a particular species as well as species assemblages under different environments.

Extreme scarcity of food leads to the cessation of growth and reproduction in certain species while the unfed specimens of certain other species grow equally well or better than the specimens that were supplied with food at regular intervals (Bradshaw, 1961; Lee et al. 1991). However, the scarcity of food lead to longer survival rate as the reproduction was delayed (Hemleben & Kitazato, 1995). The study can help to understand the reduced abundance of few species under low productivity conditions. The response to additional essential nutrients, nitrate or phosphate, was not only species specific but also was different under different light and food conditions. While *Amphistegina lobifera* does not respond to changes in nitrate and phosphate, *Marginopora kudakajimensis* showed maximum growth under illuminated, nutrient enriched and well fed conditions (Lee et al. 1991).

The type of food also affected the growth rate as well as the survival of benthic foraminifera (Lee et al. 1961; 1991). Lee and Muller (1973) noted that *Allogromia laticollaris*, *Rosalina leei* and *Spiroloculina hyalina* were selective feeders preferring only few algal species. Furthermore, these species can adjust well with the changes in the microbial community structure. The essential presence of bacteria for favorable growth was reported by Lee et al. (1991).

The response to phytodetritus has also been found to vary as per the depth at which the species are found in the ocean, thus reflecting the depth zonation of benthic foraminiferal species. Witte et al. (2003) noticed that the response of abyssal foraminifera to the phytodetritus was different than that of the continental slope species and foraminifera showed a retarded response as compared to the macrofauna. Nomaki et al. (2005a) studied the carbon assimilation rate of benthic foraminiferal species under in situ experiments and noted that shallow infaunal species (*Uvigerina akitaensis*, *Bulimina aculeata*) assimilated more carbon as compared to the intermediate (*Textularia kattegatensis*) and deep infaunal species (*Chilostomella ovoidea*). Here also, the response varied within the same species and also species to species subject to different types of food during different seasons. It thus explains the changing abundance and diversity during different seasons and in different geographical regions. The arrival of food has increased impact on the shallow infaunal species as compared to the deep infaunal species, while few species do not respond at all (Nomaki et al. 2005b).

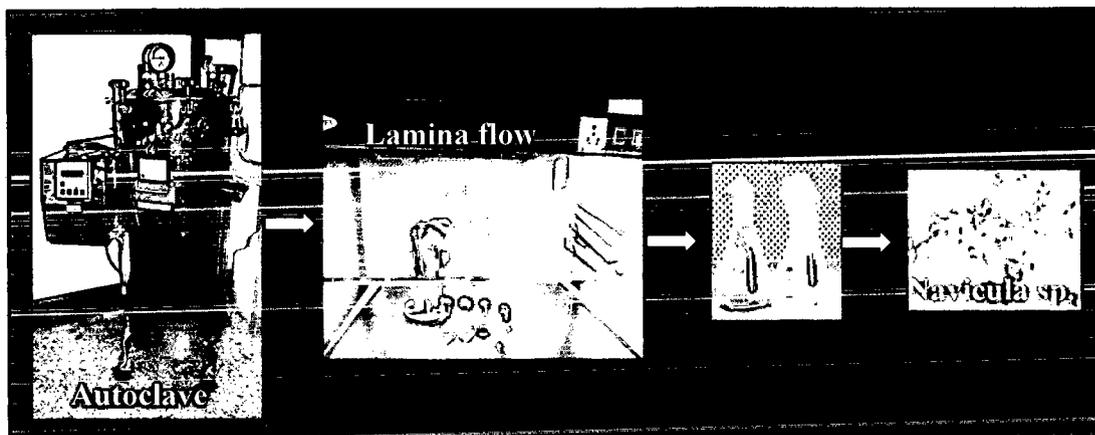
Linke, (1992) identified two survival strategies in benthic foraminifera based on the adenosine-tri-phosphate (ATP, a measure of feeding activity) content and metabolic rates, namely, the one that maintained uniform rate throughout the year and those that showed seasonally varying ATP turnover rate. The latter group of species will increase in abundance and respond well to the increased phytodetritus input. Different feeding preferences and mechanisms in benthic foraminifera were identified by Nomaki et al. (2006), who reported that benthic foraminifera prefer atleast three types of food (1) fresh phytodetritus, selectively (phytophagous species, namely *Uvigerina akitaensis*, *Bolivina spissa*, *Bolivina pacifica*); (2) fresh phytodetritus, selectively but sedimentary organic matter as well, when phytodetritus is absent (seasonal-phytophagous species, namely *Bulimina aculeata*, *Textularia kattegatensis*, *Globobulimina affinis*); and (3) sedimentary organic matter at random (deposit feeders, namely *Cyclammina cancellata*, *Chilostomella ovoidea*). The response of benthic foraminifera to phytodetritus under controlled laboratory culture studies has definitely helped to understand the changing abundance of different species in the field.

Since the type and amount of food depends on the climatic conditions, species abundance, morphology and assemblages can help reconstruct past climatic changes. This requires knowledge of food habits of different benthic foraminiferal species. In view of the significant effect of type and amount of food on benthic foraminifera, experiment was conducted to understand the food habits of selected benthic foraminifera.

## **4.2 Diatom Culture: Food for the Foraminifera**

Diatoms are unicellular organisms belonging to the class Bacillariophyceae. They are enclosed in silicate frustules which are made up of two halves. They are one of the major groups of producers (since they are photosynthetic in nature) in marine and freshwater environments. As they are photosynthetic they are limited to the photic zone of the water column. They are of two types, viz. the Pennate diatoms and the Centric diatoms. The Pinnate diatoms are elongated whereas the Centric diatoms are circular, triangular or rectangular. They reproduce asexually. The parent which consists of two valves splits across the middle and separate from each other forming

two daughter cells. Each daughter cell which takes one of the parent valves adds another valve by absorbing the silica from the ambient environment.



**Plate 4.1: Sub-culturing of diatom, *Navicula* sp.**

To use diatoms as food, a continuous laboratory culture of diatoms was maintained throughout the experiment. In order to isolate diatoms, water samples were collected from the study area. The water samples were brought to the laboratory and concentrated with the help of 10  $\mu\text{m}$  mesh. From this concentrated sample *Navicula* sp. were isolated and transferred to filtered sea water and kept under light. After a few days, only the *Navicula* sp. were again isolated and transferred to F2 media (Plate 4.1) This procedure was repeated three to four times in order to get pure culture of *Navicula* sp. F2 media was prepared following the Guillard and Ryther (1962) method and is described in the next section.

#### **4.2.1 Preparation of F2 Media**

One litre of seawater brought from the study area was filtered through Whatman 44 filter paper and was autoclaved. To this autoclaved seawater different stock solutions were added. The preparation of the stock solutions is described below (Table 4.1). Each of the major nutrient and trace metal stock solution was autoclaved separately. After autoclaving, all the components were mixed together to prepare 1l of trace metal solution. Vitamin working stock solution was prepared by dissolving 1 ml of Biotin and 0.1 ml of VitB<sub>12</sub> in 100 ml of separately prepared Thiamine HCl.

**Table 4.1: Composition of stock solutions used for preparing culture media.**

Compound	Quantity	
NaNO <sub>3</sub>	0.5 ml	Solution I
NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O	0.5 ml	Solution II
Na <sub>2</sub> SiO <sub>3</sub> .5H <sub>2</sub> O	0.5 ml	Solution III
Ferric Citrate	0.5 ml	Solution IV
Trace metal solution	0.5 ml	Solution V
Vitamin working stock solution	0.5 ml	Solution VI
Seawater autoclaved	1000 ml	

Major nutrients			
Compound	Quantity	Dissolved in	
NaNO <sub>3</sub>	15.00 g	100 ml distilled water	Solution I
NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O	1.13 g	100 ml distilled water	Solution II
Na <sub>2</sub> SiO <sub>3</sub> .5H <sub>2</sub> O (heat to dissolve)	2.27g	100 ml distilled water	Solution III
Ferric citrate	0.9 g	100 ml distilled water	Solution IV

Trace metals solution			Solution V
Compound	Quantity	Dissolved in	
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.020 g	100 ml distilled water	
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.044 g	100 ml distilled water	
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.22 g	100 ml distilled water	
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.360 g	100 ml distilled water	
NaMoO <sub>4</sub> .2H <sub>2</sub> O	0.013 g	100 ml distilled water	
Distilled water		500 ml	

Vitamins stock solution			Solution VI
Compound	Quantity	Dissolved in	
Biotin	0.01 g	100 ml distilled water	
Vitamin B <sub>12</sub>	0.01 g	100 ml distilled water	
Thiamine HCl	0.02 g	100 ml distilled water	

The F2 media was stored in the refrigerator. Before sub-culturing, F2 media was brought out of the refrigerator and kept at room temperature. In order to avoid possible contamination, conical flasks used for diatom sub-culture were autoclaved regularly. Sub-culturing was done after every 7 days. Diatom cultures were kept at four different temperatures (25°C, 27°C, 30°C and 35°C) and it was observed that, they grew well at 25°C, 27°C and 30°C temperatures, but at 35°C temperature the diatom growth and abundance was very low. The diatoms maintained in laboratory

were used as food for the foraminifera. Before feeding the foraminifera, the flask was shaken well and food was added to the different cultures of foraminifera with the help of micro pipette (Plate 4.1). The diatom cell count was made with the help of Sedgewick-Rafter.

### 4.3 Experimental Setup

The experiment was conducted on *Cymbaloporeta plana* (Cushman). The juvenile specimens of *C. plana* with 4 to 5 chambers were picked from the stock cultures maintained at different temperatures (25°C, 27°C and 30°C), as described previously. The isolated juvenile specimens were subjected to the same temperature from which it was picked, in order to prevent the experimental shock which the specimens would experience if they were directly transferred to different temperatures. The selected temperature range (25°C, 27°C and 30°C) was the same at which specimens of *C. plana* had reproduced. The salinity was kept constant (35‰) throughout the experiment. Different amount of pure *Navicula* sp. food (20cells/ml, 40cells/ml, 60cells/ml, 80cells/ml and 100cells/ml) was supplied. One set of *C. plana* specimens was not supplied with any additional food. The illumination in the form of 12h light and 12h dark intervals was provided. The experiment was conducted in replicate. A total of 5 specimens were kept in each well of the 6 well culture trays. Therefore, a total of 60 specimens were used for each temperature, this makes a total of 180 specimens used for food experiment. The salinity, temperature, dissolved oxygen and pH of the food as well as the media was measured every time the media was changed. Initially, at the onset of the experiment, coiling direction, number of chambers and maximum diameter of all the specimens was noted. Later on the maximum diameter of the individuals was noted, and a record of the number of specimens reproduced and died during the experiment was maintained.

### 4.4 Results

Initially at the start of the experiment, good pseudopodial activity along with movement was observed. During the course of the experiment, it was noticed that as the concentration of food increased, the specimens responded more quickly than the specimens with no additional food or less amount of food. With increasing amount of

food, significant growth was noted in all the specimens kept at all three temperatures (25°C, 27°C and 30°C) in both the sets (Fig. 4.1 A, B). However, the specimens subjected to 27°C temperature attained maximum growth compared to the specimens subjected to 25°C and 30°C temperature, whereas the specimens kept at 25°C temperature showed least growth (Fig. 4.1). No matter what the temperature was, the specimens with no additional food did not grow much.

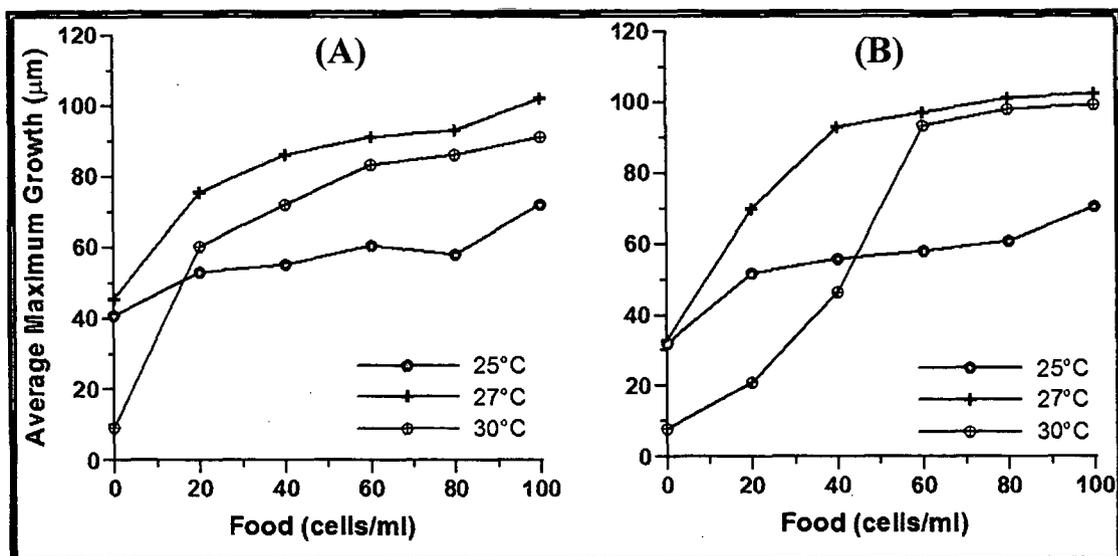


Figure 4.1: Average maximum growth at different concentration of food and seawater temperature in *Cymbaloporella plana* (Cushman).

Interestingly, all the specimens that were fed with 100cells/ml of diatoms, reproduced, irrespective of seawater temperatures (Fig. 4.2). Almost all the specimens supplied with 80cells/ml food also reproduced, except those maintained at 25°C temperature, wherein only 80% of the specimens reproduced. In case of specimens supplied with 60cells/ml, all the specimens maintained at 27°C temperature reproduced, whereas only 80% reproduction was observed in the specimens kept at 25°C and 30°C temperature (Fig. 4.2). Out of all the specimens maintained with no added food, 40% of those kept at 27°C temperature, and 20% of those subjected to 25°C temperature, reproduced (Fig. 4.2). None of the specimens, supplied with no additional food kept at 30°C temperature reproduced (Fig. 4.2).

All the specimens subjected to 30°C temperature and with no added food, died, whereas 80% specimens maintained at 25°C temperature, and 60% specimens at 27°C temperature died (Fig. 4.3).

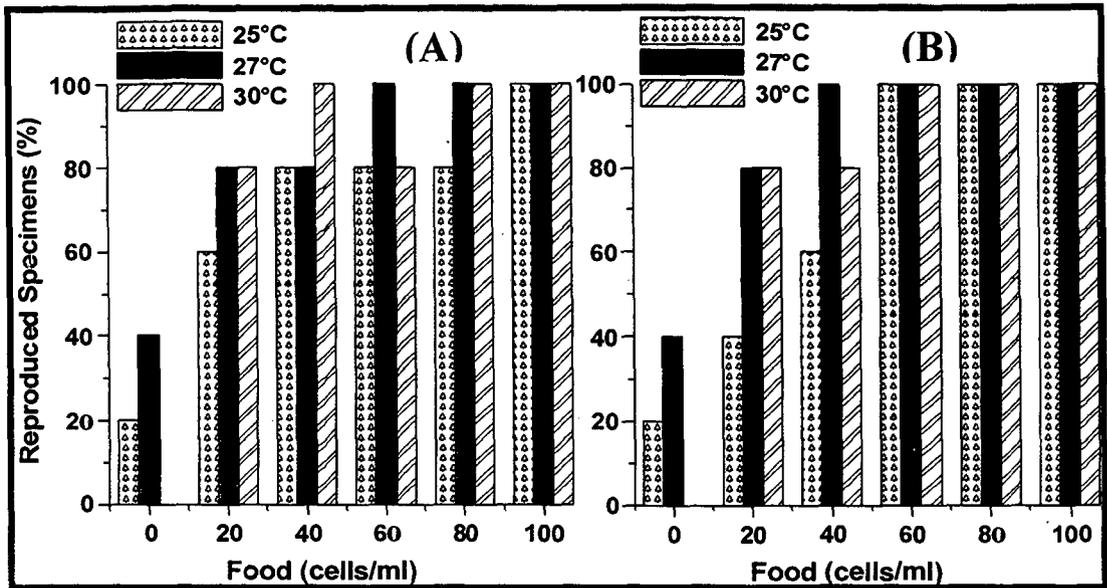


Figure 4.2: The percentage of specimens reproduced at different concentration of food at different temperatures in *Cymbaloporetta plana* (Cushman).

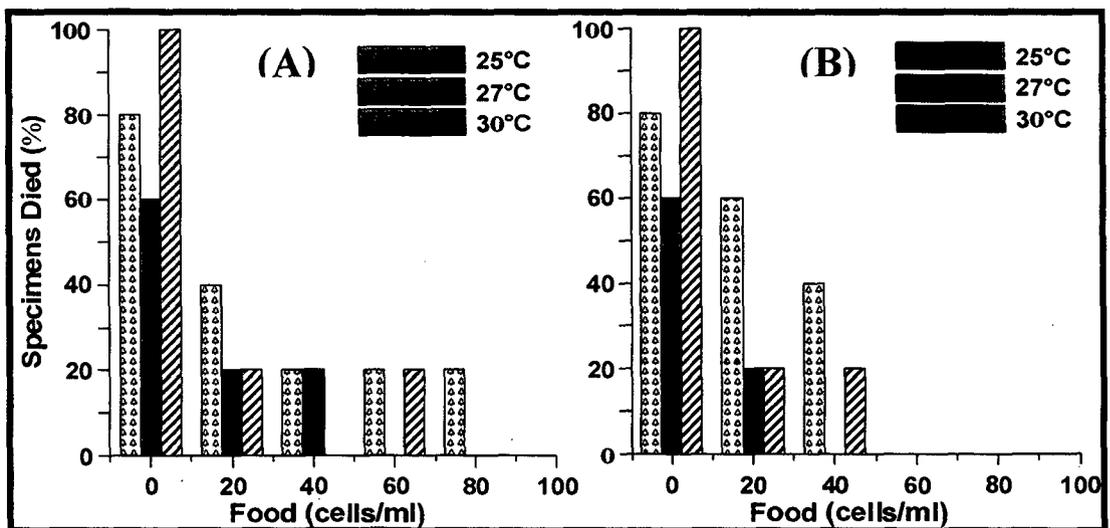
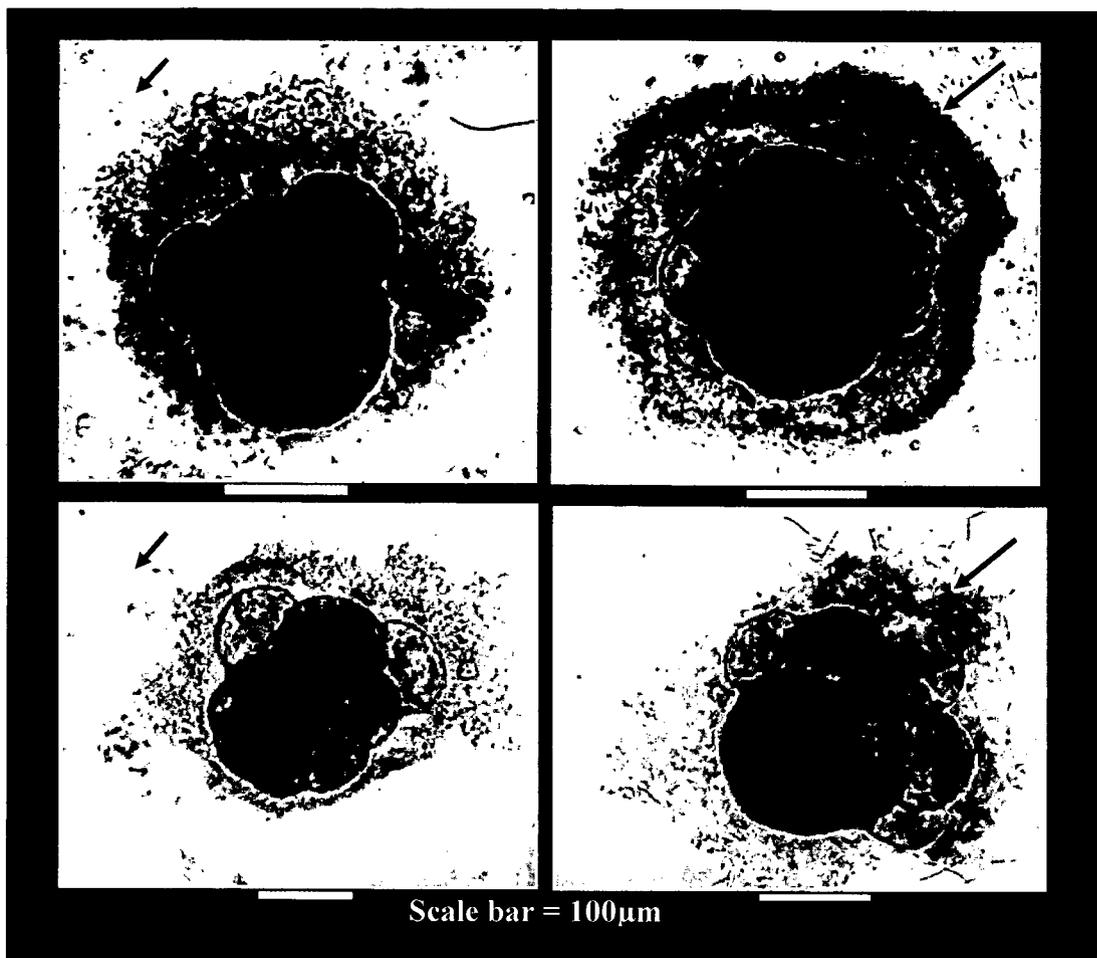


Figure 4.3: The percentage of specimens died at different concentration of food and temperatures in *Cymbaloporetta plana* (Cushman).

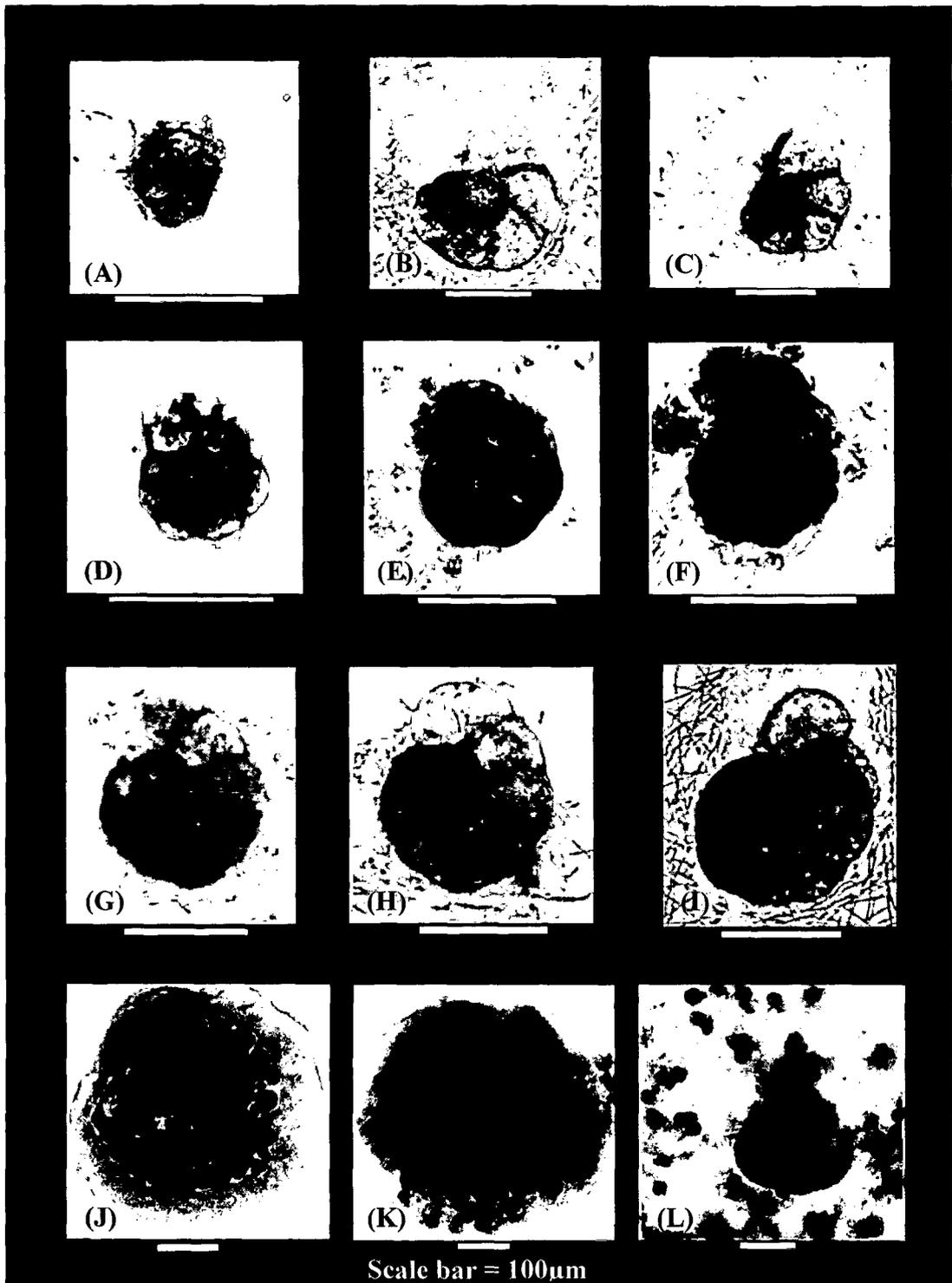
*Cymbaloporetta plana* accumulated a lot of organic matter all around its test just before addition of new chambers or when the specimen was about to reproduce (Plate 4.2). During asexual reproduction the juveniles require food to form its initial two to three chamber before being released from the parent cell or the cyst. The food material collected by the parent cell helps to feed the juveniles. Stages of growth and chamber formation in *Cymbaloporetta plana* are illustrated in Plate 4.3.



**Plate 4.2: *Cymbaloporeta plana* (Cushman) specimens formed cyst of food material with the help of pseudopodia at the time of chamber formation.**

## **4.5 Discussion**

Myers (1943b) and Bradshaw (1955) observed that growth in foraminifera is faster when plenty of food is available whereas when food is less the growth is comparatively slower. This observation is in favour of the results of this study wherein it was observed that growth was more as well as the number of specimens undergoing reproduction increased as the amount of food increased. Lee et al. (1966) also noted that in places where there are phytoplankton blooms, the foraminifera grow faster and reproduce more frequently, but whenever there is scarcity of food, growth is slower and the number of specimens undergoing reproduction also decreases. Muller (1975) also reported that the quality and quantity of the organisms used as food, affects the feeding of foraminifera.



**Plate 4.3: Stages of growth and chamber formation (A-I), Juveniles seen inside the cyst formed by the mother cell (J), Juveniles on verge to move out of the cyst (K) and Juveniles moved away from the mother cell leaving it empty (L) in *Cymbaloporetta plana* (Cushman).**

He also reported that the specimens of *Spiroloculina hyalina* flourish where the density of bacteria is high and competing species are absent. The growth may also

vary with the type of diatom species supplied as food, as noted by Muller and Lee, (1969) that *Quiqueloculina lata* grew three times faster on diets of *Nitzschia acicularis* and *Chlorococcum* sp. than on other species. Heinz et al. (2002) also reported that foraminiferal number increased when supplied with various diatoms and algal species such as *Chlorella* sp. *Dunaliella tertiolecta* and *Amphiprora* sp. Lee et al. (1966) fed foraminifera collected from shallow water environments with 50 different types of organism, and it was noticed that yeast, blue-green algae and dinoflagellates were not accepted as food. Foraminifera preferred the diatoms (*Phaeodactylum tricornutum*, *Nitzschia acicularis*, *Cylindrotheca closterium*), a chlamydomonad (*Dunaliella parva*) and a chrysomonad (*Monochrysis lutheri*). Foraminifera grow better on mixed algal and bacterial diets than on restricted diets as reported by several workers (Lee and Pierce, 1963; Lee et al., 1966, 1969, 1979; Muller and Lee, 1969; Lee and Mcenery, 1970). No such inference can however be made based on this study wherein only one type of diatom species was used as food.

The food alone was not the limiting factor. The response of *C. plana* to different amount of food also varied with the ambient temperature. Even in specimens that were fed same amount of food, both, the growth as well reproduction were more frequent in specimens kept under warmer waters. This probably reflects the tropical shallow water nature of *C. plana*. However, food seems to be significant factor in the death of the specimens, as the majority of specimens without food, died whereas no death was reported in specimens fed, with 100cells/ml of food.

## 4.6 Conclusion

Based on this experiment it is inferred that average growth in *Cymbaloporeta plana* (Cushman) increases with increased amount of food, irrespective of the seawater temperature. Out of all the specimens fed with same amount of food, higher growth was noted in specimens kept under warmer waters. Even the reproduction was favoured under warmer water. Under the experimental conditions, in general majority of the specimens subjected to 27°C temperature reproduced, which indicates that 27°C temperature is more suitable for growth and reproduction of *C. plana*. The study shows pronounced influence of food and seawater temperature on the growth, survival and reproduction in benthic foraminifera.

## Deciphering the life span of a few species

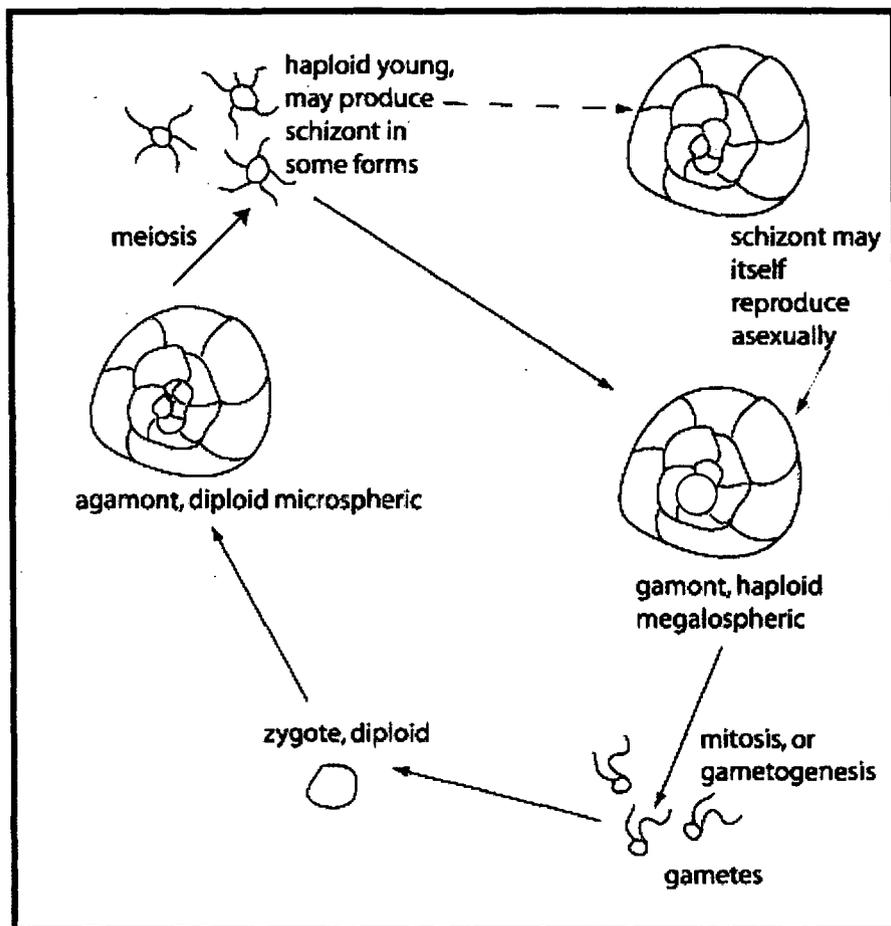
### 5.1 Introduction

Foraminifera first appeared in the Cambrian and are found in all marine environments from the intertidal regions to the deepest trenches of the ocean and from the tropics to the poles. Although unicellular, they perform all the functions performed by the multi-cellular organisms (Boltovskoy and Wright, 1976). Many studies have been conducted on live foraminifera wherein biological processes such as locomotion, growth, chamber development, calcification, reproduction, life cycles, pollution effects etc. were observed (see Lee and Anderson, 1991; Goldstein, 1999). In view of its widespread application for paleoclimatic studies, it is important to know their life-span. The species with a life-span of a few days to a few weeks will incorporate seasonal climatic signals, while the species with life spans of a several months to years will indicate annual climatic changes. It will help in selecting species to infer seasonal and annual climatic changes. The knowledge of various stages in the life cycle of foraminifera is important to measure its life span. The precise documentation of the stages in the life cycle of a species further helps in its application for paleoclimatic studies.

