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## REGENERATION AND ABNORMALITY IN BENTHIC FORAMINIFER *ROSALINA LEEI*: IMPLICATIONS IN RECONSTRUCTING PAST SALINITY CHANGES

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**Key words:** Culture experiment, *Rosalina leei*, salinity, dissolution, regeneration.

**Abstract.** A laboratory culture experiment has been conducted to assess the response of marginal marine benthic foraminifer *Rosalina leei* to salinity and associated pH changes. Live specimens of *Rosalina leei* were subjected to a range (10-35 psu) of salinity. It was observed that hyposaline condition leads to dissolution of the calcareous tests. However, if the hyposaline condition persists only for a short period, then even after considerable dissolution, specimens were able to regenerate the dissolved part of the test. Additionally, in all the specimens subjected to lower than normal salinity, the regenerated chambers were abnormal. The abnormalities included smaller or larger chambers and addition of new chambers in planes different than the normal plane of the tests. The regenerated specimens, however, attained a final size almost equal to that of control specimens that were not subjected to hyposaline conditions. The differential response of *R. leei* was attributed to decreased seawater pH under hyposaline condition. The findings can help understand the increased abundance of abnormal specimens under ecologically stressed environments.

**Riassunto.** È stato compiuto un esperimento con cultura in laboratorio per verificare la risposta delle variazioni in salinità e pH di un foraminifero bentonico di ambiente marginale, *Rosalina leei*. Esemplari vivi di *Rosalina leei* sono stati sottoposti a variazioni di salinità, da 10 a 35 psu. Si è osservato che le condizioni isoaline portano alla dissoluzione del guscio calcareo. Tuttavia, se le condizioni isoaline persistono solo per un breve periodo, in seguito anche se vi è stata dissoluzione significativa, gli esemplari sono stati in grado di rigenerare la porzione di guscio dissolta. Inoltre, in tutti gli esemplari portati a salinità inferiore al normale le camere rigenerate avevano caratteri anormali, quali dimensioni variabili o aggiunta di nuove camere su piani diversi dal normale piano di avvolgimento. Gli esemplari rigenerati tuttavia raggiungevano una dimensione finale confrontabile con gli esemplari di controllo, non sottoposti alle variazioni di salinità. La risposta differenziale di *R. leei* è stata attribuita alla diminuzione del pH in condizioni ipoaline. Questi risultati possono aiutare nel comprendere il significato dell'incremento di esemplari in condizioni di ambiente sottoposto a stress.

### Introduction

Shallow marine waters are potential sites for high-resolution paleoclimatic studies, due to relatively higher sedimentation rate. Huge aeolian and riverine flux and proximity to the land lead to high sedimentation rates in the shallow marine water, especially in regions where rivers meet the ocean. Benthic foraminifera, preferentially marine microorganisms with a hard calcareous or agglutinated exoskeleton known as the test, are one of the most abundant groups of microorganisms in the shallow marine waters. They are very sensitive to the slightest changes taking place in the ambient environment and have preservation potential. Therefore, the characteristics of benthic foraminifera have often been used to reconstruct past climatic changes from the shallow water regions (Nigam et al. 1992, 1995; Nigam & Khare 1999; Robinson & McBride 2008; Rossi & Vaiani 2008; Kemp et al. 2009). In order to decipher past climatic changes from the benthic foraminiferal characteristics, it is necessary to understand the factors affecting benthic foraminiferal distribution in the shallow marine waters. Various factors including water depth, sediment type, organic matter flux, availability of oxygen, seawater temperature, salinity, distance from the river mouth, extent of bioturbation, etc. have been proposed to affect benthic foraminiferal distribution in the shallow water regions (Mendes et al. 2004; Bouchet et al. 2009; Murray 2006; Scott et al. 2001). Out of these several biological and physico-chemical factors affecting the benthic foraminiferal distribution in the marginal marine areas, fresh water runoff related salinity changes are especially effective in shallow water areas (Bouchet et al. 2009;



Fig. 1 - Location of sampling station.

Samir et al. 2003; 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.

We have monitored changes in salinity at a shallow marine location off Goa for a period of two years (Fig. 1). The location is directly affected by the fresh water influx from the nearby land during the monsoon season. Additionally, we also recorded the changes in seawater pH at the same location. It was noted that the hyposaline waters are relatively less alkaline (Fig. 2). We opined that the change in seawater pH as a result of decreasing salinity might be one of the causes for dissolved benthic foraminiferal tests reported from the shallow water regions. Therefore, we decided to understand effect of seawater salinity related pH changes on benthic foraminifera. It is difficult to understand the effect of only salinity changes on benthic foraminifera, from field studies as many factors operate and simultaneously co-vary in the field. The changes in benthic foraminiferal characteristics might be the result of any one or a combination of a few of the physico-chemical parameters changing along with salinity.

On the other hand, the laboratory culture studies can help to understand the foraminiferal response

to varying seawater salinity and associated pH changes. The results of such studies can then be applied to the sediments collected from the field. However, so far, very limited attempts have been made to understand the effect of salinity changes on benthic foraminifera, in laboratory culture (Bradshaw 1955, 1961; Stouff et al. 1999a, 1999b; Nigam et al. 2006, 2008). Such studies on benthic foraminiferal species from Indian waters can help to reconstruct past changes in Indian monsoon. The present laboratory culture experiment was carried out to understand the effect of hyposaline water on benthic foraminiferal species *Rosalina leei* and its capability, (if any), to overcome the adverse effects of hyposaline conditions. Here our objective is to find out the effects of seawater salinity and associated changes (pH) on the hard part of the foraminifera.