### 5.2 Life cycle of Foraminifera

Life cycle in foraminifera is a complex one with alternation of generations (Fig. 5.1). D'Archiac & Haime, (1853) noticed that foraminiferal specimen belonging to the same species were morphologically different from each other. This observation was confirmed by Parker and Jones (1861). This phenomenon is known as dimorphism. This concept was confirmed by Lister (1895) and Schaudin (1895), who simultaneously and independently, explained the cause of dimorphism to be alternation of generation. Lister (1895), (1903), Schaudin (1895) and Jepps (1942) observed the complete life cycle of *Elphidium crispum* and further confirmed the presence of dimorphism in foraminifera. Figure 5.1 gives an idea of foraminiferal life cycle which is characterized by alternation of sexual and asexual generations

(Goldstein, 1999). Later on, the alternate generations were defined as microspheric and megalospheric, based on its morphological features.



**Figure 5.1: Various stages in the life cycle of foraminifera (Goldstein, 1999).**

### 5.2 A. Microspheric Forms

The adult agamont, or the Schizont form, which produces numerous young ones by multiple fission is multinucleate with tiny proloculus and a large test, is termed as microspheric. Each of these nuclei gathers small amount of protoplasm from the mother cell and forms the initial chambers of the new individual. Later these individuals are released from the microspheric test. The new individuals formed are usually megalospheric. The reproduction is thus asexual (schizogony) (Boltovskoy and Wright, 1976).

## 5.2 B. Megalospheric Forms

The adult gamont, or the sporozont form which produces gametes, has a single nucleus and a test characterized by a relatively large proloculus and small diameter. Such forms are called as megalospheric forms. It has only one nucleus but, when the specimen matures this nucleus divides into many small nuclei which carry a small quantity of cytoplasm with them when they leave the test as flagellated zoospores (gametes). When two such gametes join, a zygote is formed. This process of sexual reproduction is called gamogony wherein microspheric generation is formed (Boltovskoy and Wright, 1976).

The life cycle of foraminifera, however, may or may not have alternate generations. Observations by Arnold (1955), Phleger (1960), Frankel (1975), Kitazato (1992), Gross (2000) etc. have shed light on the complicated life cycles and biology of foraminifera. Some stages of the life cycle of *Ammonia tepida* have been studied by Schnitker (1974), Goldstein and Moodley (1993), Goldstein (1997). Though there are more than 10,000 extant species of foraminifera, complete life cycle has been documented for less than 30 species (Sen Gupta, 1999). These studies show that the life span of benthic foraminifera may vary from a few weeks to more than a year (Boltovskoy & Wright, 1976). Unfortunately, of late, not much attention has been paid to understand the life-span of benthic foraminiferal species. Therefore, it was decided to document the life span of a few benthic foraminiferal species.

## 5.3 Experimental Set-up

Individuals of *Cymbaloporetta plana* (Cushman), *Discorbina concinna* (Brady) and *Spiroloculina* sp. were isolated from the sediment and sea-grass samples collected from the coastal areas off Goa. Initially when the specimens were isolated they were kept in the conditions same as that at the time of sample collection. This was done in order to avoid the sudden stress which the specimens would experience if transferred to different conditions. After a few days, in order to observe the optimum temperature and salinity for each species, the specimens were transferred to media with different combinations of salinity and temperature. The salinity as well as the temperature was reduced or increased slowly to avoid the salinity or temperature shock which the specimen would experience if directly subjected to different

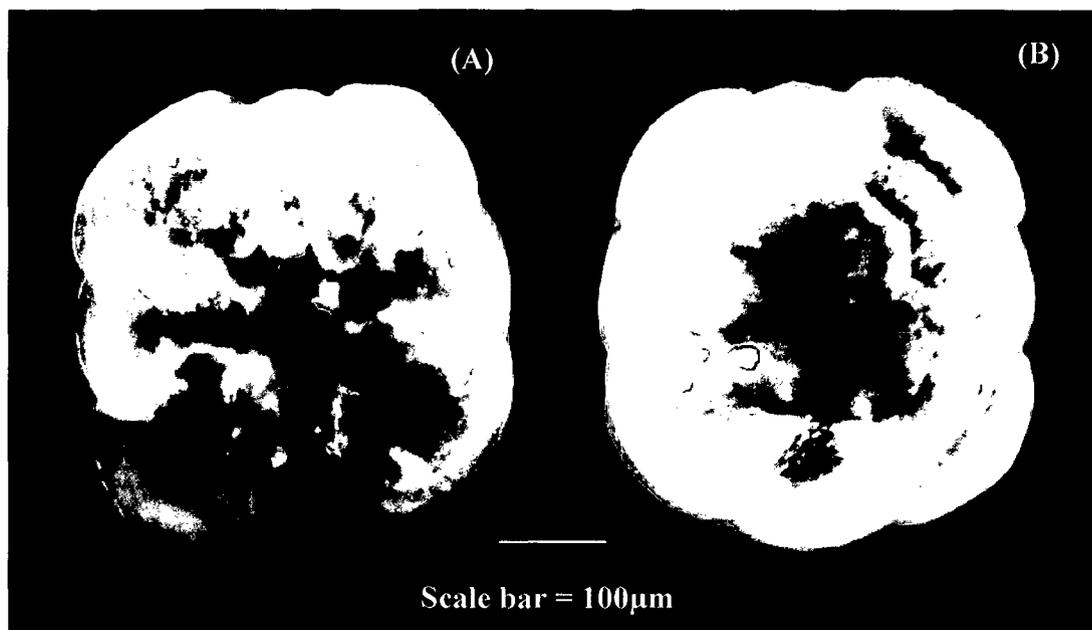
conditions. The illumination of 12 h light and 12 h dark was provided. The specimens of *C. plana* and *D. concinna* were subjected to 16 combinations of temperatures (25°C, 27°C, 30°C and 35°C) and salinities (25‰, 30‰, 35‰ and 40‰) whereas *Spiroloculina* sp. was subjected to 12 combinations of temperature (25°C, 27°C, 30°C and 35°C) and salinities (25‰, 30‰ and 35‰). 30 specimens were subjected at each combination. The above temperature and salinity range was selected as it was the same as reported from the study area in literature as well as during continuous monitoring of the physico-chemical parameters as part of this study.

The *Navicula* sp. of diatoms was used as food for these foraminiferal species and was also maintained in laboratory by sub-culturing after every seven days. Every time the media was changed, 100 µl of food (~20 cells/ml) in the form of diatoms was added to the culture media. The species were kept in six well culture dish and each well had 5 specimens. The experiment was carried out in replicate. The reproduced specimens were kept undisturbed after each time the reproduction took place. This was done to monitor the life span and different growth phases for each specimen. The media conditions, at which the reproduction took place, were maintained and finally more than 8-10 generations for each species were monitored. It was observed that not all the specimens belonging to the same species reproduce at the same time; a few of them reproduced after two days while others reproduced after four days. It was noted for most of the specimens. Further, it was also observed that during reproduction, neither all the juveniles come out of the mother specimens at one stretch, nor do all the specimens have three to four chambers. A few of the juveniles had two chambers at the time of reproduction. The life-cycle of various species is discussed individually in the next section.

#### **5.4 *Cymbaloporeta plana* (Cushman)**

It has a calcareous test which is planoconvex, trochospiral, subcircular in contour, with rounded periphery, and lobulate margins (Plate 5.1). The test wall is prominently perforate on its dorsal side which is convex. The initial chambers are off center, small somewhat inflated brownish in colour followed by larger irregular more inflated chambers. The last formed whorl comprise of four shallow, broad chambers. The ventral side is flat to concave. The central wall of four visible chambers is

perforate and is separated by rather broad radial channels at sutural area. The test size is 0.442 x 0.221 mm (McCulloch, 1977).



**Plate 5.1: Micrograph of *Cymbaloporetta plana* (Cushman) (A) Ventral view and (B) Dorsal view.**

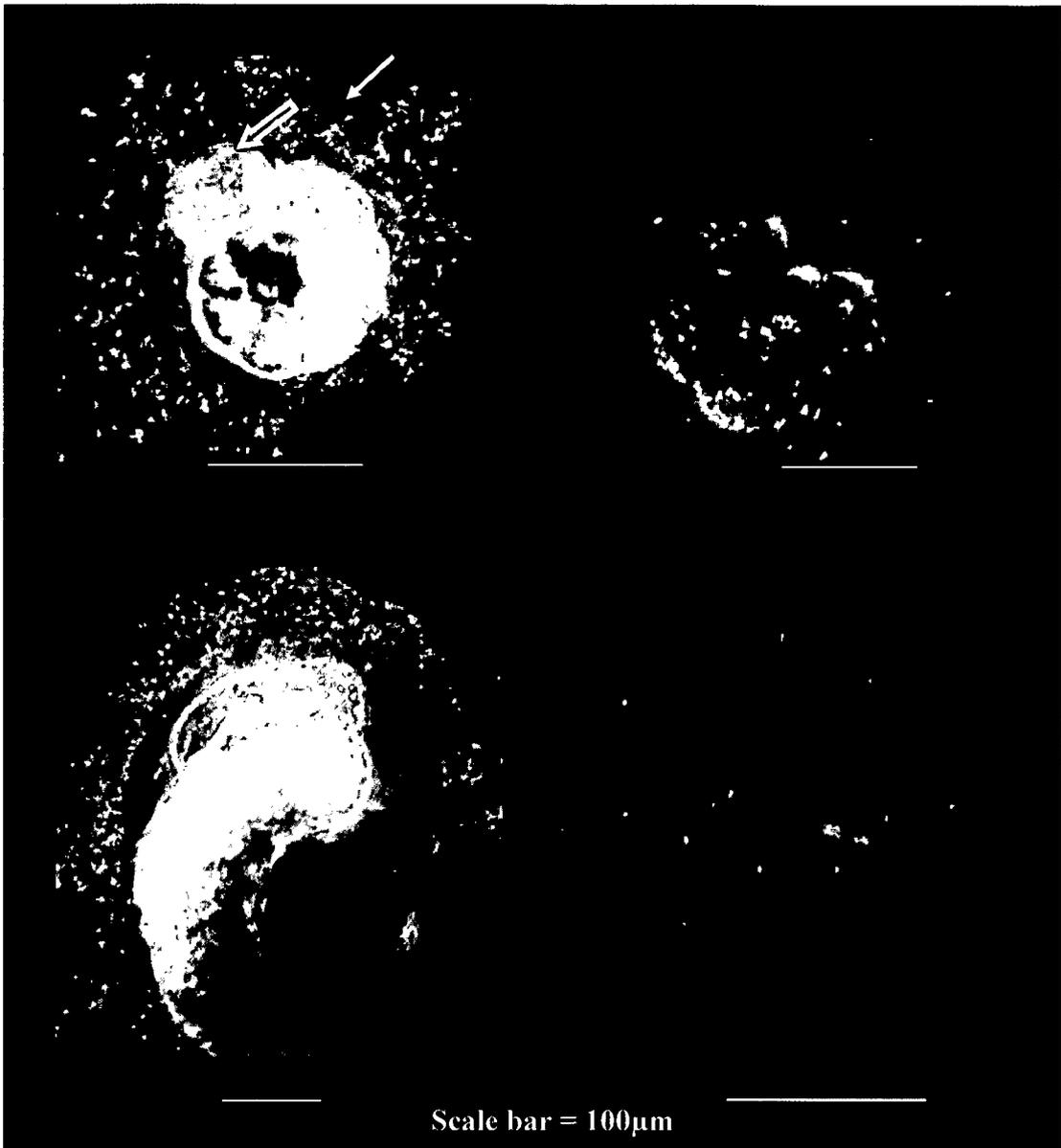
### 5.4.1 Systematic Classification

Order	FORAMINIFERIDA Eichwald, 1830
Suborder	ROTAIINA Delage and Herouard, 1896
Superfamily	PLANORBULINACEA Schwager, 1877
Family	CYMBALOPORIDAE Cushman, 1927
Subfamily	CYMBALOPORINAE Cushman, 1927
Genus	CYMBALOPORETTA Cushman, 1928
Species	<i>Cymbaloporetta plana</i> Cushman, 1924

### 5.4.2 Reproduction

*Cymbaloporetta plana* (Cushman) reproduced asexually in laboratory. Reproduction and chamber formation in this species occurs within a cyst (Plate. 5.2). The specimens build a cyst of food material and debris when it is about to reproduce or to add a new chamber. The food gathered by the mother specimen to form the cyst at the time of reproduction, is then utilised by the offspring to build its test before these

offspring's are released by breaking open the cysts. The specimens reproduced asexually at 25°C to 30°C temperature and 30‰ to 35‰ salinity in laboratory.



**Plate 5.2: Chamber formation within the cyst formed of food material by *Cymbaloporetta plana* (Cushman) (the arrow indicates the cyst and the newly formed chamber).**

The test size of the adult specimens varies but a fully formed adult consist of 20-25 chambers. The cytoplasm in the initial chambers is dark in colour compared to the later chambers, whereas the newly formed chambers are almost transparent (Plate 5.2). The time taken for reproduction varies from specimen to specimen. *Cymbaloporetta plana* took almost three to four days from the time of cyst formation to the final dispersal of the juveniles. Number of offspring's depends on the

maximum diameter of the mother specimen. The relationship is highly significant ( $R=0.88$ ) (Fig. 5.2). Since this specimen reproduced asexually, the juveniles are formed within the mother cell and are released with two, three or four chambers. Just after reproduction, once the juveniles come out of the mother test, it takes almost two days to add a new chamber depending on the availability of food. The time taken to add subsequent chambers however, increases slowly. Finally when the specimens mature, it again forms the cyst and remains dormant for one or two days. During this dormant phase the cytoplasm within the parent cell starts dividing into new specimens and then after two or more days the test of the mother specimen bursts open thus releasing the juveniles. The released juveniles are very active with an extensive pseudopodial activity. These reproduced juveniles were then again subjected to identical conditions to observe the next generation. A total of 10 such generations of this specimen were observed in laboratory. It was found that *C. plana* reproduces within 45-55 days. The different stages in the life cycle of *C. plana* including the time taken to form chambers are shown in Plate 5.3.

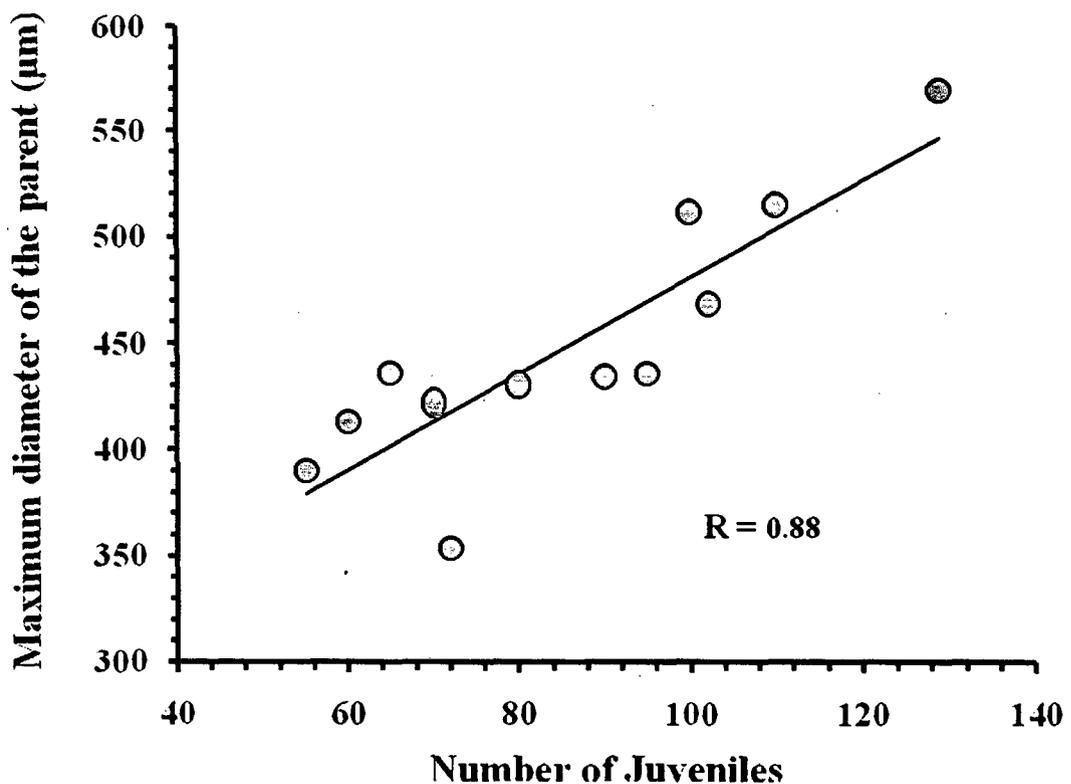


Figure 5.2: Relationship between maximum diameter of the parent cell of *Cymbaloporeta plana* (Cushman) and number of Juveniles.

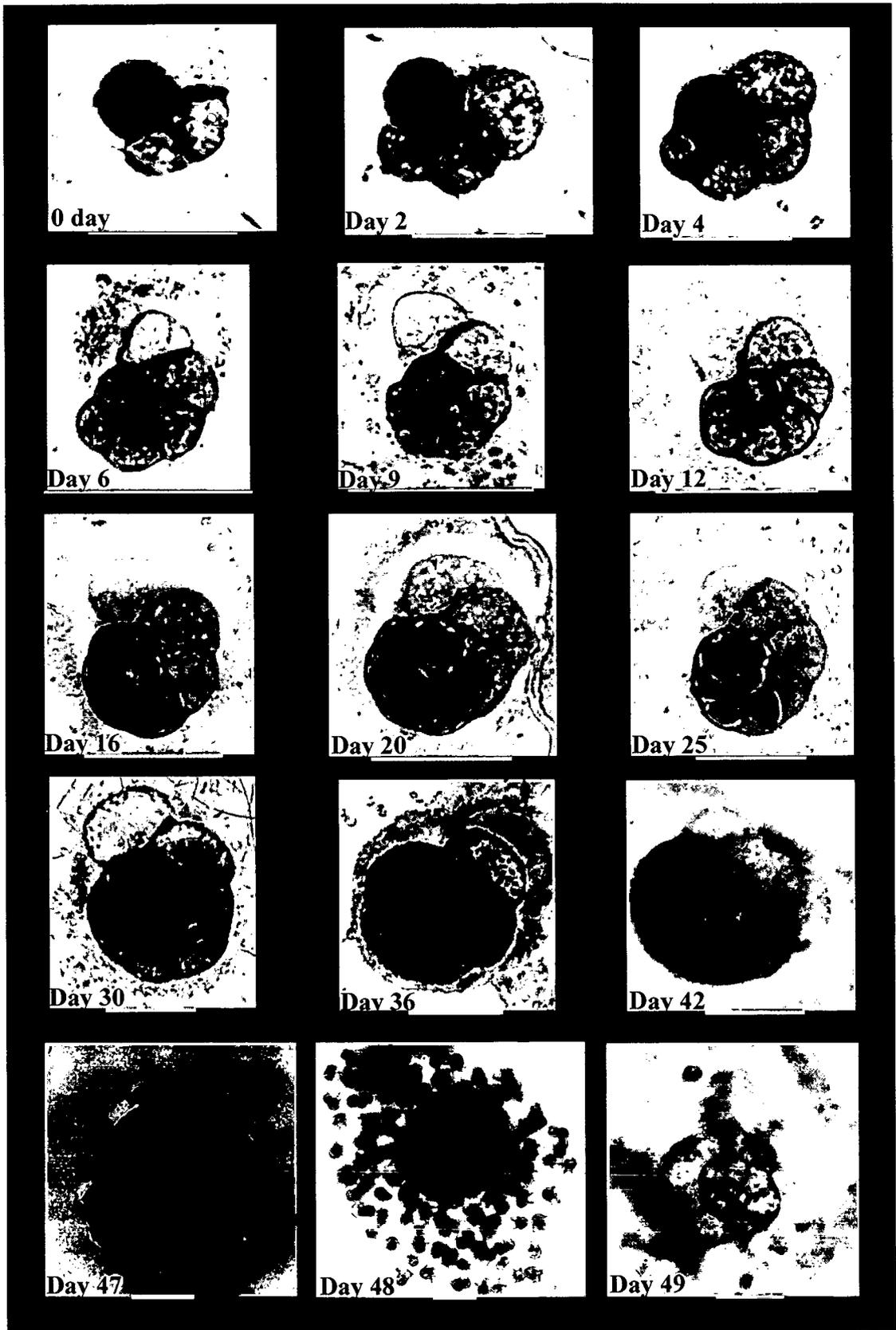


Plate 5.3: Chamber formation and reproduction in *Cymbaloporetta plana* (Cushman) Scale bar =50 $\mu$ m (0 day to day 4) and 100  $\mu$ m (day 6 to day 49).

## 5.5 *Discorbina concinna* (Brady)

The tests of *Discorbina concinna* (Brady) are circular in outline, with convex superior face (Plate 5.4). The interior of the tests is somewhat concave. The peripheral edge is angular, composed of more than two convolutions, of which the latest consists of three to four segments. On the inferior side, the final segment occupies nearly half the entire surface. The umbilical flaps are distinct but not greatly developed. The tests walls very thin and conspicuously perforate. It has sutures marked by fine lines, neither depressed nor limbate externally. The diameter of the tests is ~0.25 mm.

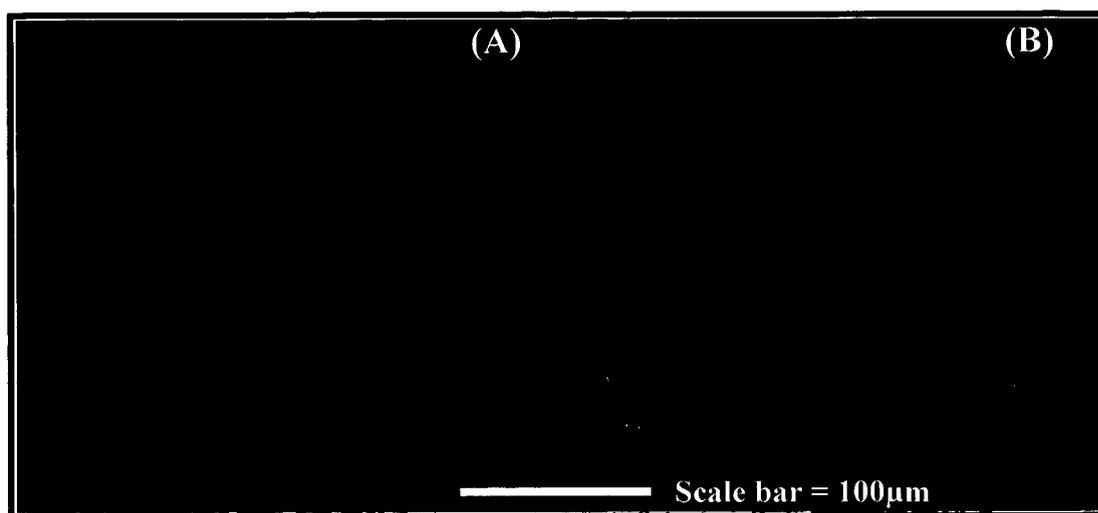


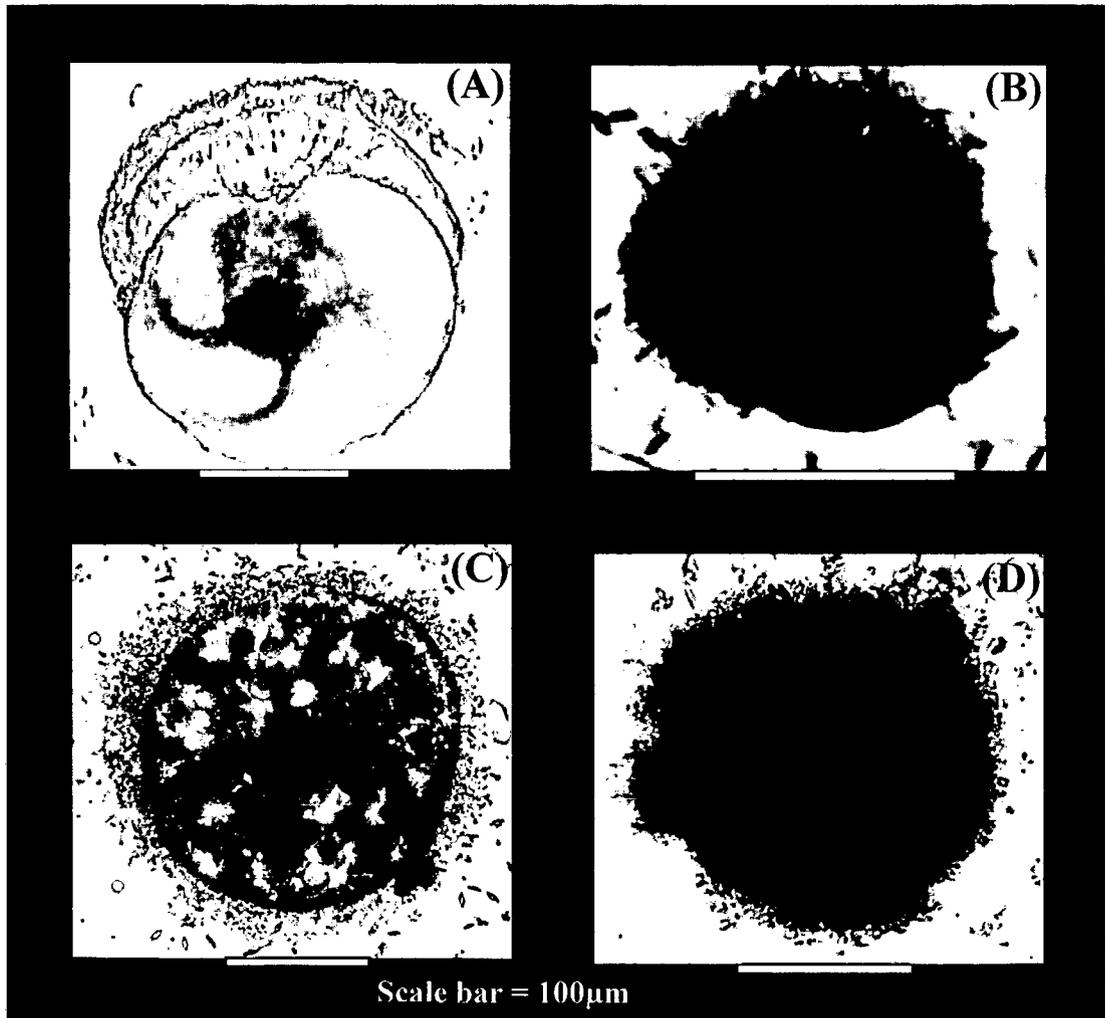
Plate 5.4: Micrographs of *Discorbina concinna* (Brady). A. Ventral view and B. Dorsal view.

### 5.5.1 Systematic Classification

<b>Order</b>	FORAMINIFERIDA Eichwald, 1830
<b>Suborder</b>	ROTAIINA Delage and Herouard, 1896
<b>Superfamily</b>	DISCORBACEA Ehrenberg, 1838
<b>Family</b>	ROSALINIDAE Reiss, 1963
<b>Subfamily</b>	PARAROTALIINAE Reiss, 1963
<b>Genus</b>	TRETOMPHALOIDES Banner, Pereira and Desai, 1985
<b>Species</b>	<i>Discorbina concinna</i> Brady, 1884

## 5.5.2 Reproduction

*Discorbina concinna* (Brady) specimens are found along the coastal areas off Goa. After keeping them at 16 different combinations of salinity and temperature, it was noted that it reproduces asexually at a temperature range of 25°C - 27°C and salinity range of 30‰ - 35‰. When the specimen is about to reproduce or to form a new chamber, it also forms a cyst of food particles and debris. When the specimen is about to add a new chamber, an extensive pseudopodial network is first formed outside the last chamber spreading to a distance corresponding with the size of the newly added chamber. When the specimen is about to reproduce it forms a cyst of food material around itself. This food material in the cyst helps the juveniles to feed on, while they are within the cyst (see Plate 5.5).



**Plate 5.5:** Cyst formed around the specimen during chamber formation (A&B) and cyst formed around the specimen during reproduction (C&D) in *Discorbina concinna* (Brady).

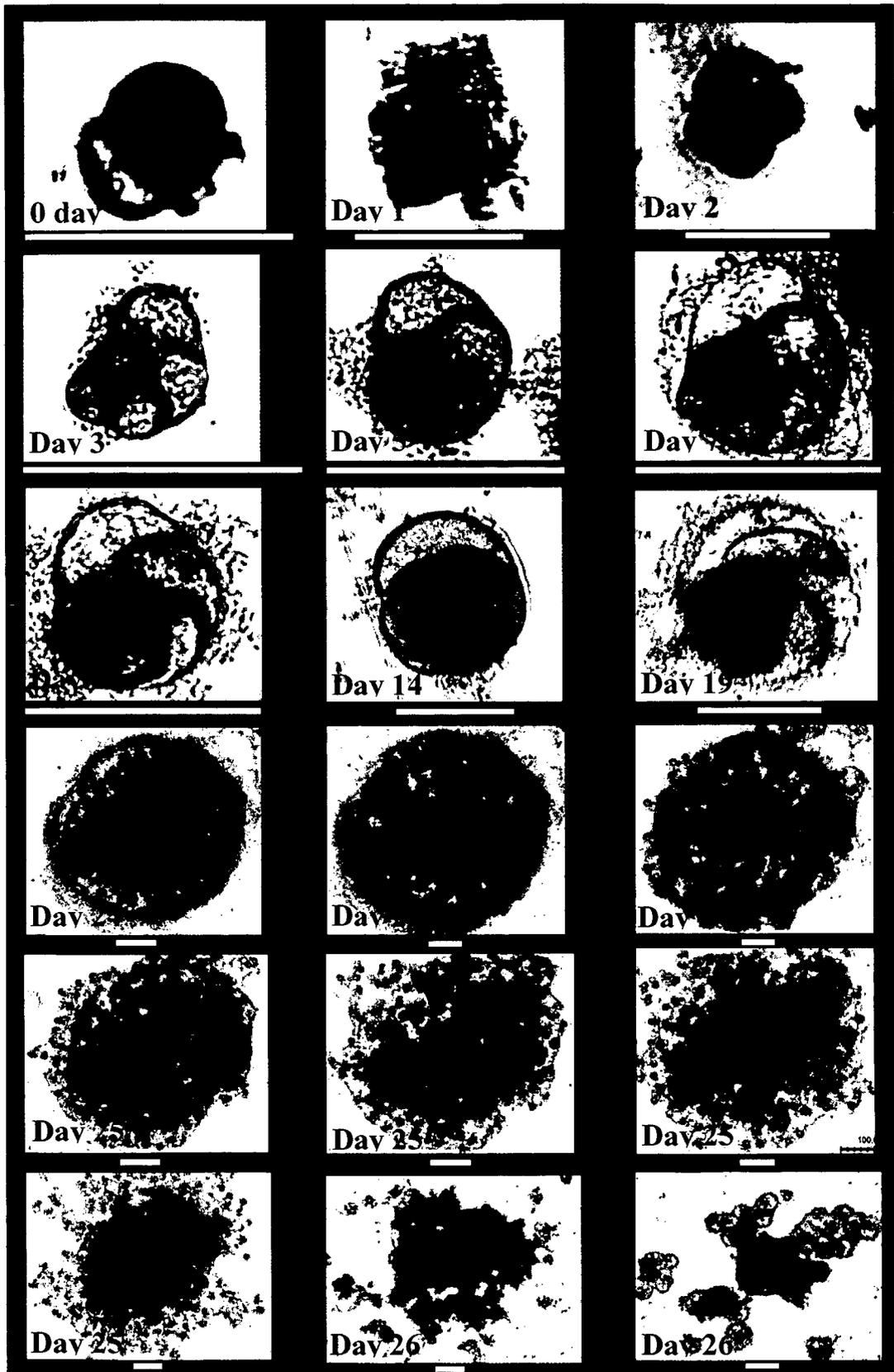
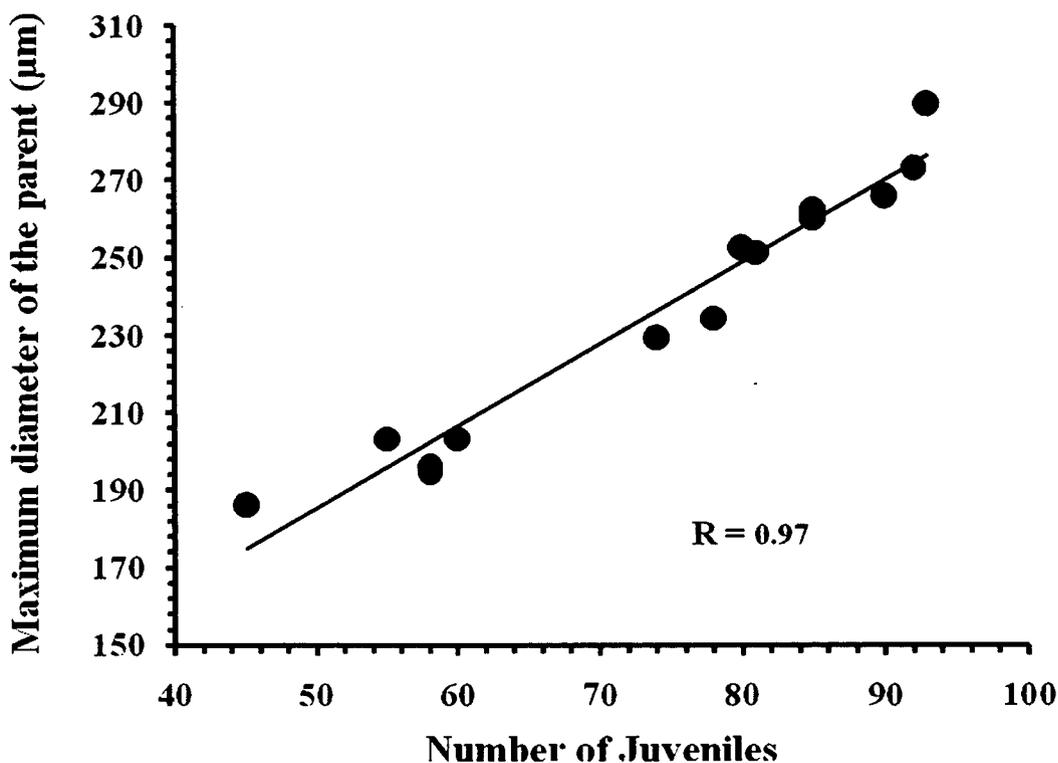


Plate 5.6: Chamber formation and reproduction in *Discorbina concinna* (Brady). Scale bar=50µm (0 day to day 2) and 100µm (day 3 to day 26).



**Figure 5.3: Relation between maximum diameter of the parent cell of *Discorbina concinna* (Brady) and number of Juveniles.**

Immediately after reproduction, the proloculus of the juveniles has a reddish ting. With the progress of addition of new chambers, the cytoplasm colour in the proloculus becomes lighter, while the newly added chambers are transparent as seen in Plate 5.6. The lifespan of this species is ~22-25 days. The life span estimates are based on a total of 10 generations as also in case of *C. plana*. The juveniles are released with three to four chambers and the total number of chambers in the adult test is around 13 to 15. Number of juveniles released during reproduction again depends on the size of the specimen, i.e. the larger the size of the parent, more the number of juveniles. The relationship is highly significant ( $R=0.97$ ) (Fig. 5.3).

Twinned specimens were also noted in *D. concinna* (Plate 5.8). However, the twinned specimens in laboratory culture were noticed only in this species. It was evident that initially the two specimens grew separate as two different individuals. But later on both the specimens formed a common chamber and thus the two different individuals joined into one test. The twinning was not seen under stressed conditions as it was noted at the same conditions, at which reproduction in this specimen took place.

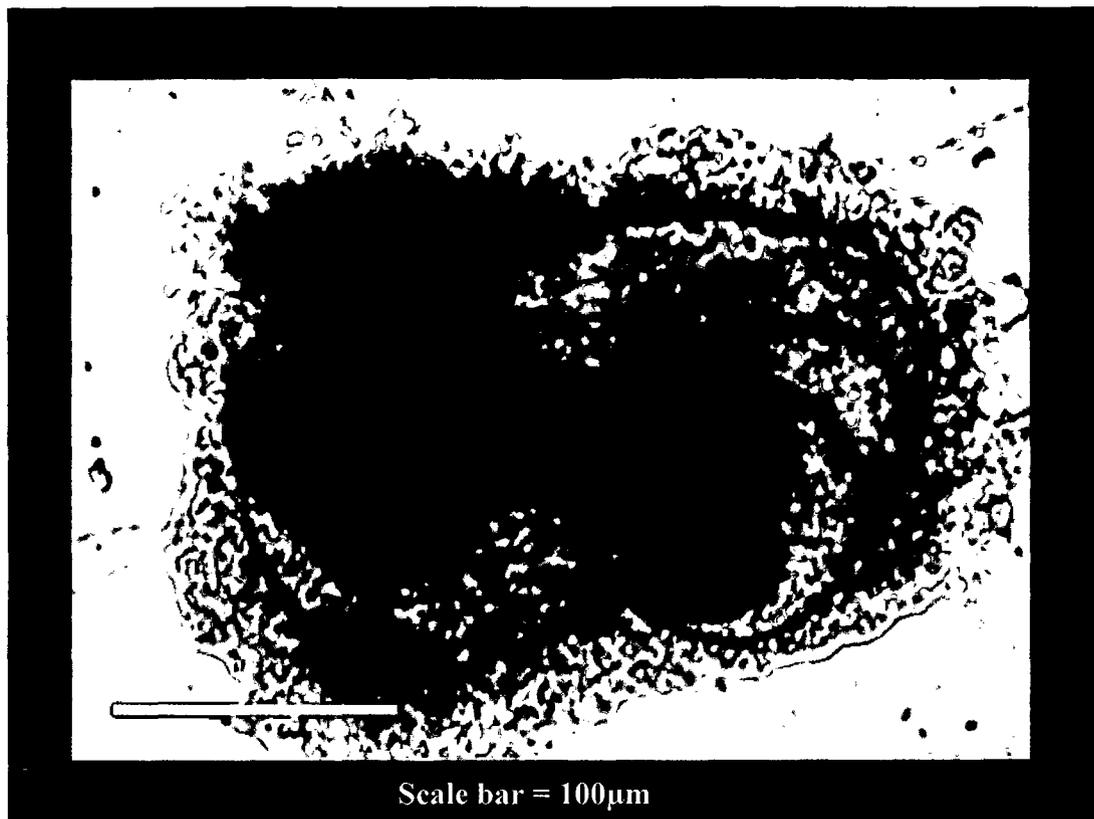


Plate 5.8: Twin specimens of *Discorbina concinna* (Brady).

## 5.6 *Spiroloculina* sp.

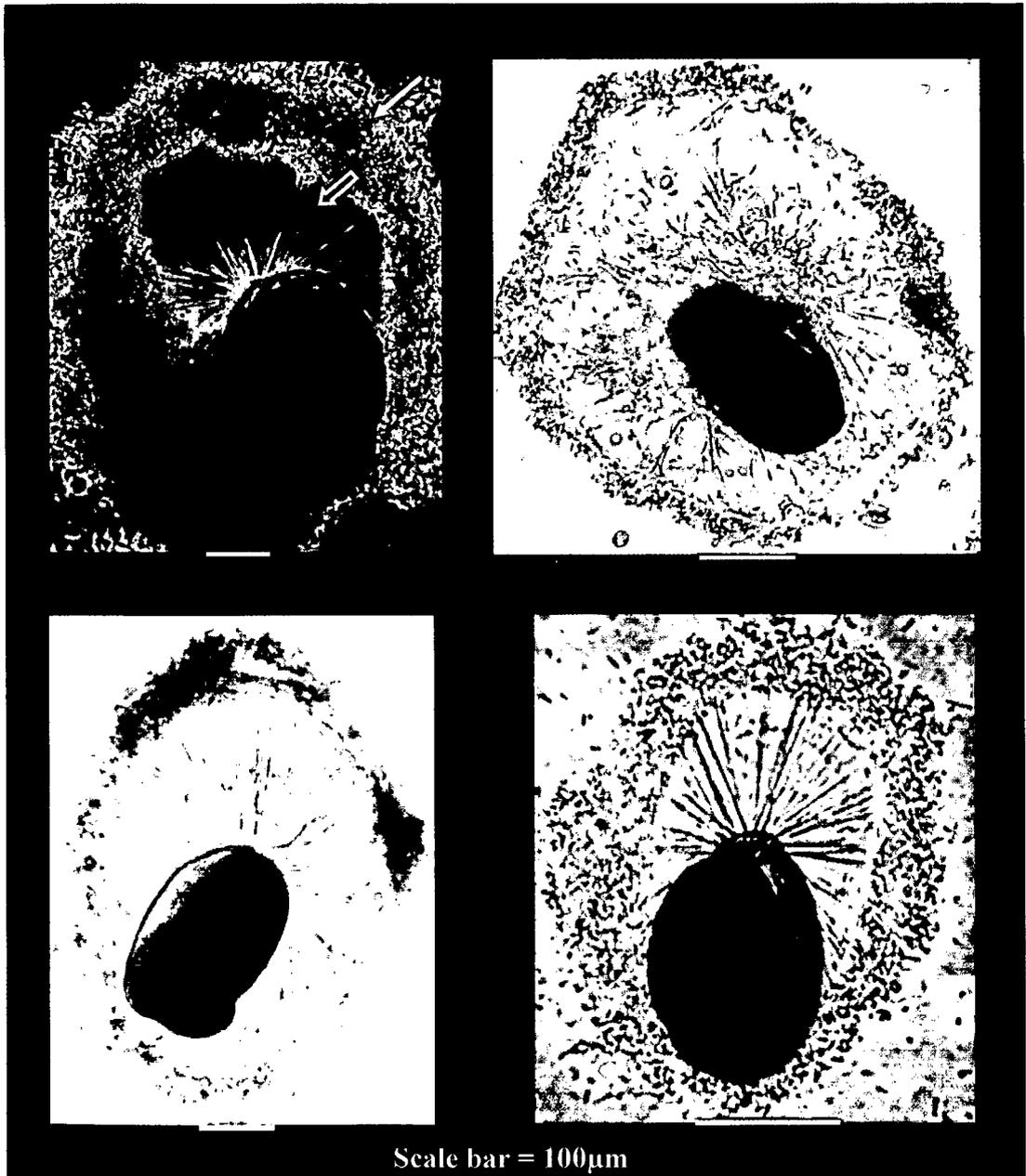
*Spiroloculina* specimens are found abundantly along the coastal areas off Goa. It is procollaneous in nature with chambers added at 180°. The test is elongated, more or less oval in shape and shiny. The periphery of the chambers is smooth and curved. It has a single aperture occupying terminal position. It was noted that *Spiroloculina* sp. tests remain in upright position spreading its pseudopodia through the terminal aperture.

### 5.6.1 Systematic Classification

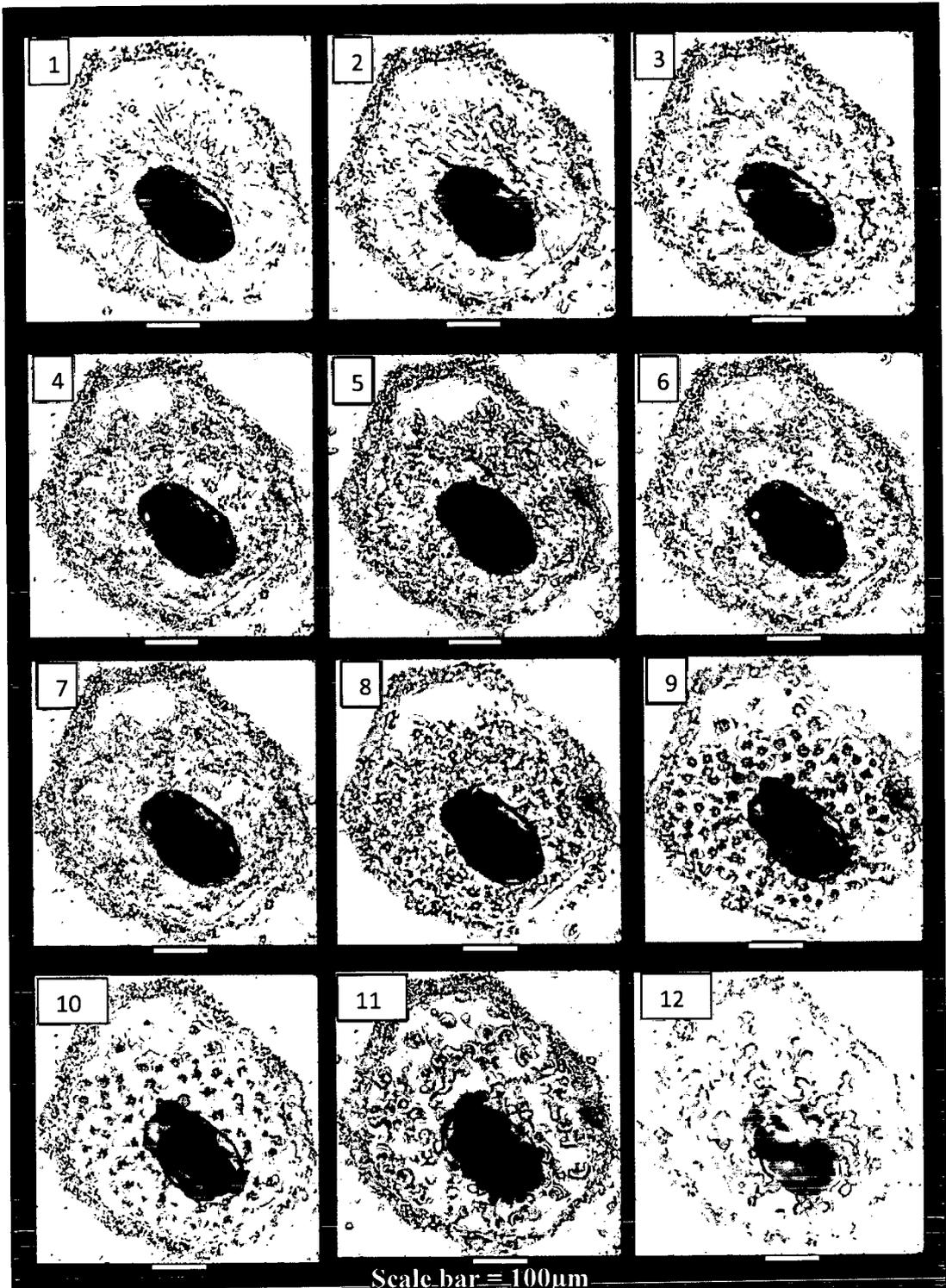
<b>Order</b>	FORAMINIFERIDA Eichwald, 1830
<b>Suborder</b>	MILIOLINA Delage and Herouard, 1896
<b>Superfamily</b>	MILIOLIACEA Ehrenberg, 1839
<b>Family</b>	SPIROLOCULINIDAE Wiesner, 1920
<b>Genus</b>	SPIROLOCULINA D'orbigny, 1826

## 5.6.2 Reproduction

The specimens of *Spiroloculina* sp. were subjected to 12 combinations of salinity and temperature. From all these temperature-salinity combinations, it was observed that these specimens reproduced at a temperature range of 25°C - 30°C and salinity range of 30‰ - 35‰. Asexual reproduction was seen in this species too. It was found that when the specimen is about to reproduce it forms a cyst with pseudopodial network slightly away from the mother test whereas in case of *C. Plana* and *D. concinna* the cyst is formed very near to the mother test (Plate 5.9).



**Plate 5.9:** Formation of pseudopodial network ( $\rightleftharpoons$ ) with a cyst of food material ( $\blackrightarrow$ ) at the time of reproduction and addition of new chamber in *Spiroloculina* sp.



**Plate 5.10: Formation of cyst before reproduction (1), cytoplasm from the parent cell moving in the cyst (2-7), formation of juveniles (8-11), fully formed juveniles in the cyst (12) in *Spiroloculina* sp.**

As part of reproduction, once the specimens complete the cyst around the test by pseudopodial network, all the cytoplasm comes out of the parent cell in the cyst area (Plate 5.10). Unlike, previous specimens, reproduction in *Spiroloculina* sp. take place outside the test (Plate 5.10). No pseudopodial activity is noted for several days once the cytoplasm comes out and starts dividing into offsprings. Then each of the nuclei, along with a small amount of cytoplasm forms the new offspring. The juveniles form the initial few chambers within the cyst formed outside the parent test.

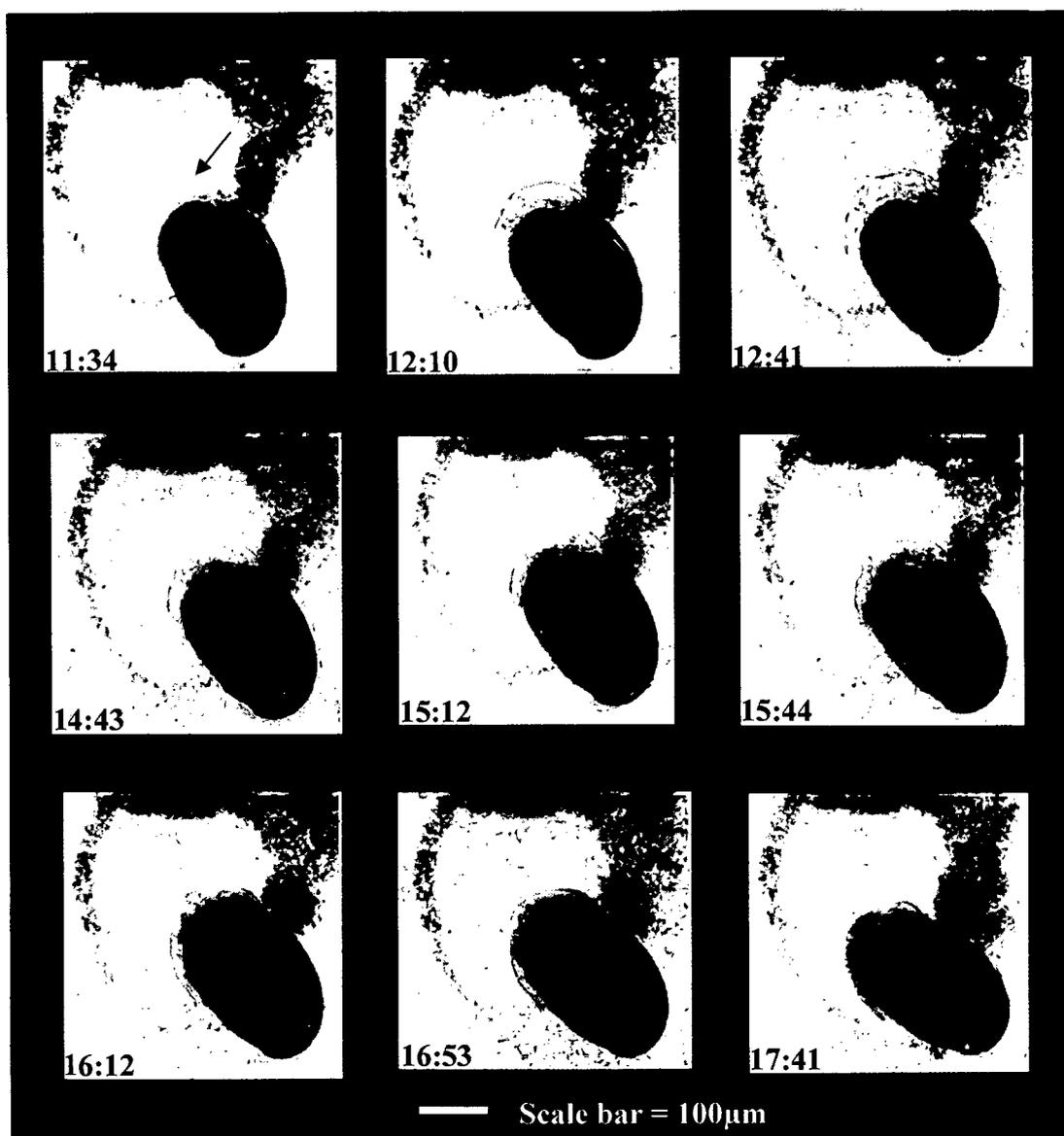


Plate 5.11: Time taken to complete a chamber in *Spiroloculina* sp.

The juveniles come out by bursting open the cyst. After observing several generations of *Spiroloculina* sp., the life span was found to be of ~25 - 30 days. The initial chambers of the specimens were brownish in colour while the last and newly formed chambers were transparent. The juveniles form 2-3 chambers while within the cyst, while additional chambers are added once they come out of the cyst. The parent test dies after reproduction as all the cytoplasm of the parent test is utilized by the offsprings. *Spiroloculina* sp. takes ~6 hours to add a new chamber. Initially when the chamber is in the process of formation, it is transparent. However, later when the chamber has been fully formed, the cytoplasm from the other chambers comes out in to the new chamber giving it a brownish colour (Plate 5.11).

## 5.7 Discussion

All the three species viz. *Cymbaloporeta plana* (Cushman), *Discorbina concinna* (Brady) and *Spiroloculina* sp. reproduced asexually in laboratory. In *C. plana* and in *D. concinna* it was noticed that the juveniles formed within the parent test, whereas in case of *Spiroloculina* sp. the juveniles formed outside the test within the cyst formed by the pseudopodial network. In case of *Heterostegina depressa*, *Amphistegina lobifera* and *A. lessonii* also it was observed that the cytoplasm flows out of the maternal test and then undergoes multiple fission (Rottger, 1974; Hallock et al. 1986). Only asexual reproduction has been recorded in most of the laboratory culture studies, the majority of which were carried out on small shallow water forms (Scott et al. 2001). Haq and Boersma (1978) noted that sexual reproduction is very likely a secondary reproductive mechanism, while asexual reproduction is the basic and the more frequent reproductive mode of the majority of foraminiferal species. Le Calvez (1939) found that megalospheric agamonts of *Planorbulina mediterranensis* can undergo repetitive asexual reproduction before producing sexually reproducing gamonts. Grell (1954, 1957a, b, 1958a, 1979), while studying the tiny *Rotaliella heterocaryotica*, *R. roscoffensis*, *Metarotaliella* sp. and *Rubratella intermedia* found no difference in the size of the proloculus of different generations. Microspheric and the megalospheric test differences have been reported only in a few species (Lister, 1895; Schaudin, 1895; Myers, 1935b, 1942; Grell, 1957a, b, 1958a, b; Boltovskoy and Wright, 1976).

The life spans of studied specimens were of the order of several weeks and varied by a few days between different generations. The reason for the slight difference in the life-span of different generations of same species is not known at present. Earlier Myers (1943a) concluded that the life span of *Elphidium crispum* is usually 1 year in temperate regions but may take longer time in deeper waters. He opined that the growth is faster in tropical waters and the life cycle is completed in 1 year. This possibility is ruled out since all the generations of same species were maintained under similar conditions. He also reported that the whole life-cycle that is both sexual and asexual reproduction is completed in 2 years in tide pools. Unfortunately, sexual reproduction was not noted in any of the specimens in this study.

Not much is known about foraminiferal reproduction in relation to environmental dynamics (Bradshaw, 1961; Buzas, 1965). Bradshaw, (1961) reported that foraminifera will reproduce only in favourable conditions. From this study also it is evident that foraminifera prefer certain set of favourable conditions for reproduction. In this study the specimens were subjected to different combinations of salinity and temperature, but reproduction took place at a narrow range of temperature and salinity.

Though, trimorphic forms have been reported in several benthic foraminifera particularly the larger, algal symbiont-bearing foraminifera (Rottger et al. 1986, 1990b; Harney et al. 1998; Dettmering et al. 1998), no such distinction could be made in the juveniles of all three studied species.

Additionally, though the reproduction in *Hastigerina pelagica* and *Globigerinoides sacculifer* is linked to lunar cycle (Spindler et al. 1979; Hemleben et al. 1987). No such lunar link was noted for the benthic foraminiferal species of the present study.

## 5.8 Conclusion

Based on this work, life spans of three species belonging to three different families viz. Spiroloculinidae, Rosalinidae and Cymbaloporidae were noted. All the three specimens reproduced asexually in laboratory. In case of *Cymbaloporetta plana* (Cushman) and *Discorbina concinna* (Brady) the juveniles were formed within the parent cell whereas in case of *Spiroloculina* sp. juveniles were formed in the cyst

built by the pseudopodial network. The life span of *Cymbaloporetta plana* is of 45 to 55 days, *Discorbina concinna* is of 22 to 25 days and *Spiroloculina* sp. is of 25 to 30 days. A slight difference of a few days was noted in the life span of different generations of the same species. In case of *C. plana* and *D. concinna* number of juveniles show significant relationship between number of juveniles and size of parent test. The study shows that these species will incorporate seasonal climatic signatures.

## Foraminiferal response to salinity changes

### 6.1 Introduction

Application of foraminifers for paleoclimatic reconstruction as well as environmental assessment work relies on the state of preservation of foraminifera after death. Factors that are responsible for the complete preservation of the foraminiferal population include, composition of test and physico-chemical parameters (rate of sedimentation, water depth, sea water upwelling, pore-water oxygen etc.) at the place of occurrence of foraminifers. In coastal areas, mixing of fresh water significantly changes the characteristics of marine water and thus affects both the living population as well as dead foraminiferal fauna (Murray, 1991; Nigam et al. 1992; Nigam and Khare, 1994, 1999; Murray and Alve, 1999a, b). Among the numerous changes brought by the fresh water influx, change in salinity is the most prominent (Liu et al. 2001). Out of several biological and physico-chemical factors affecting the benthic foraminiferal distribution in the marginal marine areas, fresh water runoff related salinity changes are specially effective in shallow water areas (Boltovskoy and Wright, 1976; Annin, 2001; Scott et al. 2001; Samir et al. 2003; Mendez et al. 2004; Hromic et al. 2006; Horton & Murray, 2007; Eichler et al. 2008; Frezza & Carboni, 2009). Changes in abundance, species assemblage, size and even the number of dissolved and distorted benthic foraminiferal tests have been assigned to various ecological parameters including salinity changes (Boltovskoy et al. 1991). However, specific effect of salinity changes on benthic foraminifera is not well understood, as it is difficult to delineate the effect of a particular parameter, from the field studies. If such specific effects of salinity changes are known, it can help decipher changes in the monsoon intensity during the geologic past. Thus by looking at the foraminiferal assemblage found in an area and its down core variation, one can infer salinity variations in the past (Nigam et al. 1992; Nigam and Khare, 1994). This information can be used to infer the climatic changes that took place in that region. But for such work it is essential to have knowledge of specific response of the species or the assemblage of species to salinity variations.

The effects of salinity on growth and overall size have been investigated by Moore (1957). He noted that lower than normal salinity is the main cause for the reduction in overall size of foraminifera. *Elphidium crispum* cultured under lower than normal salinity conditions developed relatively smaller chambers (Murray, 1963). Similar results were also obtained by Kurc (1961), Le Calvez and Le Calvez (1951), Myers and Cole (1957), Fursenko (1959), and Tufesku (1973). Though all of these studies cited, lower than normal salinity as the cause for the reduced growth rate, Wright (1968), observed similar results in case of *Miliammina fusca*, but under conditions of increased salinity. Abnormalities in tests have also been observed in the foraminiferal populations subjected to below normal salinity (Hofker, 1971; Brasier, 1975; Wang et al. 1985; Bik, 1964). Poag (1978) found comparatively larger individuals in the environments having salinities towards the lower tolerance limit. Boltovskoy et al. (1991) summarized that the effects of abnormal salinities include variation in size, reduced ornamentation and increased abnormalities of tests.

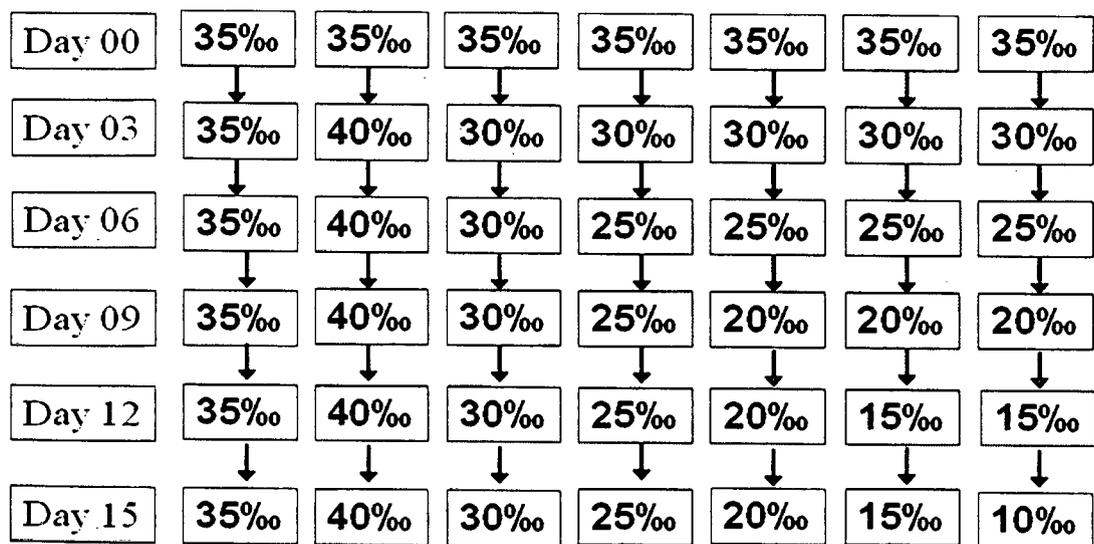
Even though a vast amount of literature, based on field observations, is available on the effect of salinity on distribution of foraminifera and specific types of foraminiferal assemblages have been reported from different types of marine environments (Sen Gupta, 1999), there is still need to validate such field-based observations by culture studies. Little work has been done in this direction (Bradshaw, 1961; Murray, 1963). Here an attempt has been made to study the effect of salinity changes on benthic foraminiferal species *Rosalina leei* (Hedley and Wakefield).

## 6.2 Materials and Method

Material for picking live specimens of *Rosalina leei* (Hedley and Wakefield) was collected from waters off Goa as per the procedure given in previous chapter. In order to understand the response of any benthic foraminiferal species to physico-chemical parameters under controlled laboratory conditions, it is advisable to begin with juvenile specimens. Since by this time juveniles produced in laboratory after reproduction, were not available, specimens isolated from the material collected from the field were used for the experiment. Care was taken to select the smallest specimens available, so as to be as close to juvenile specimens as possible. As salinity at the sampling station varied from ~11‰ to ~37‰ (Fig. 3.8, also Rodrigues,

1984) this species was subjected to seawater of salinities ranging from 10‰ to 40‰, with an interval of 5‰. Total seven sets for *R. leei* were prepared. 84 specimens were subjected to this experiment. The seawater of different salinities was prepared by adding distilled water to prepare water of less than normal salinity and by evaporating seawater for those of higher salinities. Water was brought weekly from the sampling station to prepare different salinities. Initially all the specimens were kept in normal saline water (salinity at the time of collection of material, 35‰ for *R. leei*), so as to avoid the salinity shock, which may be, if the specimens are directly transferred to different salinities. Experiment was conducted at room temperature. Media was changed every alternate day. Food was provided in the form of diatoms.

For the experiment, three days after the start of experiment, media of one set, was changed with marine water of 40‰ salinity. Similarly media of five other sets was changed with marine water of 30‰ salinity. Whereas, one set was kept at normal salinity, i.e. 35‰, and was maintained as such for the rest of the experiment. Again after three days the media of four trays, out of those five, which were kept at 30‰ salinity, was replaced with marine water of 25‰ salinity. Likewise after next three days the media of three trays, out of the four, having marine water of 25‰ salinity was changed with that of 20‰ salinity. The same procedure was followed until all seven sets were having different salinities ranging from 10‰ to 40‰, fifteen days after the commencement of experiment (Fig. 6.1).



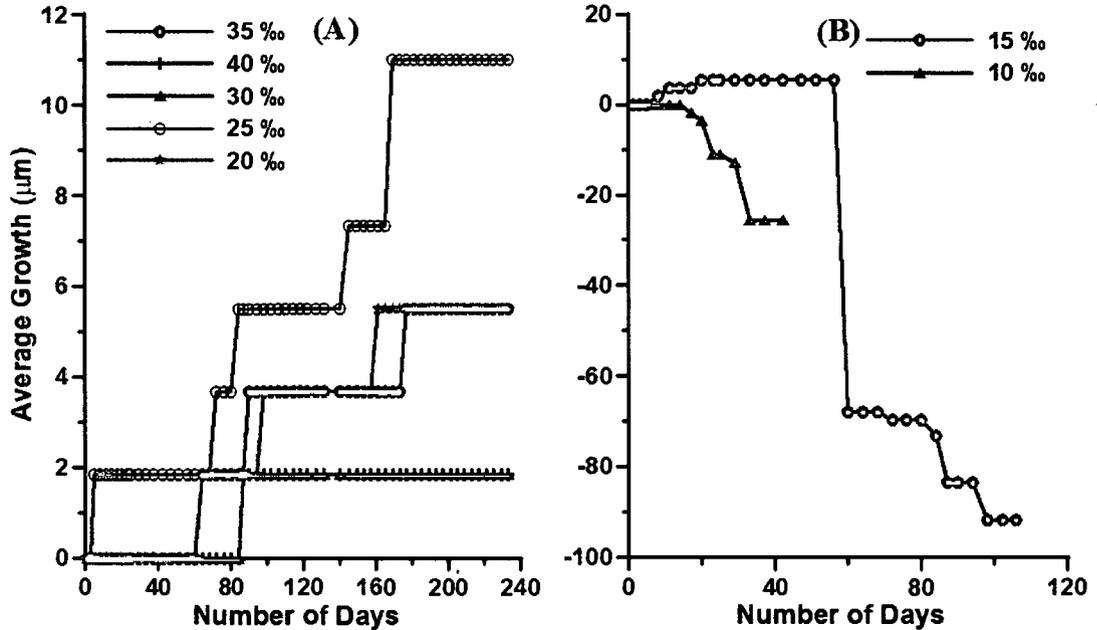
**Figure 6.1: Experimental setup for *Rosalina leei* (Hedley and Wakefield).**

This condition was maintained for the full duration (~240 days) of the experiment. However, in order to find out the maximum salinity tolerance limit, the

salinity of the seawater in the dishes with specimens at 40‰ salinity was gradually increased. Media of all the experimental sets was changed every alternate day with that of same salinity as on fifteenth day from the beginning of experiment.

### 6.3 Results

The specimens kept at 25‰ salinity showed optimum growth, while rest of the specimens kept at higher or lower salinities showed comparatively less growth (Fig. 6.2A). The specimens kept at 15‰ salinity died after 110 days of start of experiment (Fig. 6.2B). But in case of specimens kept at 10‰ salinity the survival time was comparatively very less, only ~ 45 days. No remarkable correlation was observed between size of the specimens and the survival time. After about 15 days, during the routine observation of specimens, one peculiar phenomenon in the form of increased pore spaces in the last chamber leading to the chambers becoming almost transparent was noticed in the specimens kept at 15‰ as well as 10‰ salinity. The chambers that became transparent started dissolving (Plate 6.1).



**Figure 6.2:** Average growth of *Rosalina leei* (Hedley and Wakefield) at different salinities (A). Because of dissolution of tests, 10‰ and 15‰ salinities are given separately (B). The negative values in Fig. (B) indicates reduction in size of the test with time due to dissolution. Experiment conducted on live specimens collected from field.

The dissolution process initially progressed with dissolution of one after the other chamber, starting from the last one, becoming completely transparent and ultimately dissolving fully. But later, it was noticed that after the dissolution of last few chambers the whole specimen started dissolving at the same time. The dissolution was very fast in case of the specimens kept 10‰ salinity, than those kept at 15‰ salinity.

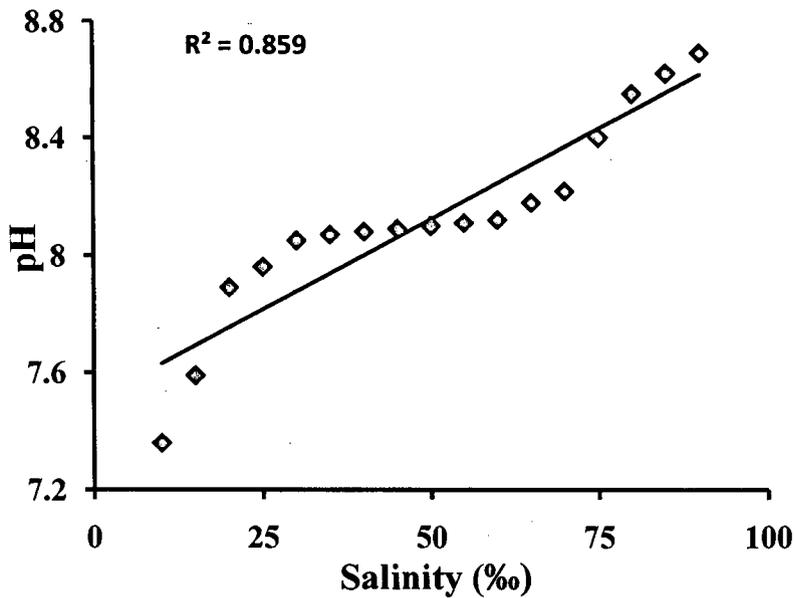
## 6.4 Discussion

As foraminifera are sensitive to salinity, change in salinity is supposed to affect the foraminiferal assemblages by affecting the growth rate of foraminifera and disturbing the preservation conditions. This study helped to understand response of a benthic foraminiferal species *Rosalina leei* to different salinities. However, it should be kept in mind that the specimens collected from the field were used for this experiment. These specimens have already spent part of their life-span in physico-chemical conditions different than those to which they were subjected in laboratory.

The presence of live specimens at 25‰, even after 240 days indicates that *Rosalina leei* can tolerate salinities as low as 25‰ for a considerable duration. Though these specimens were alive they grew only during the initial phase of the experiment. At the same time, death and subsequent dissolution of tests of the specimens kept at 15‰ and 10‰ salinity, points towards the lower limit of salinity that they can withstand. The increase in salinity to as much as 80‰ did not result in any adverse effect on the tests. Previously, deformities in the tests have been noted in response to the change in salinity, but no such change was noted in the present study. The salinity other than optimum, however lead to cessation of growth and thus stunted specimens. The survival capability was severely affected by salinity of the ambient water, as the specimens kept at 10‰ salinity died and started dissolving first as compared to those at 15‰ salinity.

Relatively lower growth in the specimens of *R. leei* kept under hypersaline conditions in laboratory culture, in the present experiment agrees with the results of Stouff et al. (1999a) who found that *Ammonia tepida* specimens showed very slow growth when subjected to higher than normal salinity conditions. But the lower growth under hyposaline conditions in the present study is probably because of unavailability of CaCO<sub>3</sub>, whose solubility is proportional to salinity of the seawater

(Boltovskoy and Wright, 1976). The adverse effect of decrease in salinity on the size of benthic foraminifers has been reported in both field-based observations (see Boltovskoy et al. 1991, for review) as well as in laboratory culture experiments (Tappan, 1951; Bradshaw, 1961).

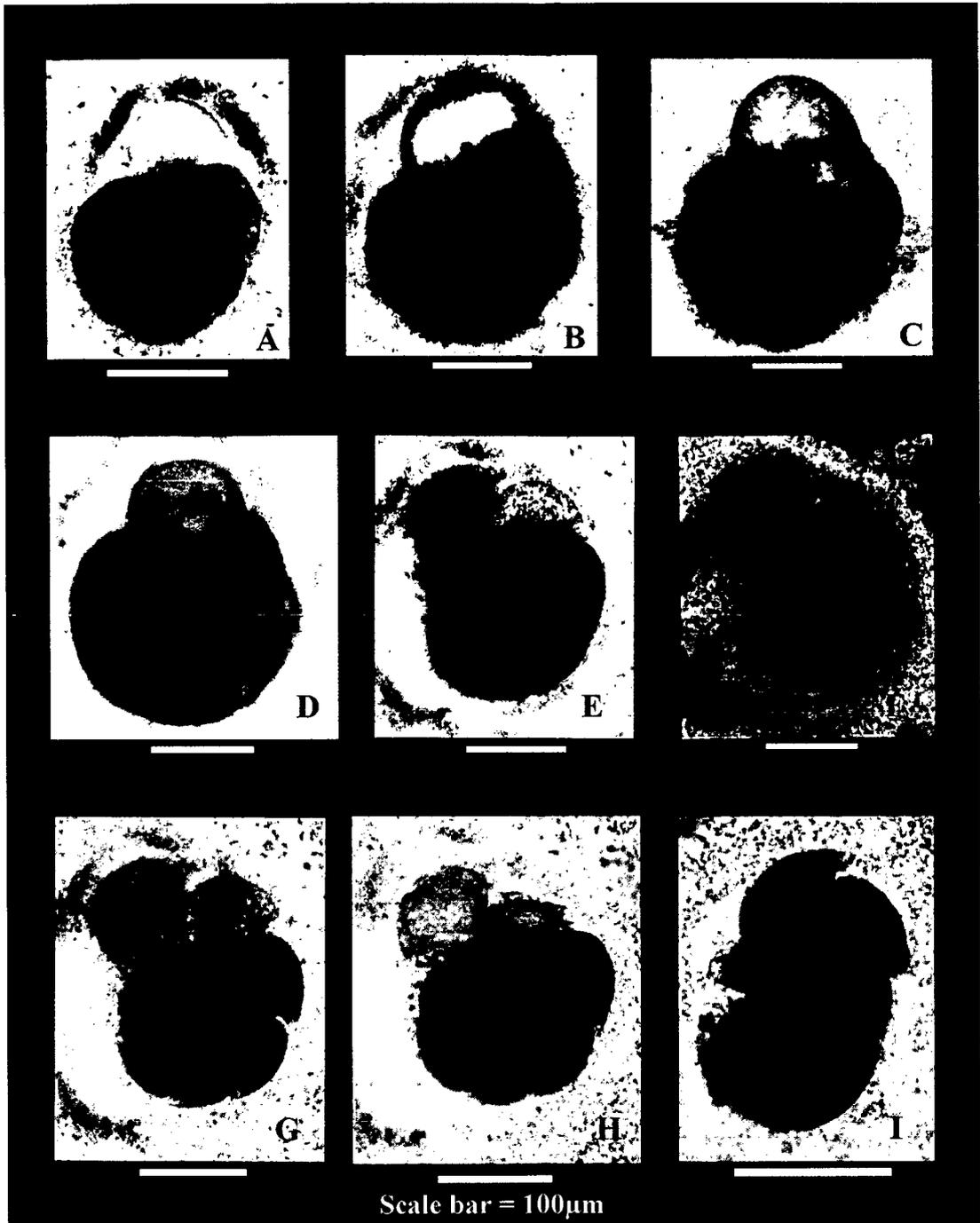


Salinity (‰)	pH
10	7.36
15	7.59
20	7.89
25	7.96
30	8.05
35	8.07
40	8.08
45	8.09
50	8.10
55	8.11
60	8.12
65	8.18
70	8.22
75	8.40
80	8.55

**Figure 6.3: Relationship between salinity and pH. The pH increases with the increase of salinity.**

The dissolution and subsequent death of *Rosalina leei* specimens below 15‰ salinity, supports the views of Boltovskoy et al. (1991), that “for survival, growth and reproduction, each benthic foraminiferal species has specific limits of tolerance to salinity”. The dissolution of foraminifers especially in shallow marine areas has been reported from several places (e.g. Murray and Alve, 1999a). Murray (1991) also noted that the dissolution is more in the water having low CaCO<sub>3</sub>, a condition prevalent in low saline water, thus confirming the results of present studies. The dissolution progresses through a series of steps, beginning with the last chamber turning transparent and gradually dissolving. Subsequent chambers follow the same pattern.

The increased dissolution of the test of the specimens kept at lower salinity probably occurred due to the decreased pH of the ambient water as the comparatively less saline water has less alkaline or more towards neutral pH (Fig. 6.3, Table: 6.1).



**Plate 6.1: Progressive stages (A-I) in the dissolution of *Rosalina leei* (Hedley and Wakefield) specimens subjected to 10% salinity. Dissolution progresses from the last chamber to the initial chambers.**

The foraminiferal test is composed of calcium carbonate, which starts dissolving once it is subjected to low pH, and as soon as the test starts dissolving it results in exposure of protoplasmic part directly to the saline water. This direct contact of the protoplasmic part with the saline water will result in removal of

essential cellular fluid out of the cell and inflow of various elements in the cell through osmosis in order to achieve equilibrium concentration between protoplasm and surrounding seawater. This loss of essential protoplasmic fluid and inflow of various elements inside the cell results in death of specimens. As the less saline water will have less alkaline pH, so the rate of dissolution of test will be more in case of specimens kept in less saline water. Thus the protoplasmic part of those specimens that are kept at low salinity will come in contact with the saline water earlier than the ones kept at comparatively higher salinity. This seems to be the possible reason for the lower survival time of the specimens kept at 10‰ salinity than those kept at 15‰ salinity.

The beginning of dissolution of test from the last formed chamber can be explained by the observation that every time a new chamber is formed, an additional calcite layer is added to the earlier formed chambers (Angell, 1967). This means that the wall of chamber keeps on thickening as one progresses from last formed chamber, towards proloculus that is the first formed chamber. Therefore the chambers formed later dissolve earlier in comparison to the earlier formed chambers.

## **6.5 Conclusion**

Here we have investigated the effects of change in salinity on the morphology, growth rate and survival time of inner shelf benthic foraminiferal species *Rosalina leei* (Hedley and Wakefield) collected from field. On the basis of this experiment it is concluded that *R. leei* can tolerate wide variations in salinity (from 25‰ to 80‰). The optimum growth was reported at 25‰ salinity. At extremely lower salinities the specimens of *R. leei* start dissolving. The dissolution starts from the last formed chamber and progresses towards earlier chambers of the tests.

## Effect of temperature and salinity changes

### 7.1 Introduction

The widespread application of foraminifera for paleoclimatic reconstruction arises from the reports based on study of foraminiferal characteristics in modern sediments, showing their high sensitivity to changes in seawater physico-chemical parameters. Physico-chemical parameters play an important role in shaping foraminiferal assemblages in marginal marine environments (Murray, 1991; Sen Gupta, 1999). The seawater temperature and salinity are the most important ecological factors, which govern the growth, reproduction and distribution of foraminifera along the coastal areas (Boltovskoy & Wright, 1976). Additional information about the differential response of foraminifera to various physico-chemical parameters is obtained by laboratory culture experiments conducted to understand the response of foraminifera to precisely known parameters (Bradshaw 1955, 1961; Nigam et al. 2006, 2008).

Boltovskoy et al. (1991) summarized that the temperature may result in morphological changes in benthic foraminifera with specimens of same species from colder areas being comparatively larger than those from the warmer regions. However, the response seems to vary from species to species with a few studies indicating that the higher temperature leads to larger tests of benthic foraminifera (Phleger and Hamilton, 1946). A few other studies showed that varying temperatures may lead to altogether different varieties of a same species (Schnitker, 1974; Miller et al. 1982). Similar observations have been made in case of salinity as well. Boltovskoy et al. (1991) opined that “for survival, growth, and reproduction, each benthic foraminiferal species has specific limits of tolerance to salinity”. Foraminiferal assemblages in coastal areas are most affected during monsoon when there is lot of freshwater influx, which in turn changes the characteristics of marine waters (Murray, 1991; Nigam et al. 1992; Nigam & Khare, 1994, 1999; Murray & Alve, 1999a, b). In general a decrease in size of the tests was noted under low saline conditions (Tappan, 1951; Morishima, 1955; Forti and Rottger, 1967; Wright, 1968).

However, again contradictory results were noted as Bradshaw (1961) reported increase in test size at lower salinities.

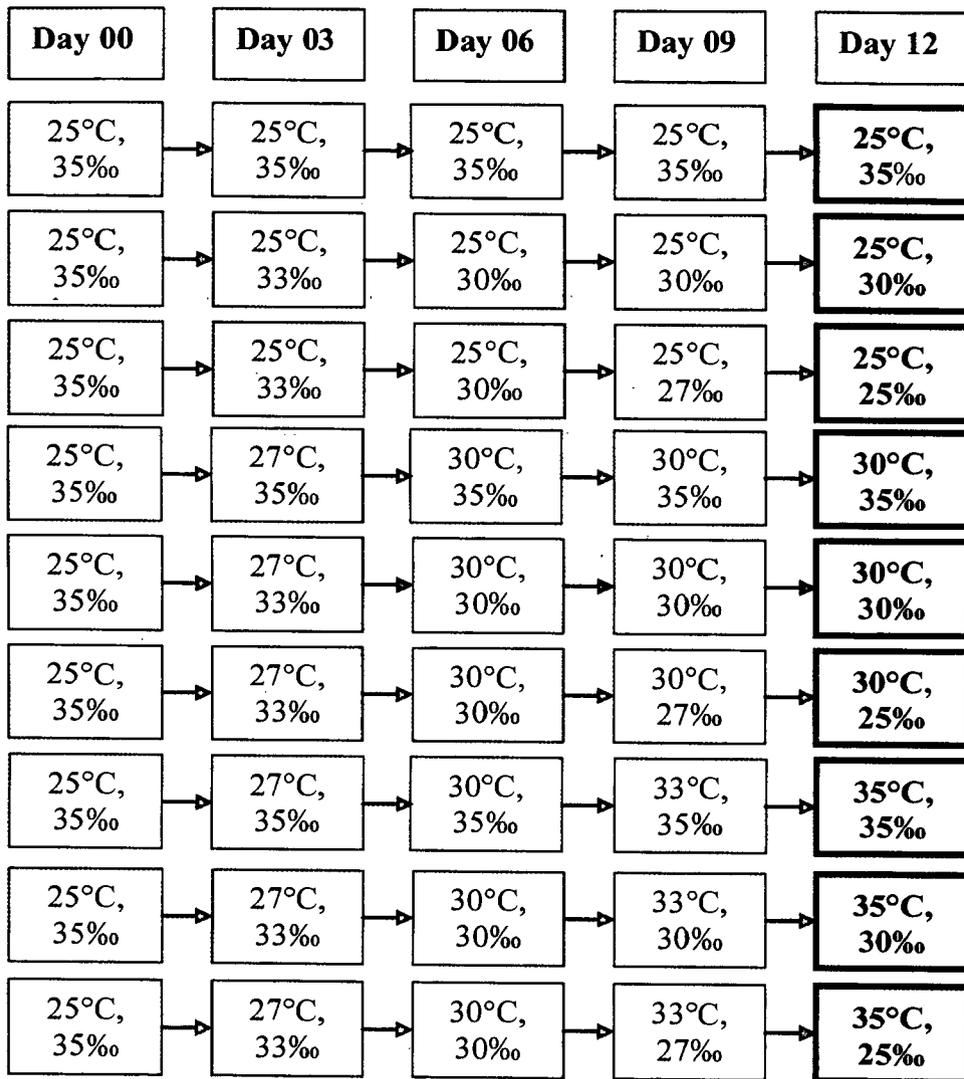
After studying the effect of seawater salinity on the foraminiferal tests, experiments were conducted to study the combined effect of temperature and salinity on the growth and rate of reproduction in shallow water benthic foraminiferal species *Rosalina leei*, *Rosalina* sp. and *Pararotalia nipponica*.

## **7.2 Experimental Setup for *Rosalina leei* (Hedley and Wakefield)**

Once live specimens required to carry out the experiment were available, they were subjected to different combinations of temperature (25°C, 30°C and 35°C) and salinity (25‰, 30‰ and 35‰). A total of 108 specimens were used for the experiment. To begin with, all the specimens were kept at 25°C temperature and 35‰ salinity, as this temperature and salinity was reported at the time of sampling. Two specimens were kept in each well of the six-welled culture tray. Thus, 12 specimens were subjected to each combination of salinity and temperature. After three days one set was kept at the temperature and salinity reported from the field while in other sets the temperature and salinity was changed according to the required combination. The schematic of the experimental set-up is illustrated in Fig.7.1.

## **7.3 Experimental Setup for *Rosalina* sp.**

A total of 6 sets, with 6 specimens in each well of the six-welled culture dish were used for the experiment. Thus a total of 72 specimens were used and the experiment was carried out in duplicate. Initially, all the specimens were kept at 27°C temperature and 35‰ salinity, as this temperature and salinity were reported at the time of collection of material for picking of live specimens. After three days the temperature and salinity in different sets were changed according to the desired combination. The schematic diagram of the experimental set-up is illustrated in Fig. 7.2. After 12 days, all the trays had final desired salinity and temperature.



**Figure 7.1: Schematic diagram of the experimental set-up for *Rosalina lei* (Hedley and Wakefield)**

#### 7.4 Experimental Setup for *Pararotalia nipponica* (Asano)

The *Pararotalia nipponica* was subjected to different combinations of temperature (25°C, 27°C and 30°C) and salinity (25‰, 30‰ and 35‰). Since *P. nipponica* reproduced in laboratory at 27°C temperature and 35‰ salinity, all the juvenile specimens were kept at this temperature and salinity at the onset of the experiment. A total of nine sets of temperature and salinity were used with 108 specimens in each set. The experiment was conducted in replicate. Two specimens were kept in each well of the six well culture tray. Therefore, 12 specimens were subjected to each combination of temperature and salinity (Fig. 7.3). Since *P. nipponica* has a very

robust test, the media in this case was changed after every eight days. The schematic of the experimental set-up is illustrated in Fig. 7.3.

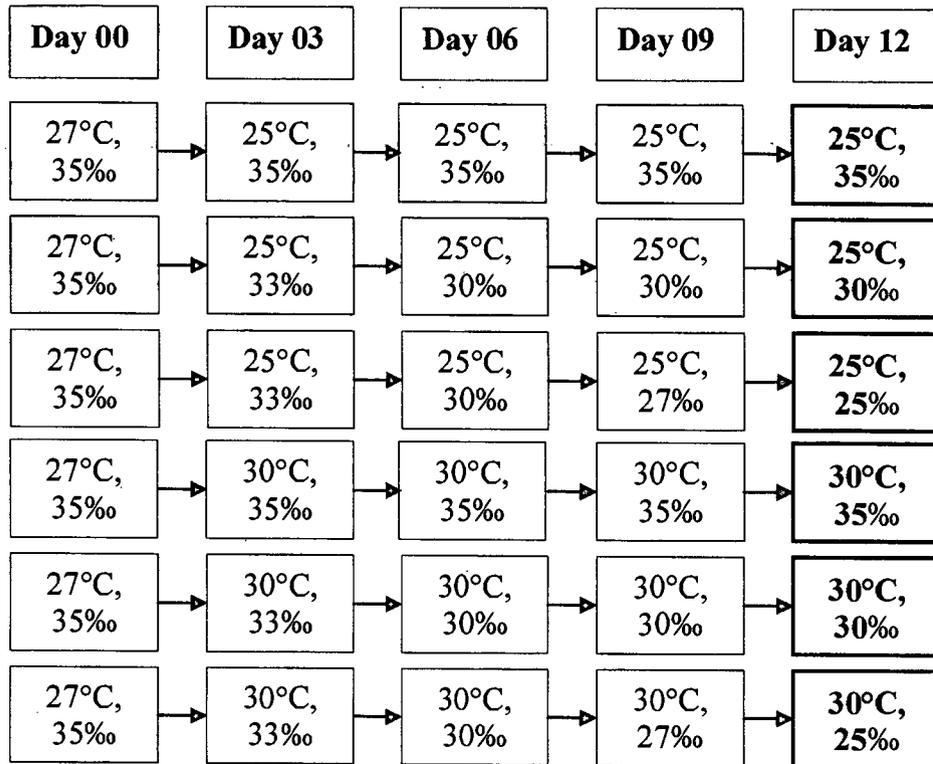


Figure 7.2: Schematic diagram of the experimental set-up of *Rosalina* sp.

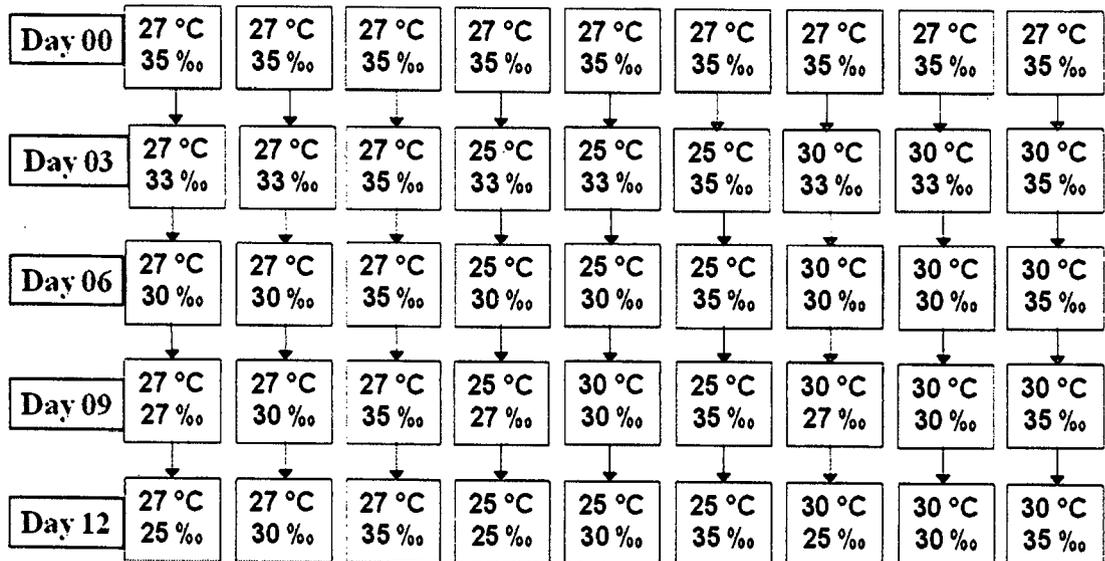


Figure 7.3: Schematic diagram of the experimental set-up of *Pararotalia nipponica* (Asano)

Media as well as the temperature in all the experiments were changed gradually, in order to prevent a sudden experimental shock, which the organisms could experience if they were transferred directly to the respective intended salinity and temperature. Seawater with salinity lower than that from the field was prepared by diluting the seawater collected from the field with distilled water. The higher saline water was obtained by controlled evaporation at 45°C temperature. The maximum diameter, number of chambers and coiling direction were noted with the help of Nikon inverted microscope at the onset of the experiment. The maximum diameter was noted and photographs were taken at regular intervals. *Navicula* species of diatoms was added every time the media was changed as food to these organisms. During the course of the experiment, all the observations regarding the growth, number of chambers and general physiological changes, including pseudopodial activity etc, were made with the help of a Nikon inverted microscope. The experiment was carried out in duplicate.

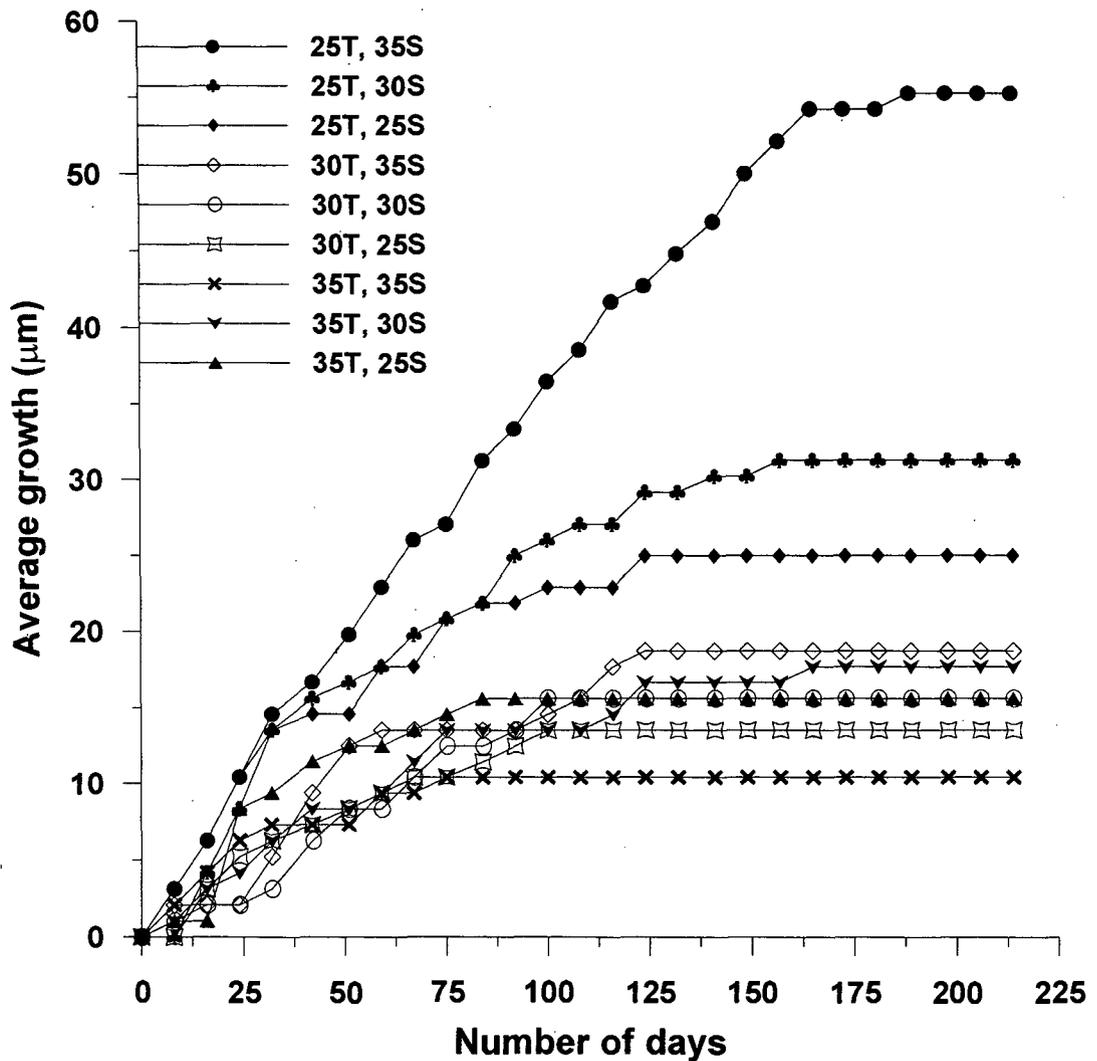
## 7.5 Result

### 7.5 A. *Rosalina leei* (Hedley and Wakefield)

All specimens showed significant growth from the beginning of the experiment till about 50 days. Maximum growth was observed in the specimens kept at 25°C temperature and 35‰ salinity (Fig. 7.4, Table 7.1). Growth was observed even till 189 days and the average growth was ~55 µm. The specimens maintained at 30‰ salinity showed growth only for 157 days with an average growth of 31 µm and in case of the specimens kept at 25‰ salinity the specimens showed growth for 92 days with an average growth of 25 µm. The specimens kept at 30°C and 35°C temperature and different salinities showed very less growth as compared to the specimens maintained at 25°C temperature and different salinities.

Of the three sets of specimens kept at 30°C temperature and different salinities, specimens kept at 35‰ salinity showed the maximum growth (Fig. 7.4, Table 7.1). The growth was reported for 122 days and the average growth was noted to be around 18 µm. Likewise the specimens maintained at 30‰ salinity showed growth only for 96 days with an average growth of 15 µm and in case of the

specimens subjected to 25‰ salinity, the growth was reported for 96 days with an average of 13  $\mu\text{m}$ .



**Figure 7.4: Average maximum growth in *Rosalina leei* (Hedley and Wakefield) at different temperatures and salinities. Experiment conducted on juveniles reproduced in laboratory**

In case of set of specimens maintained at 35°C temperature, specimens kept at 30‰ salinity showed maximum growth (Figure 7.4, Table 7.1). Growth was reported for 135 days and the average growth was noted to be around 17  $\mu\text{m}$ . Likewise the specimens at 35‰ salinity showed growth only for 159 days with an average growth of 12  $\mu\text{m}$  and in case of the specimens kept at 25‰ salinity the specimens showed growth for 95 days with an average growth of 15  $\mu\text{m}$ .

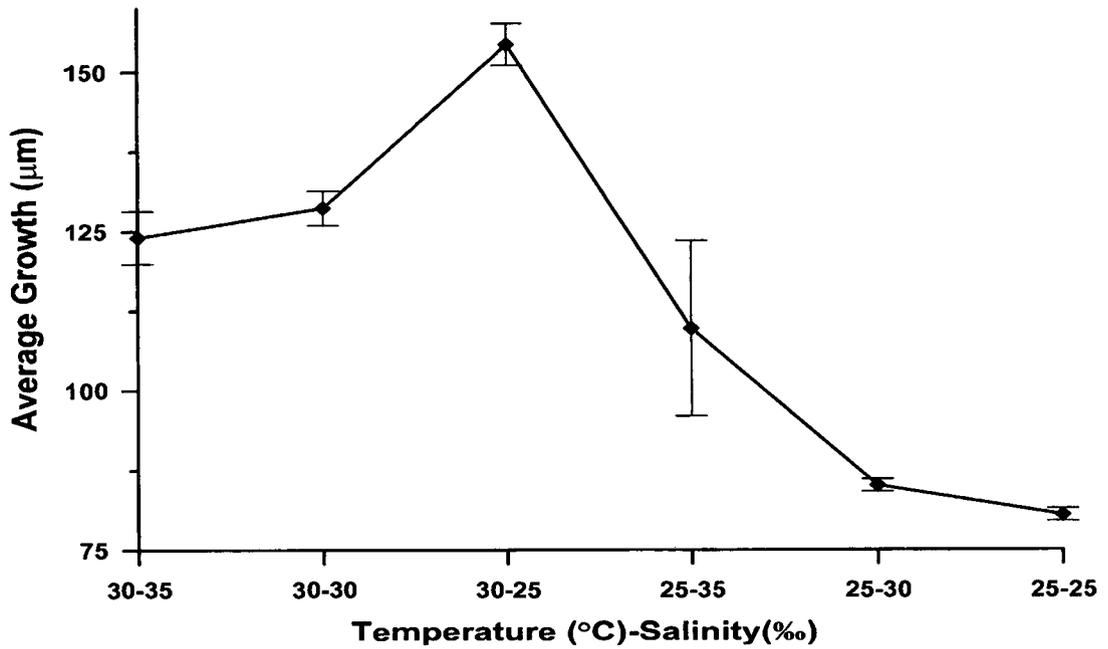
Temperature	25°C	25°C	25°C	30°C	30°C	30°C	35°C	35°C	35°C
Salinity	35‰	30‰	25‰	35‰	30‰	25‰	35‰	30‰	25‰
Average Initial Size ( $\mu\text{m}$ )	299	257	281	224	227	273	303	250	274
Average Final Size ( $\mu\text{m}$ )	354	289	306	243	243	286	316	268	290
Average Growth ( $\mu\text{m}$ )	55	31	25	19	16	14	12	18	16
Specimens kept under observation	12	12	12	12	12	12	12	12	12
Specimens responded	12	12	12	12	10	10	10	11	11

**Table 7.1: Average Initial and final size of *Rosalina leei* (Hedley and Wakefield) along with the number of specimens subjected to experiment.**

In the present experiment, growth in *R. leei* was observed to be directly proportional to salinity and inversely proportional to temperature. However, none of the specimens reproduced throughout the duration of experiment.

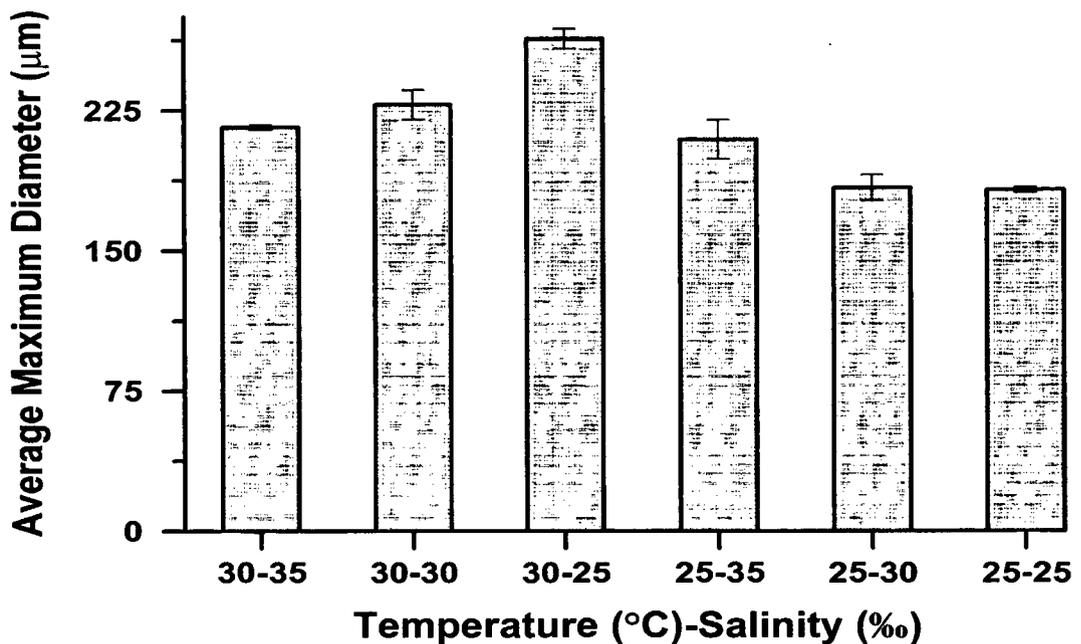
### **7.5 B. *Rosalina* sp.**

All the specimens showed significant growth throughout the experiment. The maximum growth was seen in the specimens subjected to 30°C temperature and 25‰ salinity (Fig. 7.5). Since the specimens were subjected to two sets of temperature, the results are discussed accordingly. Out of all the specimens subjected to 25°C temperatures, those kept at 35‰ salinity showed maximum growth as compared to the ones maintained at 25‰ and 30‰ salinity (Fig. 7.5). Similarly, in case of specimens subjected to 30°C temperature, out of the three sets of salinity, specimens subjected to 25‰ salinity attained maximum growth while those subjected to 30‰ salinity attained comparatively lesser growth and those at 35‰ salinity were the least to grow (Fig. 7.5).



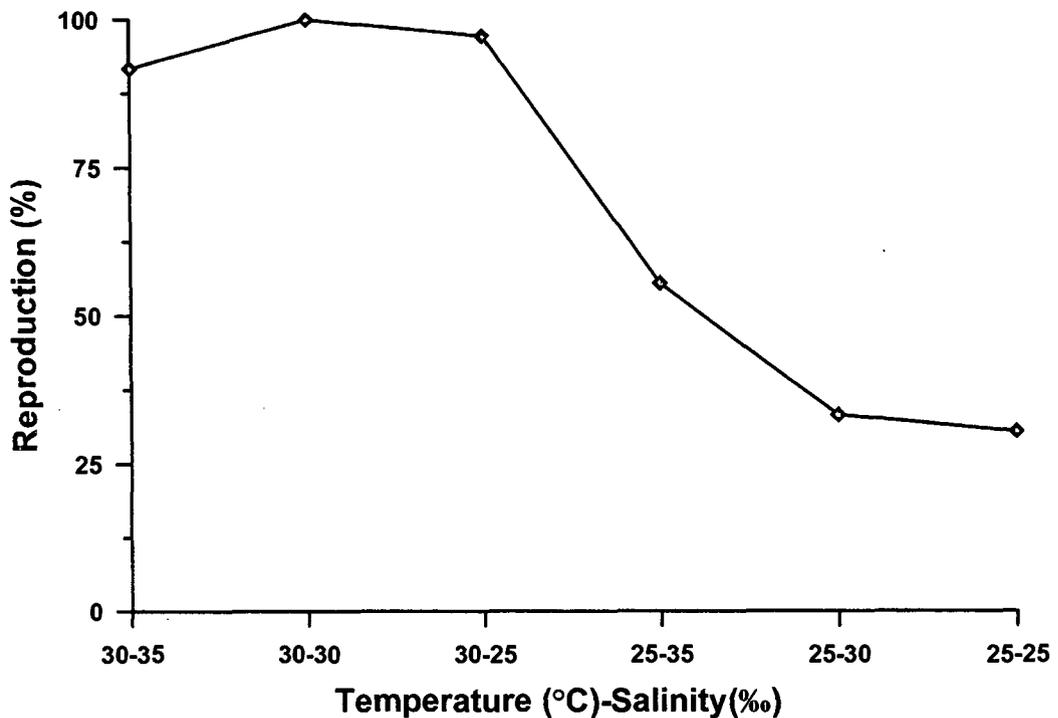
**Figure 7.5:** Average maximum growth in *Rosalina* sp. at different temperatures and salinities. Experiment was conducted on juveniles reproduced in laboratory.

Average maximum growth attained by the specimens subjected to 30°C temperatures was much higher than that of the specimens subjected to 25°C temperature. The final average maximum diameter of all the specimens subjected to 30°C temperature was much larger than that of the specimens subjected to 25°C temperature (Fig. 7.6).



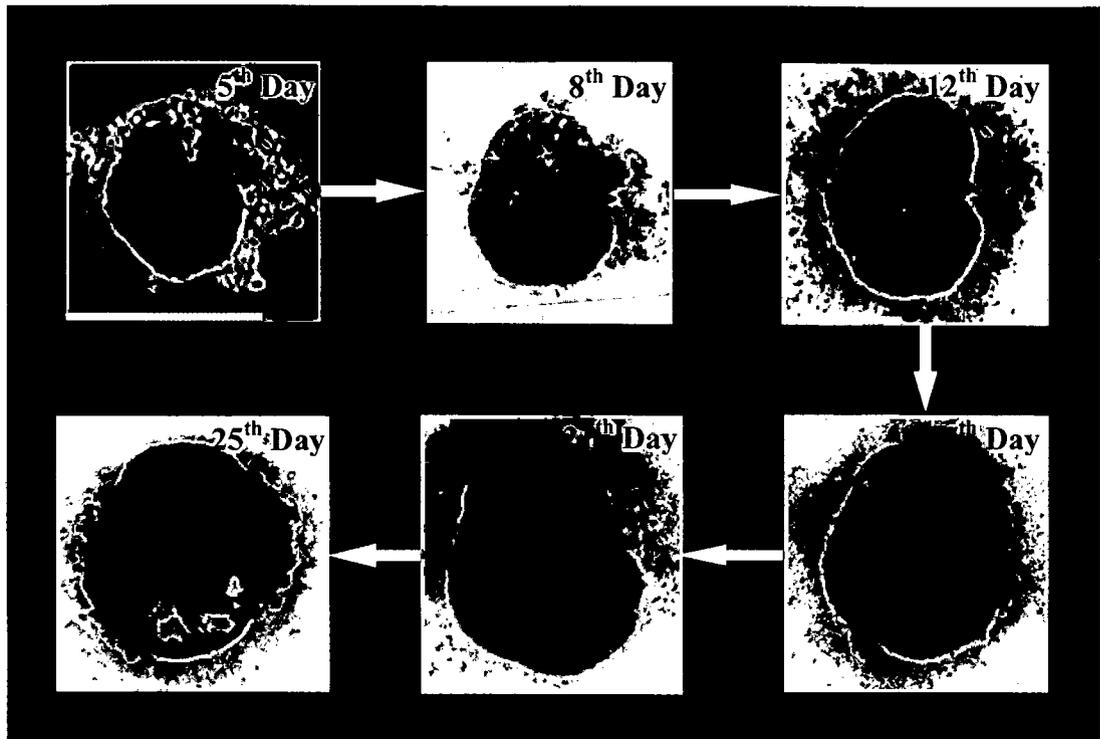
**Figure 7.6:** Average maximum diameter attained by *Rosalina* sp. at different temperature and salinities. Experiment was conducted on juveniles reproduced in laboratory.

In this case, the growth was not as much controlled by seawater salinity as by temperature. Even the response to salinity was different at different temperatures. While the maximum growth in specimens subjected to 30°C temperature was noted at 25‰ salinity, the maximum growth in specimens subjected to 25°C temperature was observed at 35‰ salinity.



**Figure 7.7: Percentage of *Rosalina* sp. specimen reproduced at different temperatures and salinities. Experiment was conducted on juvenile specimens reproduced in laboratory.**

The different temperature and salinity also affected the reproduction in *Rosalina* sp. (Fig. 7.7). Plate 7.1 shows the different growth stages and eventual reproduction. But, here a temperature related bias in reproduction was very evident. While nearly all the specimens kept at 30°C temperature reproduced, irrespective of the salinities, the number of individuals undergoing reproduction at 25°C temperature was very less. Though, the reproduction in specimens always does not match with growth as the organism reproduces under different set of favourable conditions, for *Rosalina* sp., higher temperature favoured both larger growth and near total reproduction. Many of the specimens kept at 25°C temperature were though alive but did not reproduce.



**Plate 7.1: Different stages of growth and reproduction in *Rosalina* sp.  
(Scale = 100 $\mu$ m)**

### **7.5 C. *Pararotalia nipponica* ( Asano)**

All specimens showed significant growth from the beginning of the experiment till about 50 days. Specimens maintained at 35‰ salinity and 27°C temperature showed maximum growth (Fig. 7.8), and the average growth was ~215  $\mu$ m, whereas the specimens subjected to 35‰ salinity and 25°C temperature showed an average growth of ~190  $\mu$ m for about 300 days, after which the growth stopped (Fig. 7.8). The specimens kept at 35‰ salinity and 30°C temperature showed an average growth of ~165  $\mu$ m for about 325 days after which the growth ceased as in case of specimens kept at 25°C temperature.

Specimens kept at 30‰ salinity and 27°C temperature showed an average growth of ~140  $\mu$ m for about 190 days and later the growth stopped. In case of specimens kept at 30‰ salinity and 25°C temperature the average growth was noticed to be of ~115  $\mu$ m for about 190 days whereas in case of the specimens kept at 30‰ salinity and 30°C temperature the average growth of ~105  $\mu$ m was observed for 160 days.

Out of all the specimens kept at 25‰ salinity, those kept at 27°C and 25°C grew for about 50 days with an average growth of ~80  $\mu$ m, while the specimens

maintained at 30°C temperature grew to ~40 μm for approximately 50 days. Later it was observed that the specimens started becoming whitish and ultimately dissolving the test.

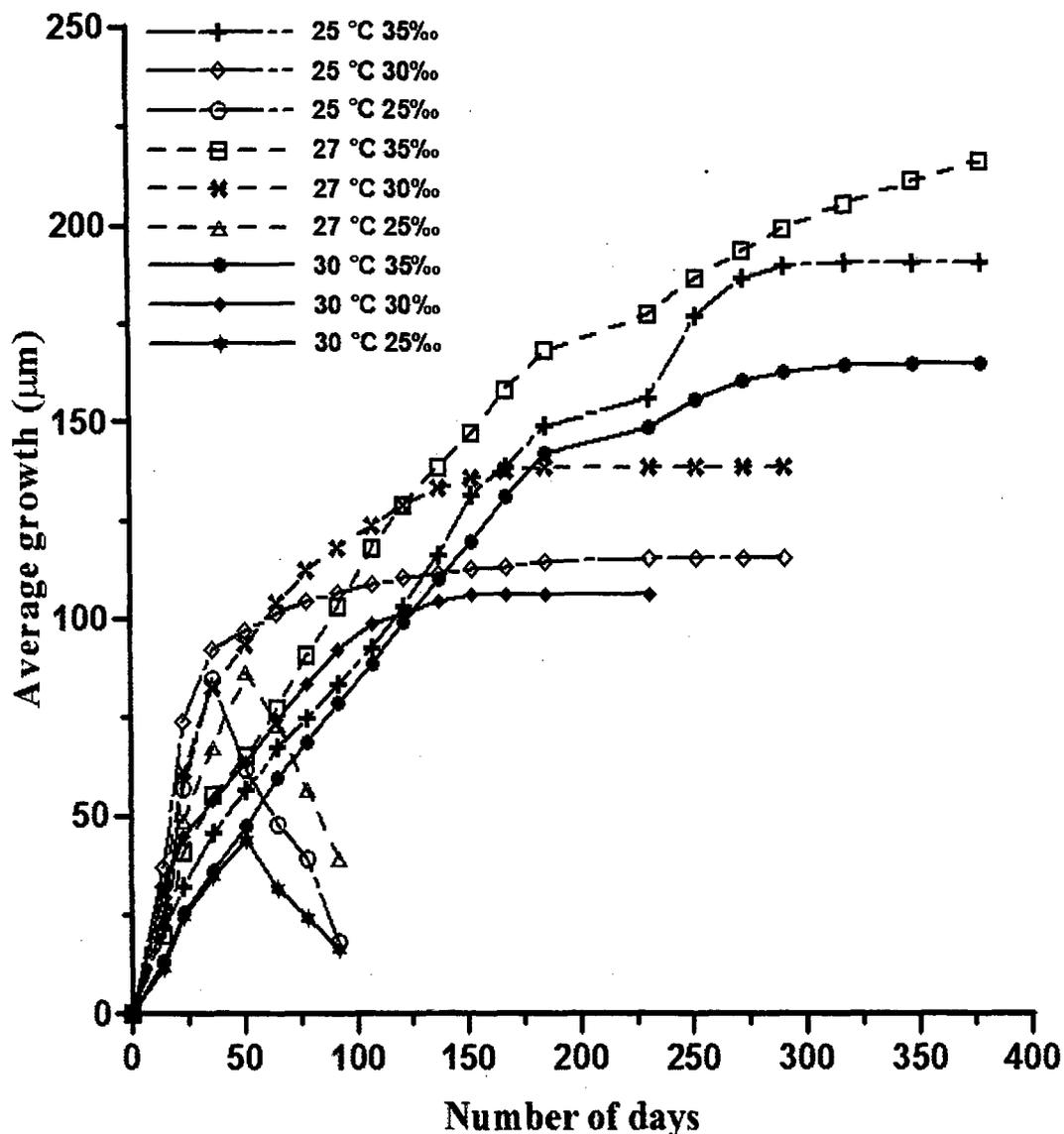


Figure 7.8: Graph showing observed average growth in response to varying temperature-salinity combinations in *Pararotalia nipponica* (Asano). Experiment conducted on juveniles reproduced in laboratory.

## 7.6 Discussion

Among all the abiotic factors, temperature and salinity are considered to be the most important factors for all marine organisms as most of the vital activities such as the tolerance limit for survival, growth, reproduction etc. are governed by these two ecological factors. As the majority of the foraminiferal tests are made up of calcium

carbonate, salinity of the seawater plays an important role in distribution, growth and abundance of foraminifera. Boltovskoy and Wright (1976) reported that the solubility of  $\text{CaCO}_3$  is proportional to the salinity of the seawater. Therefore, as the salinity decreases, the concentration of  $\text{CaCO}_3$  also decreases which in turn lowers the growth of the foraminifera.

The maximum growth in *Rosalina leei* was observed in the specimens maintained at 25°C temperature and 35‰ salinity. It indicates that at comparatively higher salinity and lower temperature, *Rosalina leei* specimens will be abundant and the test size will be large. But at relatively lower salinity, the size of the *R. leei* tests will also decrease. Bradshaw (1961) reported that the highest growth rate was observed in cultures of *Ammonia beccarii tepida* grown at normal salinity (34‰). But the growth rate decreased at lower salinity. Similar results were also reported from both fields as well as laboratory culture experiments (see Boltovskoy et al. 1991, for review; Nigam et al. 2006). Caron et al. (1987) reported that in planktic foraminiferal species *Globigerinoides sacculifer*, subjected to different temperature and salinity, maximum shell size was observed in the specimens kept at or closest to the reported temperature and salinity optima (25°C and 35.25-37.25‰). Carpenter (1856) suggested that temperature could play an important role in the morphological variations of the foraminiferal test. In the present experiment, larger specimens are noticed at relatively cooler temperatures. Rhumbler (1911) also suggested that the same species might have large specimens in cold water than in warm. Similarly, Bradshaw (1957) reported that at comparatively higher temperature (35°C), no growth was observed in *Streblus beccarii var. tepida* specimens and eventually the specimens died.

Though growth was noticed in all the specimens subjected to the experiment, reproduction did not take place at any of the several combinations. Such response is significant for the application of benthic foraminiferal characteristics for paleoclimatic studies. Comparatively cooler conditions will lead to lack or decreased reproduction rate in *R. leei*, thus decreasing its abundance. Bradshaw (1955, 1957) reported that at temperatures outside the reproductive temperature limits, the foraminifera might remain alive and yet not reproduce; he also observed that though foraminifera may have reached maturity it would only reproduce if the environmental conditions were favorable. Interestingly, the cooler conditions and

higher salinity is the result of decreased monsoon strength during glacial periods. Thus the temporal variation in the *R. leei* abundance can provide an idea about the past monsoon changes.

The study also shows a significant influence of seawater temperature on the growth of *Rosalina* sp. Previous culture studies have also revealed the influence of temperature and salinity on survival, growth and reproduction of benthic foraminifera. Bradshaw (1961) noted that at slightly lower temperatures the specimens could survive but eventually died. The findings of this work are in contradiction to Boltovskoy et al. (1991) who summarized that decrease in temperature results in larger specimens. But, the findings are supported by the work of Phleger and Hamilton (1946) who noted an increase in size of benthic species with increasing temperature. However, unlike Schnitker (1974) and Miller et al. (1982), who noted that varying temperatures may lead to altogether different varieties of a same species, within the range of temperature to which *Rosalina* sp. was subjected in this experiment; no significant morphological difference was noted. The response of *Rosalina* sp. is also different than that of the *Rosalina leei* in which maximum growth was noted in specimens subjected to 25°C temperature instead of those subjected 30°C and 35°C temperature (Nigam et al. 2008). Another species *Calcituba polymorpha* showed larger chambers when grown at 21°C - 25°C temperature than at 18°C - 19°C temperature.

It appears that at warmer conditions low salinity facilitated the growth whereas at cooler temperatures, high salinity was helpful for growth. Previous studies have shown that the salinity also plays an important role in growth and reproduction of benthic foraminifera. Experiment carried out on *Rosalina leei* (Nigam et al. 2008) showed increase in growth at higher salinities. However the tolerance limits of benthic foraminifera are very wide, though it may vary from species to species. In cultures of *Ammonia beccarii tepida*, the highest growth rate was observed at 34‰ salinity, but the growth rate decreased at lower salinity (Bradshaw, 1961). Boltovskoy et al. (1991) summarized that effect of abnormal salinity consists of size changes, loss of ornamentation and increase in percentage of aberrant forms. However, no change in ornamentation or an overall abnormality in test was noted in this experiment. In another experiment on *Pararotalia nipponica*, dissolution of the tests and an eventual death was noted in specimens subjected to

salinity below 15‰ (Nigam et al. 2006). However, this species seem to have a wide salinity tolerance range.

In *Pararotalia nipponica* (Asano), maximum growth was attained by the specimens maintained at 27°C temperature and 35‰ salinity. The growth in specimens maintained at 25°C temperature and 35‰ salinity was higher than that of the specimens kept at 30°C temperature and 35‰ salinity, which indicate that at comparatively lower temperature and higher salinity, *P. nipponica* specimens will be abundant and the test size will be large although Boltovskoy and Wright (1976) reported that large size and thick walls of specimens are found in warm tropical waters. It was also observed that lower the salinity smaller the size of these organisms. Bradshaw (1961) reported highest growth rate in cultures of *Ammonia beccarii tepida* grown at normal salinity (34‰). While at lower salinity the growth rate decreased. Dissolution was observed in the specimens kept at 25‰ saline water no matter the temperatures. The reason for this could be the juvenile specimens used for the experiment which were not able to tolerate the lower salinity for a longer duration.

Bradshaw (1955, 1957) reported that at temperatures outside the reproductive temperature limits, the foraminifera might remain alive and yet not reproduce. He also observed that though foraminifera may have reached maturity it would only reproduce if the environmental conditions were favorable. Murray (1963) also noted that though benthic foraminiferal species could flourish in a range of salinities from 0-34‰, reproduction occurred only during the warmer months.

## 7.7 Conclusions

- Out of the set of temperature-salinity conditions, 25°C temperature and 35‰ salinity appeared to be the best suited for the growth of *Rosalina leei* (Hedley and Wakefield). The specimens subjected to higher temperature (>25°C) and lower salinity (<35‰) or same temperature (25°C) and lower salinity (<35‰) as well as same salinity (35‰) and higher temperature (>25°C) showed comparatively lower growth. Therefore, we conclude that at comparatively lower temperature and higher salinities the growth rate of *R. leei* increases, but as the temperature increases and salinity decreases the growth rate also decreases. *R. leei* does not reproduce when subjected to seawater temperature higher than the optimum temperature (17-20 °C) required for reproduction. However, the specimens survived for longer time and significant growth was also noticed.

- Out of the combinations of seawater temperatures (25°C and 30°C) and salinities (25‰, 30‰ and 35‰) used in case of *Rosalina* sp., maximum average growth occurred at 30°C temperature and 25‰ salinity. Majority of the specimens subjected to this combination of seawater salinity and temperature also reproduced. Relatively less growth was noted in case of specimens subjected to 25°C seawater temperature. Very less number of specimens subjected to 25°C seawater temperature, reproduced.
- *Pararotalia nipponica* (Asano) prefers a narrow range of temperature and salinity for reproduction as well as growth. It was observed that the maximum average growth and reproduction in *P. nipponica* occurred at 27°C temperature and at 35‰ salinity. Comparatively less growth was observed at temperatures higher as well as lower than 27°C and salinity lower than 35‰. Prolonged exposure of the specimens to lower salinity (25‰) no matter what temperature they were at, proved to be detrimental to these species.

These findings are significant as the response of benthic foraminifera to different temperature and salinity is often used for paleoclimatic reconstruction.

## Effect of cyclic salinity changes

### 8.1 Introduction

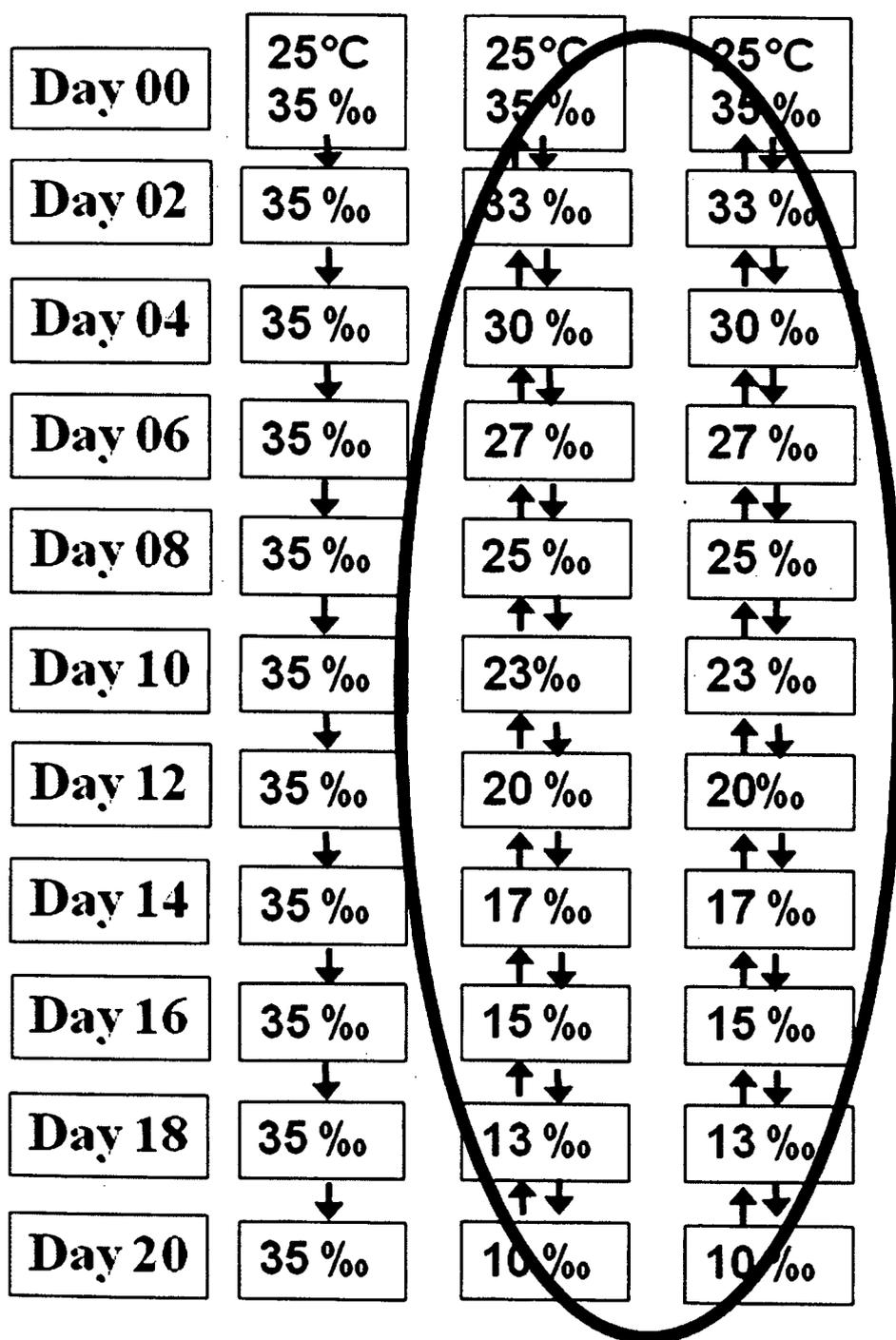
As mentioned in the previous chapters, it was noted that the foraminiferal tests dissolved if subjected to hyposaline conditions for long duration. However, persistence of hyposaline condition in the field depends on intensity and duration of monsoon. During long periods of strong monsoon, salinity in the shallow water coastal regions decreases and remains so for long time, whereas, if the intense monsoon phase remains only for a short period, salinity soon increases to pre-monsoon levels. Similar salinity fluctuations are also seen if the intense monsoon phases occur after long breaks. Under such circumstances, hyposaline condition will prevail only for short duration. Therefore, an experiment was carried out to understand the effect of short-term hyposaline condition on benthic foraminiferal species *Rosalina leei* (Hedley and Wakefield) and *Pararotalia nipponica* (Asano) and their capability, (if any), to overcome the adverse effects of hyposaline conditions. Here the objective was to find out the effects of seawater salinity and associated changes on the hard part of the foraminifera.

### 8.2 Experimental Setup

#### 8.2 A. *Rosalina leei* (Hedley and Wakefield)

Live specimens of *R. leei* were picked under Stereozoom microscope. A total of six sets, each consisting of 6 specimens were used for the experiment. Out of the total six sets, two sets were maintained at 35‰ salinity, the salinity same as that at the time of collection of samples from the field. These were considered as control sets (CS-A, CS-B). The salinity of the control sets was maintained constant (35‰) throughout the experiment. The remaining four sets of specimens, considered as treatment sets (TS-A, TS-B, TS-C and TS-D), were subjected to salinity varying from 10‰-35‰. In each of the four treatment sets, salinity was gradually decreased from 35‰ to 10‰, in alternative steps of 3‰ and 2‰, every second day (Fig. 8.1). Once the effect of lower than normal saline water became evident, the salinity was

increased gradually again till it reached to the initial level (35‰, same as at the time of collection



**Figure 8.1: Experimental Set-up for *Rosalina leei* (Hedley and Wakefield).**

of material from the field). All the culture trays were maintained at 25°C temperature under incubators and under 12 hour light 12 hour dark condition throughout the experiment. In order to avoid evaporation, culture trays were wrapped in thin polythene film, immediately after changing the culture media. The culture media was

changed every alternate day. Food was added in the form of diatom *Navicula* sp. The change in salinity and pH of the media after adding food was negligible. The pH of culture media was routinely measured, before and after changing the media. The seawater of different salinity was prepared as discussed in chapter 6. The growth, abnormality of test, if any, and pseudopodial activity were observed every alternate day.

### **8.2 B. *Pararotalia nipponica* (Asano)**

The juvenile specimens of *P. nipponica* were used for this experiment. Since adequate juvenile specimens were available, experiment was conducted at four different temperatures, viz. 25°C, 27°C, 30°C and 35°C. The experiment was conducted in replicate (A and B) with one control set (salinity was constant with 35‰ saline water) and two treatment sets (salinity was decreased from 35‰ to 10‰ and again increased from 10‰ to 35‰ saline water). Illumination in the form of 12h light and 12h dark was provided. A total of 144 specimens were subjected to the experiment. The seawater of salinity lower than normal was prepared by adding distilled water to the media. The pH of seawater as well as that of the distilled water was measured routinely. Ten milliliters of media was used in each well of the culture plate of 3.5 cm diameter and 1.7 cm height which can hold 15 ml of media. Food in the form of ~20cells/ml of diatoms was provided every time the media was changed. The pH as well as salinity of the feed was noted.

Initially, all the specimens were kept at 27°C temperature and 35‰ salinity as this was the condition at which these specimens reproduced in laboratory. The media was changed after every three days. The salinity as well as the temperature was increased or decreased after an interval of 8 days. The salinity was decreased in alternate steps of 2‰ and 3‰ (Fig. 8.2). The temperature was decreased from 27°C to 25°C and increased from 27°C to 30°C. Later again after 8 more days, in one set the temperature was further increased to 33°C and finally after 8 more days it was increased to 35°C temperature (25°C, 27°C, 30°C and 35°C). Therefore after about 35 days all the four sets had desired temperature. However, it took almost 68 days to decrease the salinity to 10‰. After a gap of 8 days interval the salinity was increased in the same order. Initially measurements in the form of maximum diameter were noted after an interval of 15 to 20 days, finally the duration was increased to 30 day.

The measurements for growth were stopped once growth stagnated or specimen died. Therefore, measurements were made for ~515 days for experimental set at 25°C temperature, ~461 days at 27°C temperature, ~237 days at 30°C temperature and ~135 days at 35°C temperature. Measurements and photographs were taken with the help of Nikon inverted microscope. The pH was measured with LABINDIA make pH meter.

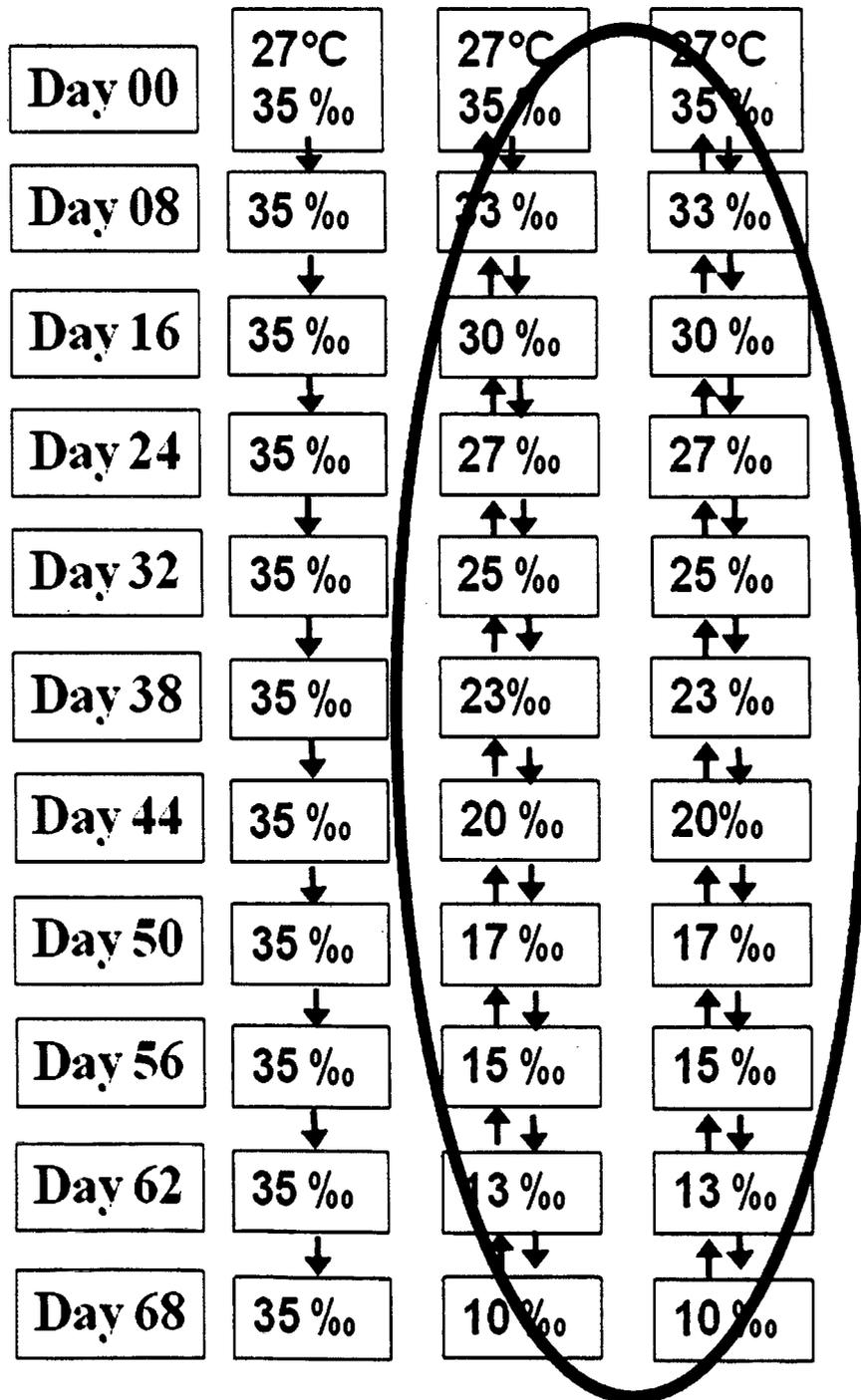


Figure 8.2: Experimental set-up for *Pararotalia nipponica* (Asano) at 27°C temperature. Similar change in salinity was made for specimens at 25°C, 30°C and 35°C temperature.

## 8.3 Results

### 8.3 A. *Rosalina leei* (Hedley and Wakefield)

Since the beginning of experiment till the salinity was lowered to 23‰, all the specimens were very active showing visible signs of being alive. Growth was observed in all the specimens of both the control as well as treatment sets till 23‰ salinity (Fig. 8.3). Once the salinity was lowered below 23‰, it resulted in decreased pseudopodial activity and dissolution of foraminiferal tests. However, the effect was not uniform on all the specimens.

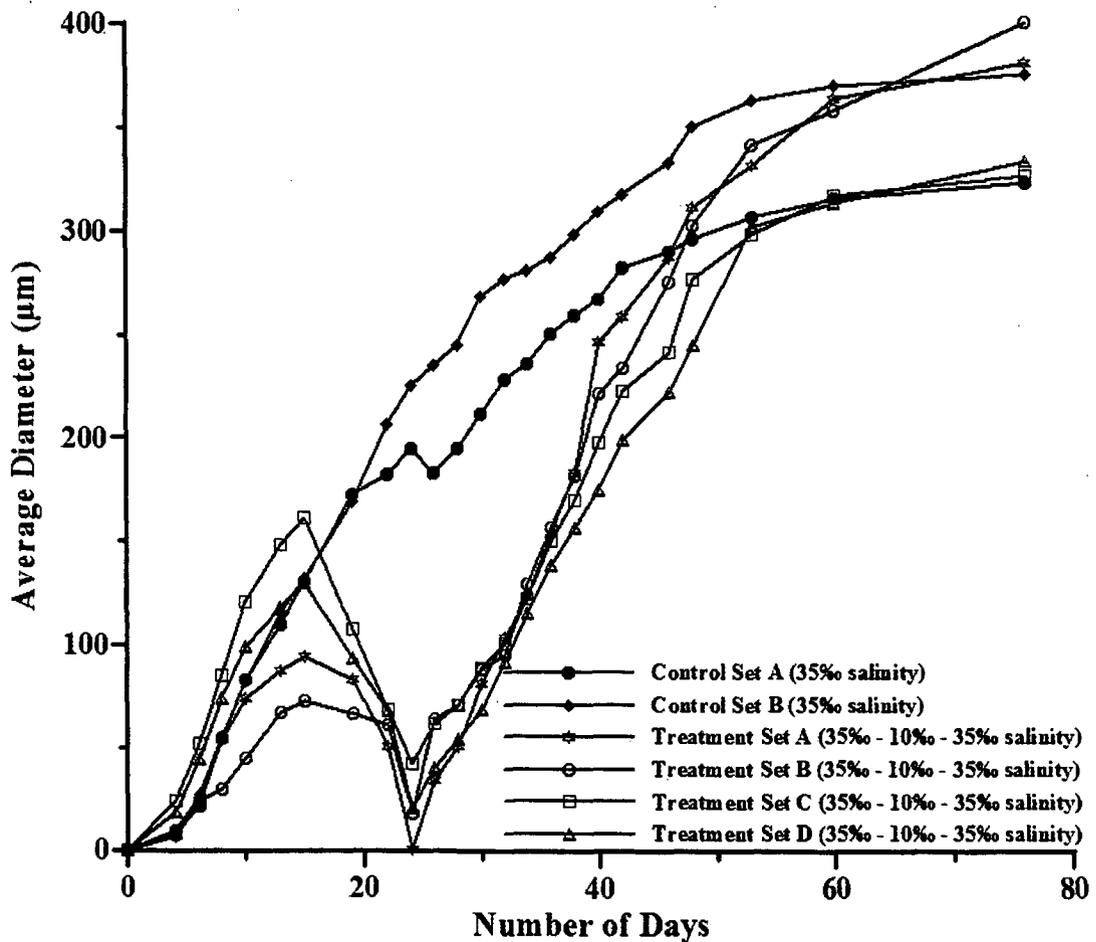
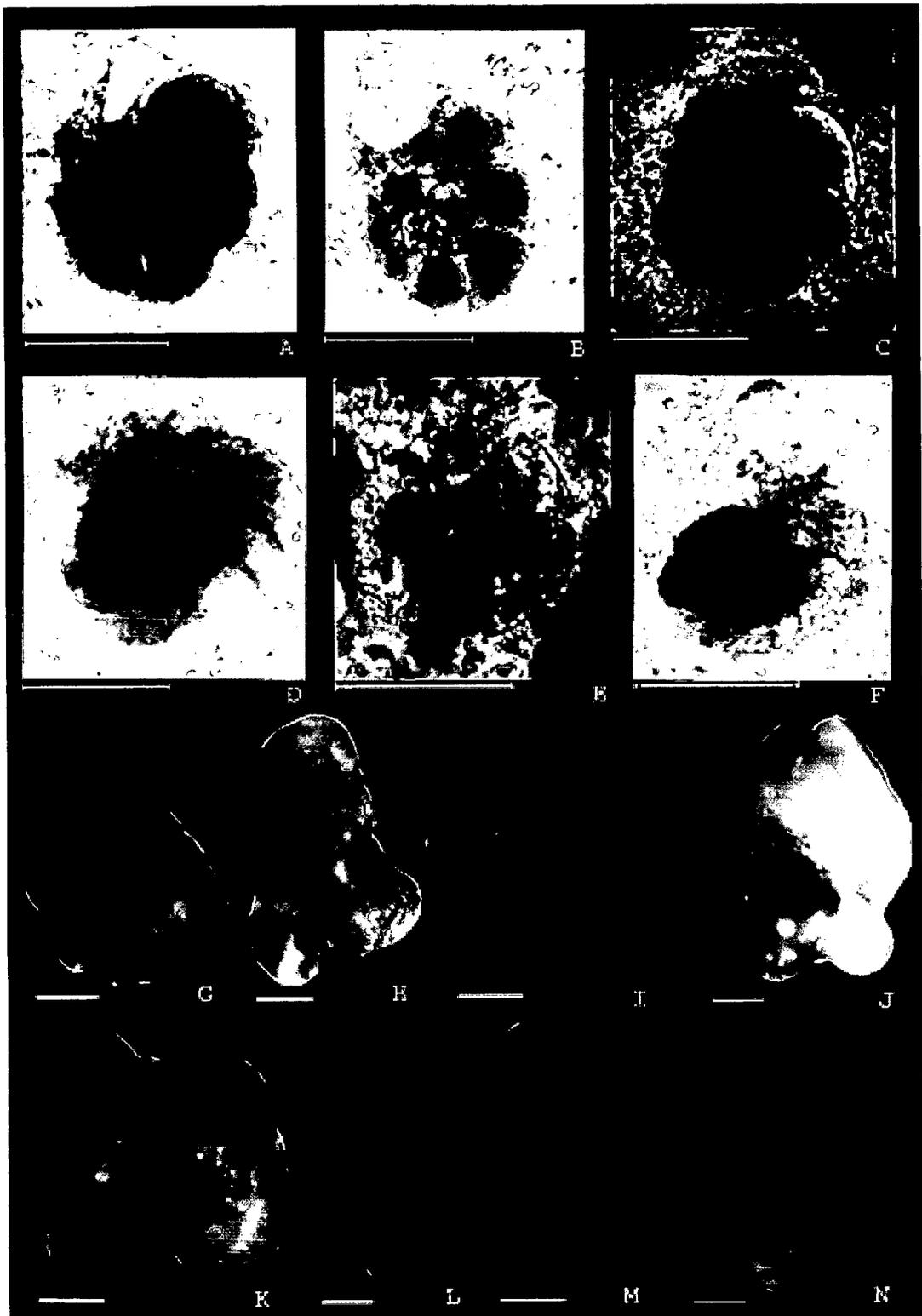


Figure 8.3: Average size and dissolution in specimens of *Rosalina leei* (Hedley and Wakefield) from control and treatment sets. The specimens from the control sets showed continuous growth. However, the test of specimens in treatment sets underwent significant dissolution, as shown by the dip in the average size curve. The experiment was conducted on juveniles reproduced in laboratory.

Test of 8 specimens (2 in TS-A, 3 in TS-B, 1 in TS-C and 2 in TS-D) started dissolving once the salinity was decreased to 20‰. A further decrease in salinity to 17‰ resulted in dissolution in 9 more specimens (1 each in TS-A and B, 5 in TS-C and 2 in TS-D). Test of additional 6 specimens (3 in TS-A, 2 in TS-B and 1 in TS-D) started dissolving once the salinity was further lowered to 15‰. One specimen showed visible signs of dissolution only when the salinity was decreased to 13‰ (Table 1). All the specimens continued to dissolve till they were subjected to seawater of 10‰ salinity. The specimens were alive as evident from the collection of food but the pseudopodial activity was very limited. The dissolution though started from the last chamber, only partial dissolution of chambers was noted. Outline of almost all the dissolved chambers remained visible (Plate 1).

Although the response of specimens varied when the salinity was lowered, all the specimens started rebuilding the test, once the salinity was increased from 10‰ to 13‰. The increase in salinity not only resulted in recalcification of partially dissolved chambers and addition of new chambers but also in increased pseudopodial activity. Specimens completely regenerated the dissolved chambers and attained the size almost equal to or slightly lower than that of the specimens in control set (Fig. 8.2). At the end of the experiment an average growth of  $\sim 357 \mu\text{m}$  (varying from 328-394  $\mu\text{m}$ ) was noted in all the six sets. The final size attained by the specimens in both the control and treatment sets was comparable.

The most interesting feature was abnormalities in the regenerated chambers (chambers added after dissolution). All the 24 specimens subjected to hyposaline seawater developed abnormalities after regeneration (Plate 1), whereas only one out of the 12 specimens in the control sets developed abnormal test in the course of experiment. The abnormality in case of specimen from the control sets was very minor with slightly bigger chamber and without any change in the plane of orientation of newly added chambers, whereas the abnormalities in specimens of treatment set included bigger than normal, disoriented and differently shaped chambers. Not all the specimens subjected to hyposaline conditions could recover; three specimens died during the experiment.



**Plate 8.1: Dissolution (A-F) and abnormalities (G-N) in *Rosalina leei* (Hedley and Wakefield) specimens subjected to hyposaline seawater. In most of the specimens, hyposaline seawater resulted in partial dissolution of chambers (A-C) while in others (D-F), last few chambers got completely dissolved. Almost all of the specimens regenerated the dissolved chambers, but became abnormal (G-N). Abnormalities included addition of larger or smaller chambers, in planes other than the normal plane of addition of chambers (Scale bar = 100 $\mu$ m).**

**Table 8.1: Size of the specimens of *Rosalina leei* (Hedley and Wakefield) in control and experimental sets during the experiment. The pH at the time of measurement is also given.**

(A)			Size of the Specimen ( $\mu\text{m}$ )											
Days	Salinity	pH	Control A						Control B					
0	35	8.08	123	138	126	160	129	137	136	143	128	163	134	128
4	35	7.88	136	149	146	163	135	140	166	149	138	166	135	133
6	35	7.92	156	155	162	176	145	146	175	194	152	163	160	149
8	35	8.04	215	185	197	216	146	184	219	249	182	184	168	161
10	35	8.03	277	197	204	275	159	202	249	295	216	201	189	175
13	35	8.04	279	215	231	326	180	239	301	337	249	216	211	209
15	35	8.03	334	237	248	328	190	259	318	344	256	238	225	242
19	35	7.92	418	249	252	448	208	273	380	352	327	279	239	266
22	35	8.10	420	269	264	448	228	275	407	395	395	324	258	289
24	35	8.03	421	277	282	450	256	293	415	410	442	339	275	302
26	35	8.01	426	292	289		271	311	423	413	447	348	297	311
28	35	8.02	429	308	301		291	322	425	415	462	371	307	319
30	35	7.92	445	329	315		318	325	508	418	483	391	321	320
32	35	7.92	479	332	326		343	336	513	420	490	408	336	322
34	35	7.90	487	333	331		358	346	511	422	494	419	345	323
36	35	8.04	510	336	354		368	358	515	423	500	426	365	324
38	35	7.85	512	337	386		375	363	529	428	502	446	371	347
40	35	7.86	513	339	399		388	374	531	446	510	460	386	354
42	35	8.04	529	341	429		398	390	534	470	514	462	400	359
46	35	7.86	530	341	440		419	397	546	490	518	467	416	391
48	35	7.88	541	341	446		436	393	565	515	525	477	430	421
53	35	7.94	553	343	449		460	405	577	520	538	482	440	449
60	35	7.91	561	344	452		473	426	579	524	542	485	456	463
76	35	7.93	572	347	456		484	434	580	527	543	487	460	486

(B)			Size of the Specimen ( $\mu\text{m}$ )											
Days	Salinity	pH	Treatment Set A						Treatment Set B					
0	33	8.08	133	129	146	118	105	146	114	116	150	132	124	137
4	30	8.05	142	147	149	120	109	154	114	130	176	139	124	145
6	27	8.01	160	150	177	121	155	163	123	134	194	163	126	162
8	25	7.99	186	171	222	127	191	208	141	193	215	244	149	168
10	23	7.69	211	208	224	143	221	216	141	198	292	256	154	182
13	20	7.79	216	211	232	163	258	222	144	176	305	290	163	205
15	17	7.77	203	203	246	201	271	222	186	175	250	235	170	210
19	15	7.64	176	187	262	260	166	227	203	173	236	202	171	204
22	13	7.60	182	171	247	129	157	196	136	161	223	150	118	198
24	10	7.59	98	167	173	78	149	116	130	146	211	119	115	155
26	13	7.16	182	172	201	115	153	158	134	153	231	132	131	201
28	15	7.47	183	171	227	151	183	167	147	183	254	171	139	208
30	18	7.47	221	191	270	217	177	190	180	195	249	213	135	226
32	20	7.56	222	196	283	277	218	201	181	206	269	252	169	233
34	23	7.70	232	210	305	293	250	226	187	237	280	283	207	267
36	23	7.78	277	247	322	319	275	259	188	262	320	297	258	294
38	27	7.82	297	292	361	327	312	287	189	301	369	326	298	318
40	30	7.84	469	335	403	360	375	311	190	341	417	336	327	359

42	33	7.80	476	369	428	368	377	312		367	461	389	352	371
46	35	7.95	483	419	471	399	398	329		380	521	416	368	413
48	35	7.88	504	422	473	463	426	359		427	529	426	379	440
53	35	7.94	518	426	506	480	450	391		422	550	476	384	479
60	35	7.91	521	513	519	532	481	393		518	562	517	421	495
76	35	7.92	527	525	534	561	520	396		567	562	527	435	538

(C)			Size of the Specimen ( $\mu\text{m}$ )											
Days	Salinity	pH	Treatment Set C						Treatment Set D					
0	33	8.08	108	151	119	132	134	103	143	121	111	142	145	119
4	30	8.05	150	156	145	134	170	138	157	140	141	167	150	140
6	27	8.01	172	187	155	165	211	170	195	177	183	185	150	156
8	25	7.99	216	213	199	197	228	209	238	204	195	221	186	187
10	23	7.69	274	274	232	201	256	237	245	244	228	242	215	204
13	20	7.79	257	293	266	273	271	276	251	256	244	262	232	244
15	17	7.77	263	290	283	296	290	294	296	249	256	246	254	260
19	15	7.64	238	264	233	250	199	212	220	194	267	209	256	197
22	13	7.60	186	184	207	201	198	184	180	169	211	196	259	176
24	10	7.59	159	176	184	186	151	145	144	103	150	188	183	137
26	13	7.16	191		201	195	174	172	182	129	162	198	196	159
28	15	7.47	201		213	208	179	177	187	138	194	206	199	183
30	18	7.47	221		221	235	199	194	196	142	229	225	202	199
32	20	7.56	234		229	241	211	212	218	156	243	253	258	205
34	23	7.70	257		266	254	224	236	258	165	259	293	275	226
36	23	7.78	288		298	300	232	258	282	180	279	322	282	267
38	27	7.82	295		317	311	252	296	310	185	295	341	290	299
40	30	7.84	313		319	346	290	344	349	191	307	358	303	319
42	33	7.80	345		363	368	308	352	387	196	312	364	333	383
46	35	7.95	395		374	381	326	355	414	197	317	383	369	432
48	35	7.88	435		375	415	348	435	468	199	338	384	388	470
53	35	7.94	437		387	459	365	467	498		355	389	403	514
60	35	7.91	449		400	484	376	500	509		369	392	426	522
76	35	7.92	451		410	486	383	527	587		375	396	429	534

### 8.3 B. *Pararotalia nipponica* (Asano)

In the beginning of the experiment, the specimens did show good growth in the control as well as the treatment set at all the four temperatures (25°C, 27°C, 30°C and 35°C). As the salinity was decreased in the treatment set (from 35‰ to 10‰ and again increased from 10‰ to 35‰ saline water) it was observed that the test of the specimens started becoming opaque first and further decrease in salinity lead to dissolution of the test. The dissolution initially started from the last chamber and proceeded towards the proloculus (initial chamber).

In case of specimens subjected to 25°C temperature, it was observed that the specimens of control set (salinity 35‰), grew normally attaining an average growth of ~250  $\mu\text{m}$  (Fig. 8.4). However, the specimens of treatment set showed an initially

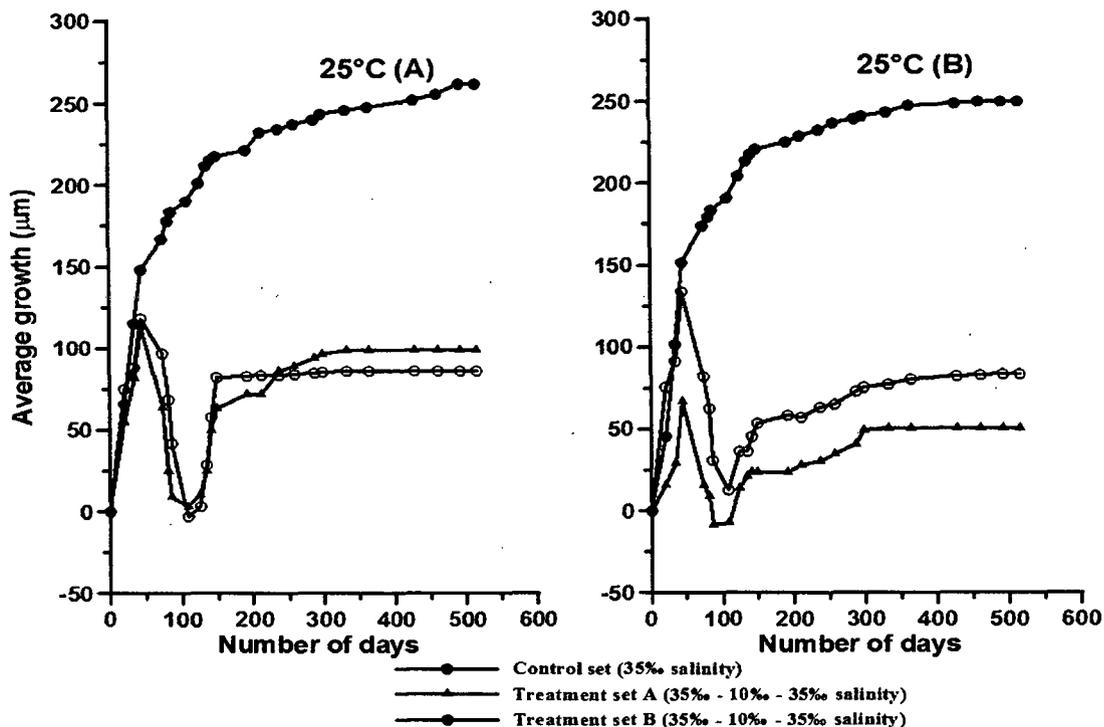


Figure 8.4: Average growth and dissolution in specimens of *Pararotalia nipponica* (Asano) from control and treatment sets at 25°C temperature. Experiment was conducted on juveniles reproduced in laboratory.

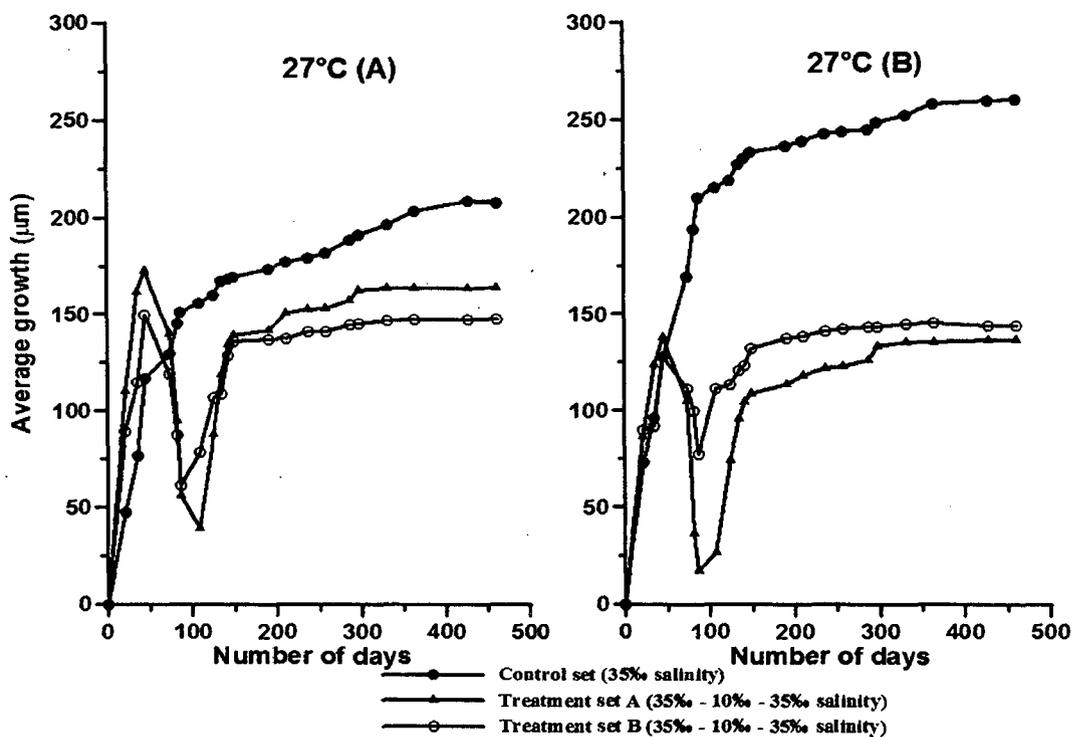


Figure 8.5: Average growth and dissolution in specimens of *Pararotalia nipponica* (Asano) from control and treatment sets at 27°C temperature. Experiment was conducted on juveniles reproduced in laboratory.

growth only till the salinity was lowered up to 20‰. With a further decrease in salinity below 20‰, the specimens become opaque (Figure 8.4) and subsequently dissolution was noted. Later, when the salinity was increased, the specimens started rebuilding the dissolved test. The average growth in case of the specimens of the treatment set varied from 50  $\mu\text{m}$  to 100  $\mu\text{m}$ , which is smaller than the average growth attained by the specimens of control set. Similar results were also obtained in case of specimens subjected to 27°C temperature. The average maximum growth attained by the specimens in control set varied from ~200  $\mu\text{m}$  to ~250  $\mu\text{m}$ , while that of the treatment sets was ~150  $\mu\text{m}$  (Fig. 8.5).

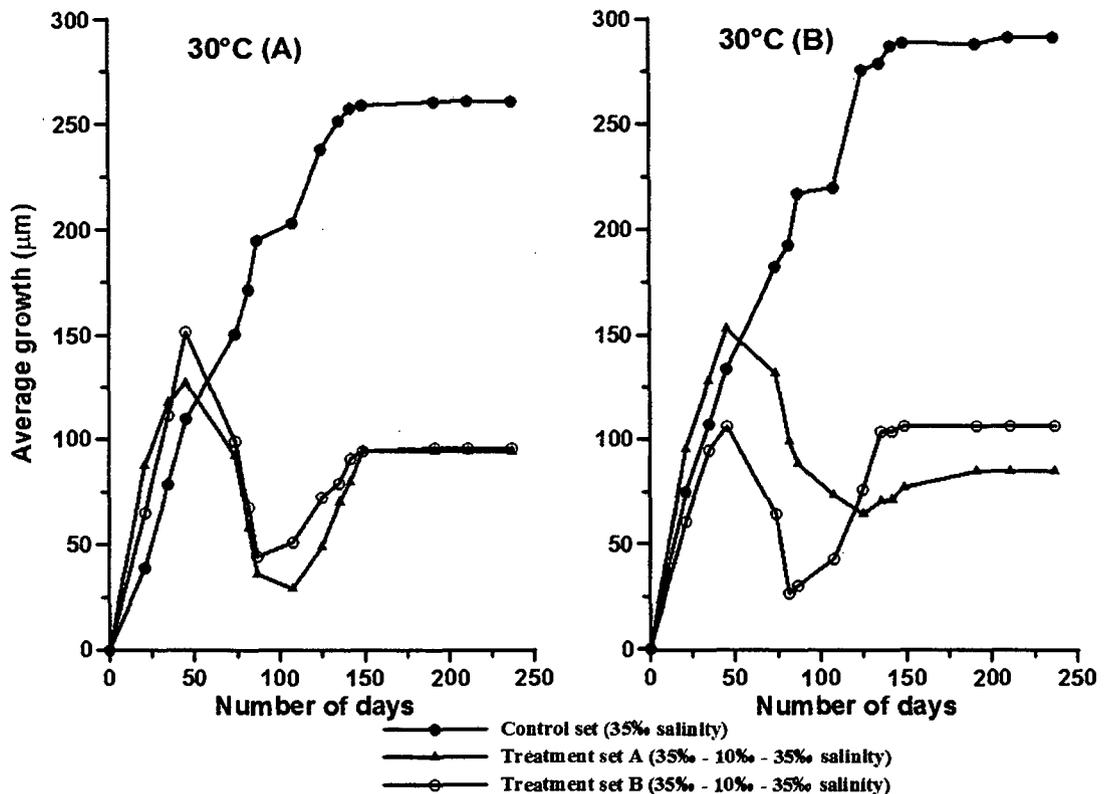


Figure 8.6: Average growth and dissolution in specimens of *Pararotalia nipponica* (Asano) from control and treatment sets at 30°C temperature. Experiment was conducted on juveniles reproduced in laboratory.

Substantial growth occurred in specimens of control sets maintained at 30°C temperature (Fig. 8.6). The response of the specimens of the treatment sets in this case was almost same as in case of specimens subjected to 25°C as well as 27°C temperatures, during the phase when salinity was decreased as well as when the salinity was initially increased. The regenerated specimens were not able to grow up

to the size same as before dissolution started. Additionally, the regenerated specimens survived only for ~200 day (Fig. 8.6).

The response of the specimens of both the control set as well as the treatment set was very poor at 35°C temperature. Initially growth was observed in control set specimens. The average growth of the control set specimens varied from ~100 μm in set (A) to ~125 μm in set (B). The average growth of the control set specimens at 35°C temperature was very less as compared to the growth of the specimens at other three temperatures (25°C, 27°C and 30°C). The control set specimens survived only for ~150 days, much less than the days for which specimens of control set survived at other three temperatures (Fig. 8.7). A short lived initial growth phase was observed in specimens of the treatment set. But later as the salinity was decreased, the growth stopped and dissolution started, ultimately resulting in death of the specimens. In all the specimens of treatment set a small recovery was noted when the salinity was again increased (Plate 2). The regenerated specimens survived only for ~100 days.

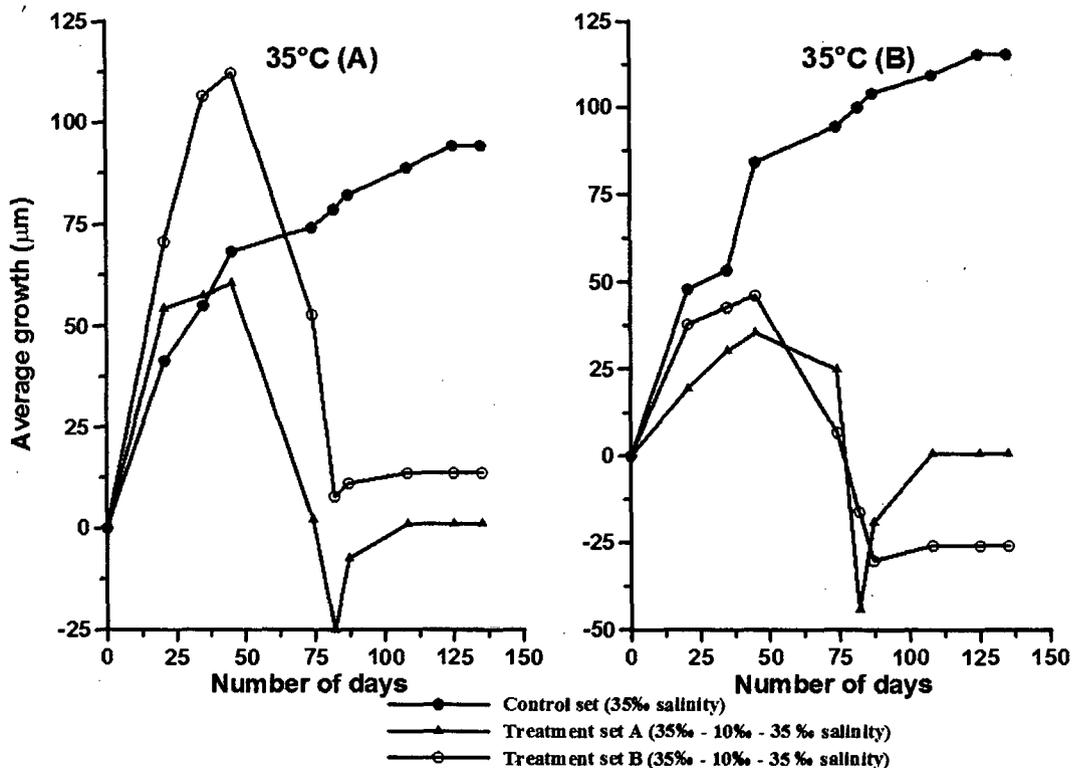


Figure 8.7: Average growth and dissolution in specimens of *Pararotalia nipponica* (Asano) from control and treatment sets at 35°C temperature. Experiment was conducted on juveniles reproduced in laboratory.

## 8.4 Discussion

The dissolution of tests in treatment sets of both the specimens (*Rosalina leei* and *Pararotalia nipponica*) probably took place because of decreased pH of seawater, associated with the decreased salinity. It was observed that pH decreased with the decreasing salinity, in both the experiments (Fig. 8.8 & 8.9). Similar observations are made in the field as part of this study as well as previous reports (Brown et al., 1999). Earlier Boltovskoy and Wright (1976) noted that pH lower than 7.8 induces dissolution of calcareous tests, whereas Bradshaw (1961) and Angell (1967) observed dissolution of the selected foraminiferal species only under acidic pH conditions. Similarly, Stouff et al. (1999b) also reported that dissolution in *Ammonia beccarii* started when the seawater pH decreased below 5. However, the dissolution in case of *R. leei* started at pH lower than 7.5 (20 ‰ salinity) and in case of *P. nipponica* it started at 7.3 pH which was at 15‰ salinity.

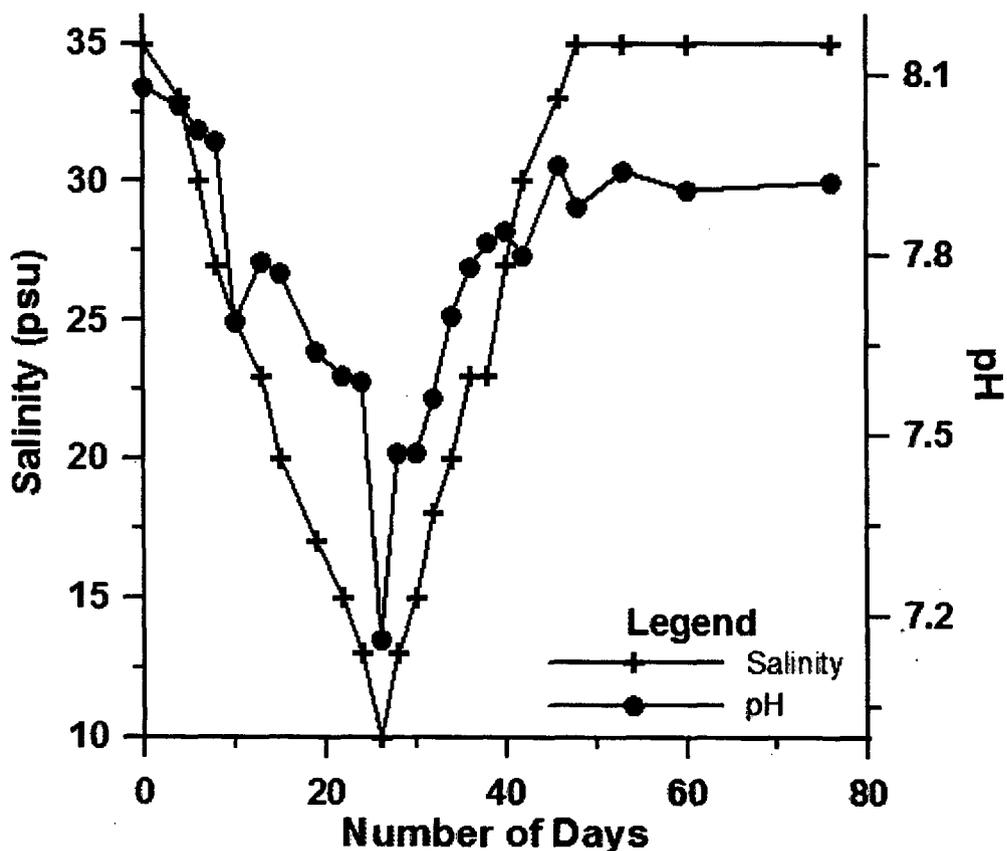


Figure 8.8: Relationship between seawater salinity and pH as observed during the course of the experiment on *Rosalina leei* (Hedley and Wakefield). The pH decreased with the lowering of salinity.

The differential response to decreased salinity may be due to the difference in thickness of the test wall of *R. leei* and *P. nipponica*. The test is comparatively very thin in case of *R. leei* and thick and robust in case of *P. nipponica*.

This study helped to refine the seawater pH value at which dissolution begins in *R. leei*, and *P. nipponica* as against the large range proposed by earlier workers (Bradshaw, 1961; Cadre et al. 2003). Cadre et al. (2003) reported that the dissolution for *Ammonia beccarii* started at the neutral pH. The study however shows that the dissolution of calcareous foraminiferal tests can take place at much alkaline seawater pH than reported before.

Another possible reason for the dissolution of the tests might be the decrease in the concentration of the calcium carbonate at lower salinities as it has been cited as a potential cause for the dissolution of foraminiferal tests in numerous paleoclimatic reconstruction studies (De Rijk, 1995; Murray and Alve, 1999a; Kimoto et al. 2003). However, unfortunately we don't have any measured values for the calcium carbonate concentration of the differently saline media.

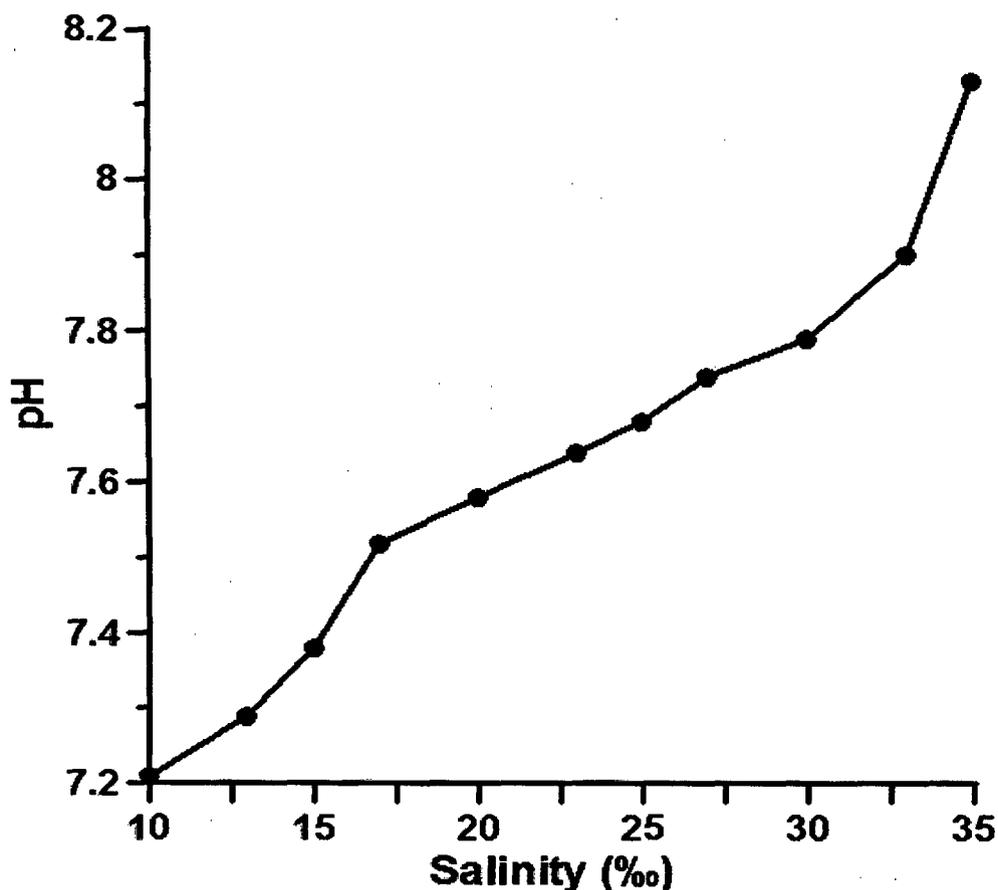


Figure 8.9: Relationship between seawater salinity and pH as observed for the media prepared in the laboratory for the experiment on *Pararotalia nipponica* (Asano).

The series of stages followed during dissolution under low salinity in case of *R. leei* (Plate 1) and *P. nipponica* (Plate 2), from the present experiment starting with tests becoming slightly opaque and then dissolution starting from last chamber, has also been reported by Cadre et al. (2003) while observing the effects of low pH on *Ammonia beccarii*. Opaque tests have also been reported from field (Murray, 1989). However, the dissolution does not progress chamber-by-chamber, except a last few chambers. After near complete dissolution of the last one or two chambers, almost all the chambers of the last whorl were equally affected by the dissolution. Though the differences in the degree of resistance to dissolution of individual tests were also noted, it probably shows the slight differential individual response.

The recalcification of dissolved and addition of new chambers after increasing the salinity, resulted because of revival of favorable conditions. This recalcification confirms the views expressed by Boltovskoy and Wright (1976) that foraminifera can repair and/or regenerate their tests after damage arising out of either physical injury or chemical effects. The results are significant in view of the reported presence of abnormal tests near river mouths, where the possibility of breakage and later abnormal regeneration has been expressed (Vilela, et al. 2002). The rate of regeneration of test was comparable with that of the dissolution. The thickening of wall of decalcified chambers took place contemporaneously with the addition of new chambers. Angell (1967) also reported similar observations for *Rosalina floridana*, where a hydrochloric acid decalcified specimen recovered once transferred to normal saline water. He explained this recalcification as a result of addition of calcium carbonate layer to the whole test, every time a new chamber is formed, rather than any healing mechanism. However, such regeneration of dissolved chambers by the specimens, not through any specific mechanism to recover damage induced to the chambers, rather as a result of normal process of addition of calcitic layer to the whole test in certain foraminiferal species, every time a new chamber is added, was not noticed in the present experiment.

Another interesting finding of the present experiment was the abnormality in the chambers added during the regeneration of the tests. Since the 19<sup>th</sup> century, morphological deformities in fossil foraminiferal test were observed from anthropogenically polluted sites (Carpenter, 1856; McCrone & Schafer, 1966; Setty & Nigam, 1984; Naidu et al. 1985; Bhalla & Nigam, 1986; Stouff et al. 1999a, b;

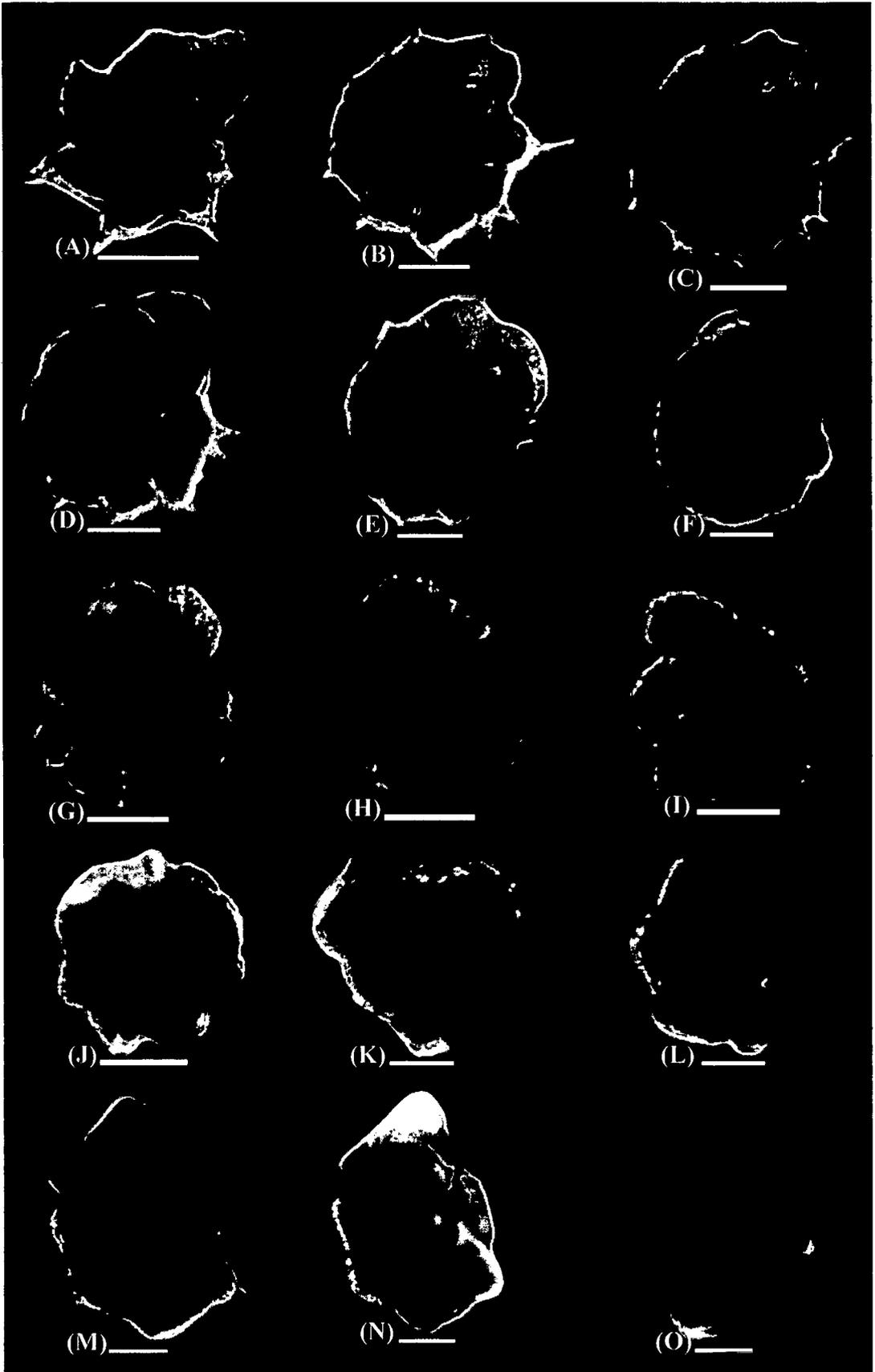


Plate 8.2: Describing the stages of growth (A-E), dissolution (F-I) and regeneration (J-O) in *Pararotalia nipponica* (Asano). Scale bar = 100 $\mu$ m

Yanko et al. 1999; Naidu et al. 2000; Debenay et al. 2001; Frontalini & Coccioni, 2008; Romano et al. 2008) as well as naturally ecologically stressed regions (Boltovskoy et al. 1991). However, in this experiment, deformed tests were the result of ecological stress:

The chambers added after regeneration, were abnormally oriented away from the normal plane of orientation of the earlier chambers formed under normal conditions (Plate1, Plate2). These findings can help explain the increased abundance of abnormal specimens in areas subjected to short-term ecological variations, especially salinity variations (Murray, 1989). This abnormality in the chambers added during regeneration of the tests probably arises because of either physiological or structural damage during dissolution of the tests, Interestingly, the size of specimens subjected to hyposaline condition was as big as that of control specimens, even after considerable dissolution of the test in case of *R. leei* specimens, but, in case of *P. nipponica* specimens it was not the case.

## 8.5 Conclusions

Based on the laboratory culture experiment carried out to understand the response of inner shelf benthic foraminiferal species *Rosalina leei* and *Pararotalia nipponica* to short-term decrease in salinity we conclude that:

- Lower than normal saline water leads to dissolution of the tests in both the specimens.
- Although these specimens are capable of recovering from short-term salinity changes, the signatures of lower than normal saline water are retained by the test in the form of visible morphological abnormalities.
- *Pararotalia nipponica* is able to tolerate higher temperatures (35°C) for a short span of time. Prolonged exposure to warmer waters leads to the death of the specimen in both the normal set as well as the treatment set.

The results can help in understanding the cause of anomalously high abundance of abnormal specimens as well as in changes in abundance of certain species in the samples collected from marginal marine areas in the field.

## Effect of temperature and salinity changes on $\delta^{18}\text{O}$

### 9.1 Introduction

As mentioned in the previous chapters, the near shore shallow water regions in front of the river mouths experience wide seasonal salinity changes due to changes in fresh water influx from the rivers. The changes in fresh water influx over the seasons are also accompanied by variation in the amount of terrestrial sediments being brought by the river into the oceans. It is then obvious that the changes in monsoon strength over the years will also be reflected in the variation in salinity and sediment load being brought by the rivers in the shallow water regions. Therefore, such regions are ideally suited to study the past changes in monsoon intensity. Benthic foraminifera are abundant in shallow water regions (Boltovskoy & Wright, 1976; Murray, 1991). The temporal changes in the abundance, diversity and morphology of benthic foraminifera from shallow water regions have been used to infer past monsoon changes (Zwaan et al. 1999; Nigam, 2005). This requires study of response of different species to salinity changes as discussed in previous chapters. However, this approach helps document qualitative changes in the monsoon intensity during the geologic past. A more precise estimate of past monsoon changes can be obtained from the study of temporal variation in the stable oxygen isotopic composition of the selected benthic foraminiferal species.

The application of stable oxygen isotopic composition of benthic foraminiferal species to infer past monsoon changes, however, requires, understanding the influence of various physico-chemical factors on the stable oxygen isotopic composition. Previously, field and laboratory culture studies have shown that the changes in seawater temperature, salinity, pH,  $\text{CO}_3^{2-}$  ion concentration, etc., affect the stable oxygen isotopic composition of planktic foraminiferal species (Bé et al. 1977; Spero et al. 1997; Bijma et al. 1999; Saraswati, 2007). The studies further show that the relationship between oxygen isotopic composition and seawater parameters is species specific. Similar observations have also been made about the

benthic foraminifera. Such studies on benthic foraminifera are, however, limited and mostly done on deep water species. Further, in view of comparatively short life span of planktic foraminifera, possibility of significant seasonal isotopic bias is more in planktic foraminifera, as compared to benthic foraminifera, which live longer, spanning a few seasons and even a few years. But, recently, a few studies show that selected benthic foraminiferal species secrete the test preferentially during particular seasons, thus bringing in significant isotopic bias (Cage & Austin, 2008). Therefore, in order to get reliable paleoclimatic signatures, it becomes all the more important to understand the effect of various factors on the benthic foraminiferal oxygen isotopic composition.

Here, we have subjected two shallow water benthic foraminiferal species, viz., *Rosalina leei* (Hedley and Wakefield) and *Pararotalia nipponica* (Asano) to a combination of seawater salinity and temperature to understand the effect of these parameters on its stable oxygen isotopic composition.

## 9.2 Experimental Set-up

The juvenile specimens with initial 2-3 chambers were subjected to the experiment. The approach ensured that bulk of the carbonate was secreted under controlled laboratory culture conditions. The specimens of *Rosalina leei* were subjected to 25°C, 30°C and 35°C temperatures and 25‰, 30‰ and 35‰ salinities, whereas *Pararotalia nipponica* specimens were subjected to 25°C, 27°C and 30°C temperatures and 30‰, 35‰ and 37‰ salinities. Therefore, nine combinations of temperature and salinity were used for the experiment. The experiment was carried out in replicate. To begin with, all the specimens were at 25°C temperature and 35‰ salinity, as this was the temperature and salinity at the time of sampling. After three days, one set was left at the temperature and salinity reported from the field, while the temperature and salinity in other sets was changed according to the desired combination. The schematic of the experimental set-up is illustrated in Fig 9.1, 9.2. The salinity and temperature was changed gradually, in order to prevent a sudden experimental shock, which the organisms could experience if they were transferred directly to the respective intended salinity and temperature.

At the onset of the experiment, number of chambers and maximum diameter for each specimen subjected to the experiment were noted. The seawater with salinity

lower than that from the field was prepared by diluting the normal seawater with distilled water.

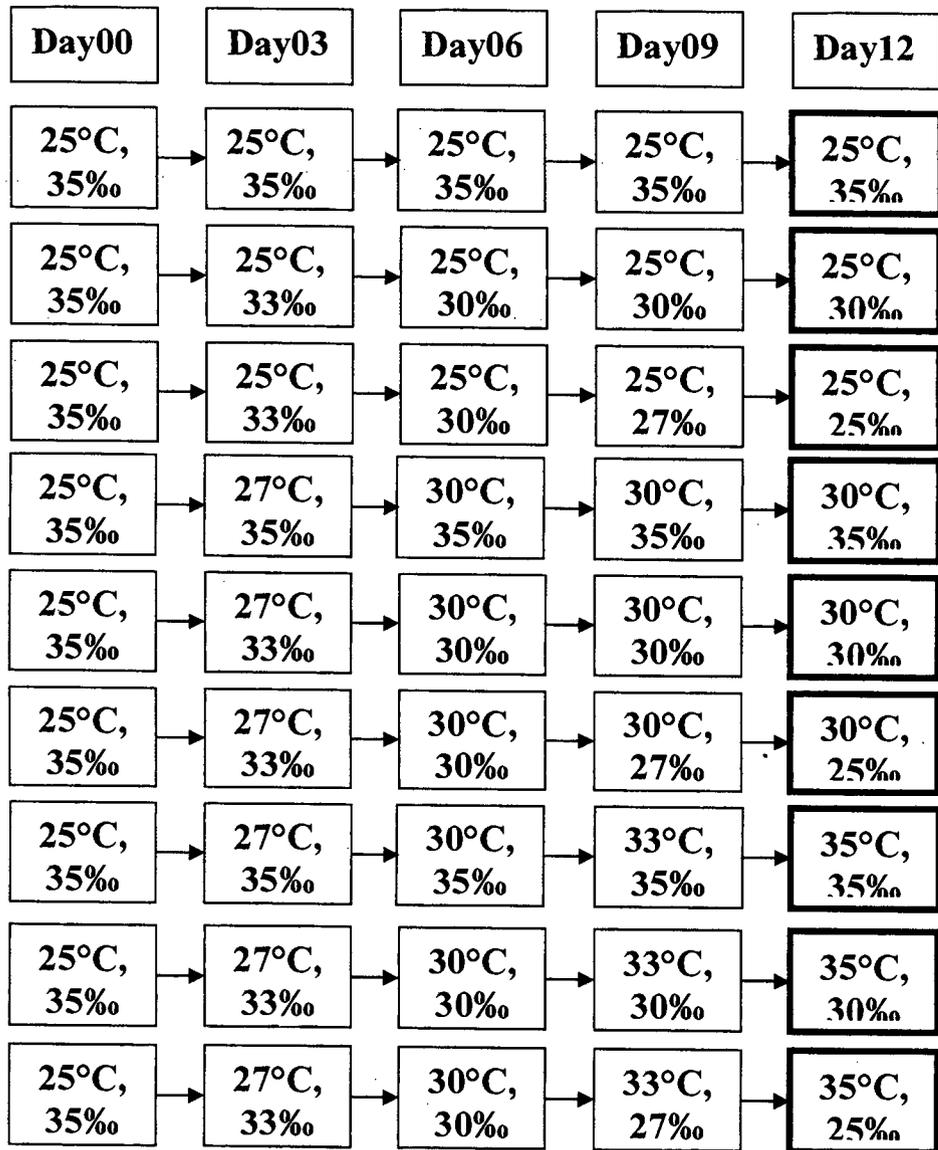


Figure 9.1: The experimental set-up as and how the salinity and temperature was changed for benthic foraminifera *Rosalina leei* (Hedley and Wakefield).

The higher salinity water was prepared by controlled evaporation at 45°C temperature. The seawater with different salinity was prepared in bulk, in the beginning of the experiment and kept in fridge to avoid evaporation. This seawater was used throughout the experiment, to avoid any change in the oxygen isotopic composition of the seawater. The culture dishes were wrapped in poly films to prevent evaporation. The salinity of the seawater was measured every time before

changing the media. The salinity of the used media was also measured in order to check for any evaporation. Equal amount of food in the form of *Navicula* sp. of diatoms culture was added in each culture dish, every time the media was changed. All the observations were made with the help of Nikon inverted microscope. The experiment was stopped when specimens were no more growing. The specimens were washed in distilled water and preserved for isotopic analysis.

The isotopic analysis was done at Leibniz-Laboratory for Radiometric Dating and Stable Isotope Research, Kiel University, Germany on Finnigan MAT 251 mass

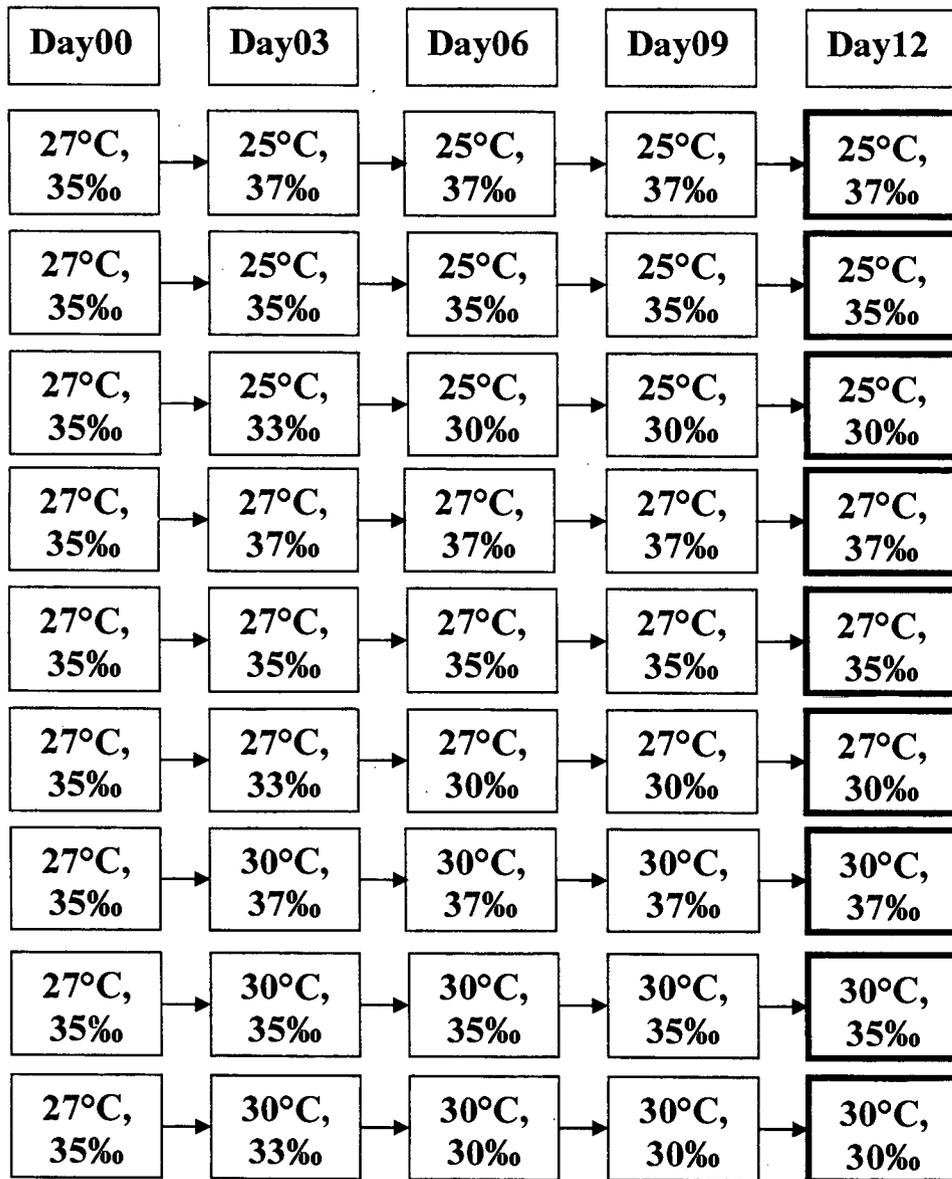


Figure 9.2: The experimental set-up as and how the salinity and temperature was changed for benthic foraminifera *Pararotalia nipponica* (Asano).

spectrometer. The precision of  $\delta^{18}\text{O}$  based on analysis of internal standard was  $\pm 0.07\%$ . The stable oxygen isotopic values are expressed with VPDB.

### 9.3 Results and Discussion

The stable oxygen isotopic values of *Rosalina leei* are relatively depleted than that of the *P. nipponica* (Table 1, 2). At 25‰ salinity, no relationship is noted between  $\delta^{18}\text{O}$  *R. leei* and seawater temperature (Fig. 9.3). At 30‰ salinity  $\delta^{18}\text{O}$  *R. leei* has a strong

Sr. No.	Temperature (°C)	Salinity (‰)	$\delta^{18}\text{O}$
1	25.0	25	-0.33
2	25.0	30	-0.58
3	25.0	35	-0.14
4	30.0	25	-0.39
5	30.0	30	-0.52
6	30.0	35	-0.40
7	35.0	25	-0.22
8	35.0	30	-0.39
9	35.0	35	-0.49

Sr. No.	Temperature (°C)	Salinity (‰)	$\delta^{18}\text{O}$
1	25.0	30	1.43
2	25.0	35	1.44
3	25.0	37	2.17
4	27.0	30	-0.05
5	27.0	35	0.59
6	27.0	37	2.08
7	30.0	30	0.45
8	30.0	35	0.10
9	30.0	37	0.51

negative correlation (0.97) with seawater temperature, whereas at 35‰ the relationship between  $\delta^{18}\text{O}$  *R. leei* and seawater temperature is highly positively correlated (0.96).

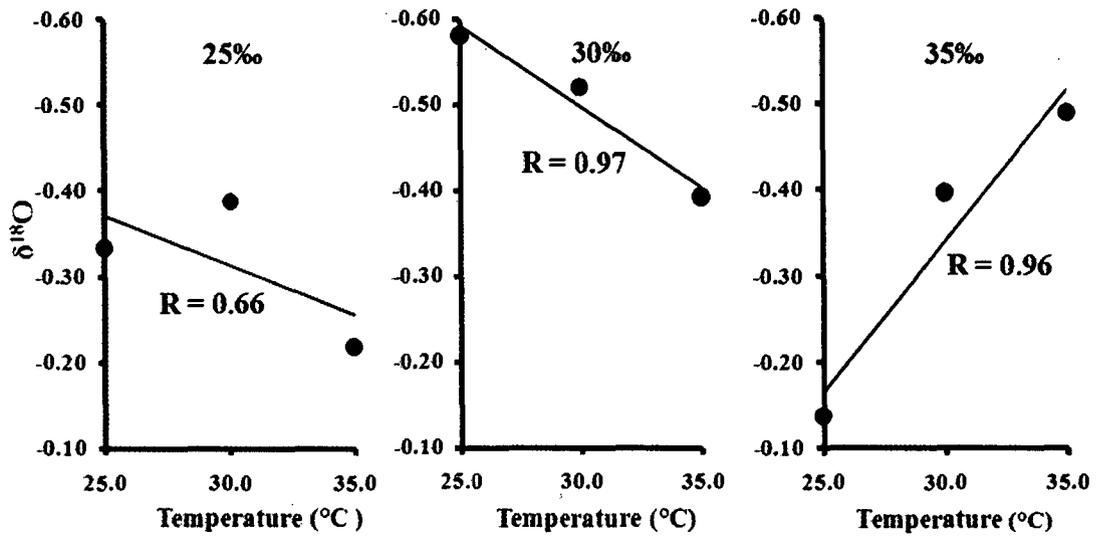


Figure 9.3: The relationship between seawater temperature and  $\delta^{18}\text{O}$  of *Rosalina leei* (Hedlev and Wakefield) at different salinities.

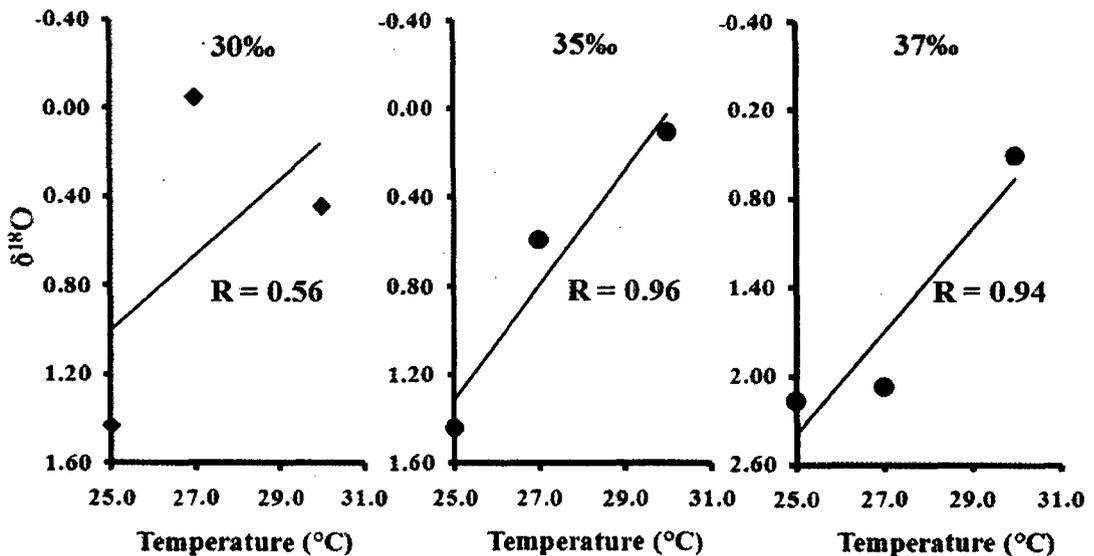


Figure 9.4: The relationship between seawater temperature and  $\delta^{18}\text{O}$  of *Pararotalia nipponica* (Asano) at different salinities.

In case of *P. nipponica* poor correlation is noted between  $\delta^{18}\text{O}$  and seawater temperature at 30‰ salinity, whereas at 35‰ and 37‰ salinity, the  $\delta^{18}\text{O}$  *P.*

*nipponica* and seawater temperature have a strong positive correlation (0.96 and 0.94, respectively) (Fig. 9.4).

$\delta^{18}\text{O}$  *R. leei* is poorly correlated with the seawater salinity in case of specimens subjected to 25°C and 30°C seawater temperatures. The  $\delta^{18}\text{O}$  *R. leei* is, however, strongly positively correlated with seawater salinity at 35°C temperature (Fig. 9.5). In case of *P. nipponica*, poor correlation is noted between  $\delta^{18}\text{O}$  and seawater salinity at 30°C seawater temperature, whereas at 25°C and 27°C seawater temperatures,  $\delta^{18}\text{O}$  *P. nipponica* is highly negatively correlated with seawater salinity (0.72 and 0.89, respectively) (Fig. 9.6).

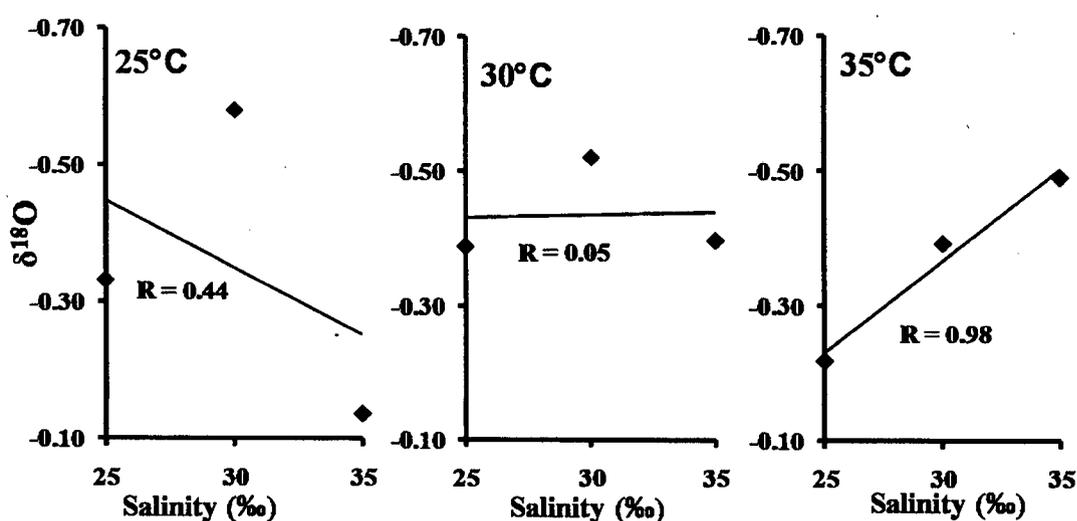


Figure 9.5: The relationship between seawater salinity and  $\delta^{18}\text{O}$  of *Rosalina leei* (Hedley and Wakefield) at different temperatures.

Note the positive correlation means increasingly depleted  $\delta^{18}\text{O}$  with increasing seawater salinity or temperature, whereas negative correlation means increasingly enriched  $\delta^{18}\text{O}$  with increasing seawater salinity or temperature. There is no consistent relationship between the stable oxygen isotopic composition and seawater temperature in case of *R. leei* (Fig. 9.3) but  $\delta^{18}\text{O}$  *P. nipponica* decreases with increasing seawater temperature at all the studied salinities (Fig. 9.4). The relationship between  $\delta^{18}\text{O}$  *P. nipponica* and seawater temperature is line with that observed in case of several deep water benthic as well as planktic foraminiferal species (Bijma et al. 1999). The slope of the line between  $\delta^{18}\text{O}$  *P. nipponica* and seawater temperature, however, varies with seawater salinity.

Similarly, the relationship between seawater salinity and  $\delta^{18}\text{O}$  also does not remain constant at different seawater temperatures. Only at 35°C temperature, very strong positive correlation (0.98) is noted between  $\delta^{18}\text{O}$  *R. leei* and seawater salinity, whereas in case of *P. nipponica*,  $\delta^{18}\text{O}$  and salinity show a strong negative correlation (0.89) at 27°C temperature. Interestingly, the seawater salinity does not result in any significant change in  $\delta^{18}\text{O}$  of both *P. nipponica* and *R. leei* at 25°C and 30°C seawater temperatures.

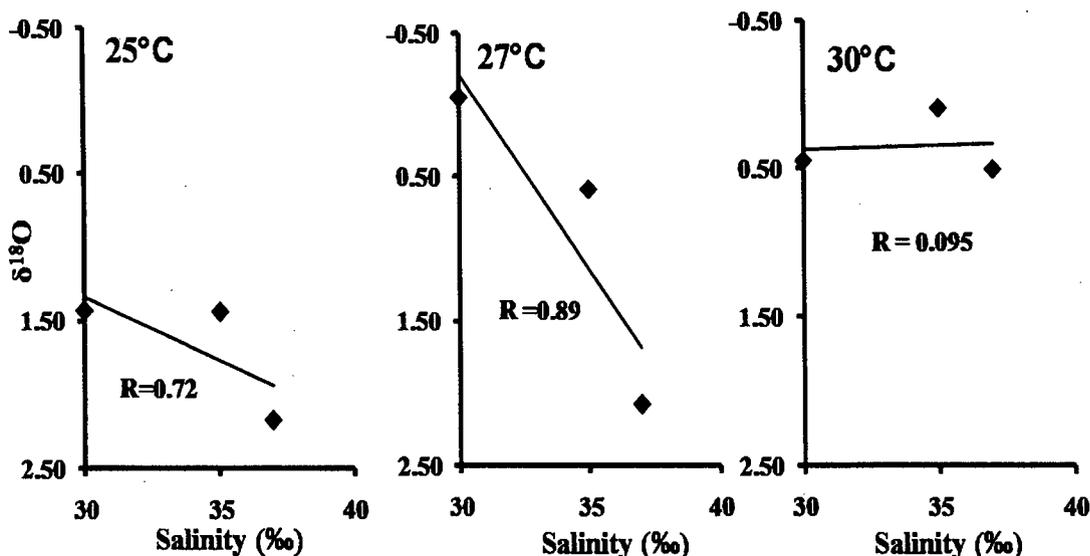


Figure 9.6: The relationship between seawater salinity and  $\delta^{18}\text{O}$  of *Pararotalia nipponica* (Asano) at different temperatures.

We have also calculated the expected  $\delta^{18}\text{O}$  calcite values by using the seawater salinity and  $\delta^{18}\text{O}$  seawater relationship of Delaygue et al. (2001) for the Bay of Bengal (this equation was chosen as it is based on a number of low salinity data points and the paleotemperature equation of Shackleton (1974)). The plot of expected versus measured  $\delta^{18}\text{O}$  values is very scattered for *R. leei* with a very poor correlation (0.02). The correlation, however, improves significantly (0.70), by removing one data point (25°C, 35‰) (Fig. 9.7). This seawater temperature and salinity combination is the extreme to which *R. leei* was subjected in this experiment. Therefore, it appears that the said extreme salinity and temperature combination might have lead to some adverse effect on the normal physiology of *R. leei*. The measured  $\delta^{18}\text{O}$  *P. nipponica* values are also significantly offset from the expected  $\delta^{18}\text{O}$  values. But the correlation between expected and measured  $\delta^{18}\text{O}$  for *P.*

*nipponica* is much better (0.77) than that for *R. leei*, with all data point plotted. Interestingly, though the measured  $\delta^{18}\text{O}$  *R. leei* values become progressively enriched when it is expected to be more depleted, the measured  $\delta^{18}\text{O}$  *P. nipponica* values are directly related with the expected values, but with an offset. The average offset of  $\delta^{18}\text{O}$  *P. nipponica* from the expected  $\delta^{18}\text{O}$  is -3.28‰ with a standard deviation of 0.58‰. A constant offset of measured  $\delta^{18}\text{O}$  foraminifera from the expected  $\delta^{18}\text{O}$  has previously been reported for a number of benthic foraminiferal species (Shackleton and Opdyke, 1973; Duplessy et al. 1984). It indicates that the relationship between  $\delta^{18}\text{O}$  *P. nipponica* and seawater salinity and temperature is different than that of the previously studied species. A more number of data point with several combinations of seawater salinity and temperatures are, however, required to precisely estimate the paleotemperature equation for *P. nipponica*. A very large standard deviation (1.18‰) in the offset of expected minus measured  $\delta^{18}\text{O}$  values for *R. leei* indicates that the oxygen isotopic composition of this species is not very reliable for estimating seawater temperature and salinity.

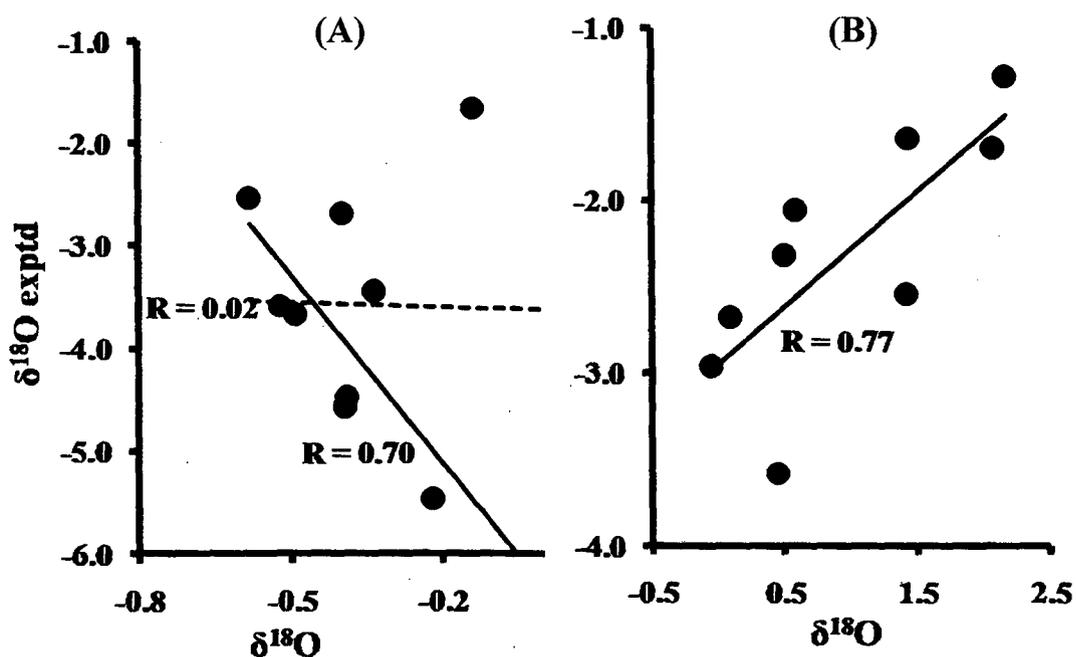


Figure 9.7: The plot of expected versus measured  $\delta^{18}\text{O}$  for *Rosalina leei* (Hedley and Wakefield) (A) and *Pararotalia nipponica* (Asano) (B). The correlation values are also given. For the plot of *Rosalina leei*, the dotted trend line is based on all the data points, while the solid trend line is based on all data points except one (-0.14, -1.38).

## 9.4 Conclusion

Based on the laboratory culture study wherein two shallow water benthic foraminiferal species, namely *R. leei* and *P. nipponica* were subjected to different combinations of seawater salinity and temperatures, we conclude that the stable isotopic ratio of the studied species shows no consistent relationship with seawater salinity. The relationship between  $\delta^{18}\text{O}$  foraminifera and seawater temperature is more consistent for *P. nipponica* than for *R. leei*. As compared to the seawater temperature, salinity appears to have little control on  $\delta^{18}\text{O}$  foraminifera, within the studied salinity and temperature range. The study shows that the changes in  $\delta^{18}\text{O}$  *P. nipponica* can potentially be used to infer past seawater temperature changes.

## Conclusions and Future Scope

### 10.1 Conclusions:

The present work as described earlier was carried out on live specimens of benthic foraminifera found along the Dias beach, which is located near the Dona Paula Bay, Goa. Effect of food, life span of few benthic foraminifers found in our study area, effect of single physico-chemical parameter, combination of parameter, effect of cyclic salinity changes and effect of temperature and salinity changes on  $\delta^{18}\text{O}$  was studied.

The present study is based on separation, identifications, maintenance and measurements of ~2000 live (no staining, live with pseudopodia) specimens of foraminifera.

The main findings of the present work are summed up as follows:

#### 10.1 A. Effect of food on growth and reproduction

- Average growth in *Cymballoporetta plana* (Cushman) increases with increased amount of food.
- Most of the specimens reproduced at 27°C temperature indicating that 27°C temperature is most suitable for growth and reproduction of *C. plana*.

#### 10.1 B. Deciphering the life span of a few species

- Life span of three different species of benthic foraminifera was noted. All the three species reproduced asexually in laboratory.
- Specimens of *Cymballoporetta plana* (Cushman) and *Discorbina concinna* (Brady) the juveniles formed within the parent cell whereas in *Spiroloculina* sp. juveniles were formed in the cyst built by the pseudopodial network.
- The life span of *C. plana* is of 45 - 55 days, *D. concinna* is of 22 - 25 days and *Spiroloculina* sp. is of 25 - 30 days.
- In case of *C. plana* and *D. concinna* number of juveniles show significant positive relationship between number of juveniles and size of parent test.

### 10.1 C. Foraminiferal response to salinity changes

This experiment was conducted on live specimens collected from the field and following observations were made:

- The effect of salinity variations was observed on benthic foraminifera *Rosalina leei* (Hedley and Wakefield). On the basis of this experiment it is concluded that *R. leei* can tolerate wide variations in salinity (from 25‰ to 80‰).
- At extremely lower salinities, the specimens of *R. leei* start dissolving. The dissolution starts from the last formed chamber and progresses towards earlier chambers of the tests.

### 10.1 D. Effect of temperature and salinity changes

These experiments were conducted on juvenile specimens reproduced in laboratory of *Rosalina leei* (Hedley and Wakefield), *Rosalina sp* and *Pararotalia nipponica* (Asano). They were subjected to different combinations of temperature and salinity and the following observations were made:

- In case of specimens of *R. leei*, at comparatively lower temperatures and higher salinities the growth rate of *R. leei* increases, but as the temperature increases and salinity decreases the growth rate also decreases. *R. leei* does not reproduce when subjected to seawater temperature higher than the optimum temperature (17°C - 20°C) required for reproduction. However, the specimens survived for longer time and significant growth was also noticed.
- Maximum average growth in case of *Rosalina sp* occurred at 30°C temperature and 25‰ salinity. Majority of the specimens subjected to this combination of seawater salinity and temperature also reproduced. Relatively less growth and reproduction was noted in case of specimens subjected to 25°C seawater temperature.
- In case of *Pararotalia nipponica* specimens the maximum average growth and reproduction occurred at 27°C temperature and at 35‰ salinity. Comparatively less growth was observed at temperatures higher as well as lower than 27°C and salinity lower than 35‰. Prolonged exposure of the specimens to lower salinity (25‰) no matter what temperature they were at, proved to be detrimental to these species.

### 10.1 E. Effect of cyclic salinity changes

Short-term salinity change experiment was conducted on benthic foraminifera *Rosalina leei* (Hedley and Wakefield) and *Pararotalia nipponica* (Asano), where in it was observed that:

- Lower salinity water (<20‰) leads to dissolution of the tests in both the specimens.
- After showing the dissolution at lower salinity when salinity was increased, they survived and showed recalcification. However most of such specimens developed morphological abnormalities.
- The result is significant and can be used to decipher such situations in past through the estimation of number of abnormal specimens in sediments from core (subsurface sediment samples).

### 10.1 F. Effect of temperature and salinity changes on $\delta^{18}\text{O}$

- Specimens of *Rosalina leei* (Hedley and Wakefield) and *Pararotalia nipponica* (Asano) were subjected to different combinations of seawater salinity and temperatures after which these specimens were sent for stable isotopic analysis.
- The relationship between  $\delta^{18}\text{O}$  foraminifera and seawater temperature is more consistent for *P. nipponica* than for *R. leei*.
- As compared to the seawater temperature, salinity appears to have little control on  $\delta^{18}\text{O}$  foraminifera, within the studied salinity and temperature range.
- The study shows that the changes in  $\delta^{18}\text{O}$  *P. nipponica* can potentially be used to infer past seawater temperature changes.

## 10.2 Future Scope:

Attempts have been made to study as much as possible within the limited time and infrastructure available to complete present thesis. In India, laboratory

culturing of benthic foraminifera is still in formative years. Some suggestions are listed below for the type of work that can be taken up in future.

- Life span and growth phases of few more benthic species found in our study area are to be studied.
- Stable oxygen isotopic studies, elemental analysis and molecular studies have to be carries out on different specimens cultured in laboratory under controlled conditions.
- Specimens from different areas and under different conditions are to be studied. Attempts to study the growth phases of deep sea benthic foraminifera have to be made.
- Effects of different type of pollutions on foraminifera need to be taken up to develop foraminifera as monitoring tool.

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### **Abstracts published**

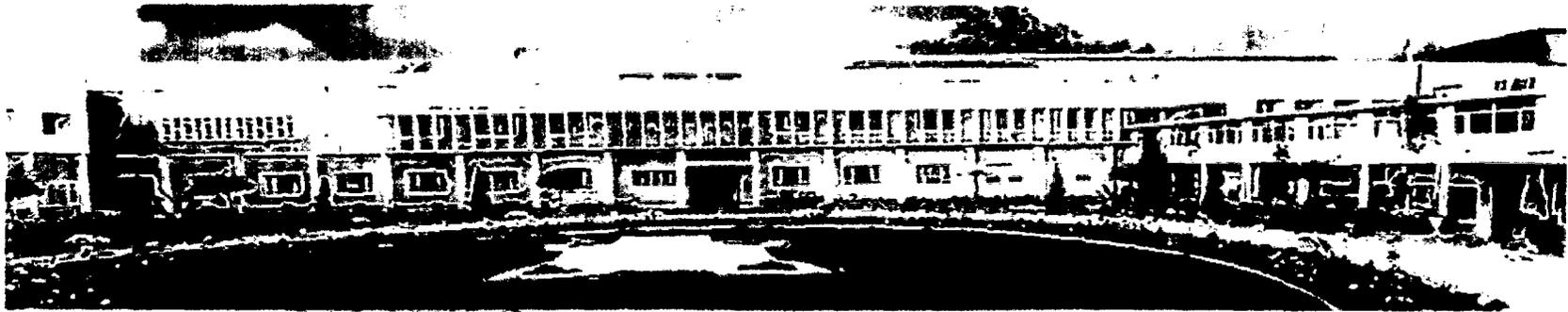
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(Rahul Garg)  
Convener