### Materials and Methodology

Samples containing live specimens were collected from the waters off Goa, where the salinity shows large seasonal fluctuation, varying from 11 psu to 36 psu (Fig. 1). The location has two major estuaries namely Zuari and Mandovi draining huge amount of fresh water during southwest monsoon, which leads to large-scale changes in the seawater salinity within short time period. The floating as well as attached (to rocks submerged in seawater) algal material was collected and transferred to plastic tub having filtered seawater. The algal material was shaken vigorously to detach foraminifera. After vigorous shaking, complete material was transferred on to the sieves of size 1000  $\mu\text{m}$  to get rid of extraneous material and subsequently over to 63  $\mu\text{m}$  sieve to remove finer material including clay and silt. The >63  $\mu\text{m}$  material was collected in beakers containing seawater and brought to the laboratory.

Live specimens of *R. leei* were picked under reflected light microscope. A total of six sets, each consisting of 6 specimens (total 36

Fig. 2 - Relationship between seawater salinity and pH as observed in the field.

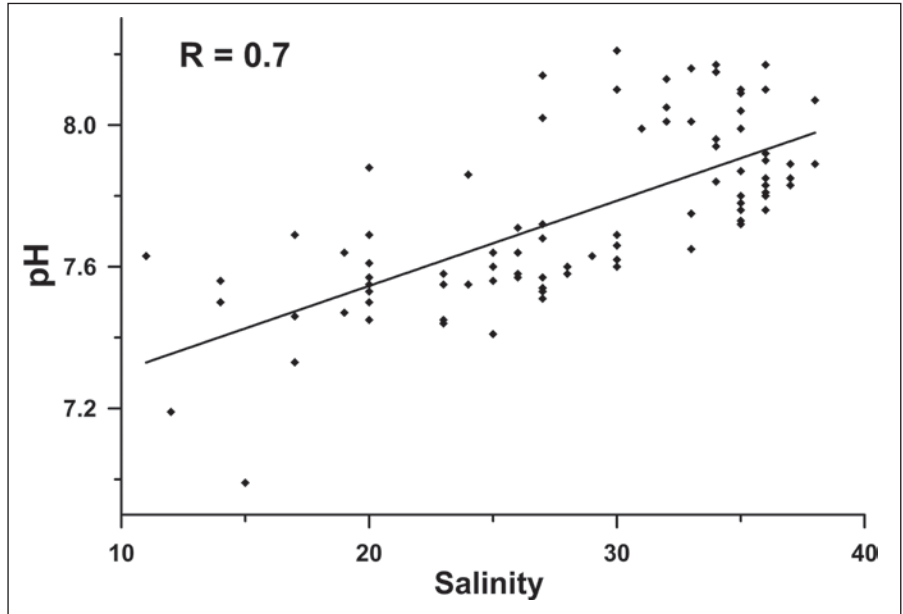
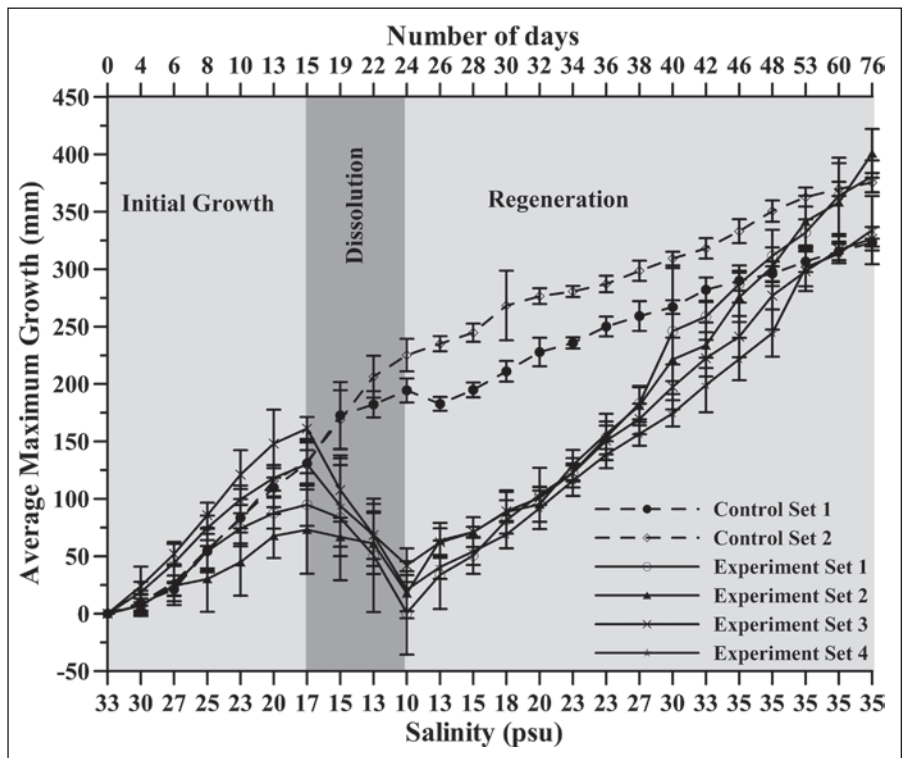


Fig. 3 - Changes in average maximum growth of control and treatment set specimens in response to salinity changes. The control sets specimens showed continuous growth as they were maintained at constant salinity (35‰). In experimental sets, growth took place initially and subsequently when the salinity was lowered below 17‰, the tests started dissolving, as evident from the downward trend in growth. Later on, when the salinity was increased again, the average growth also increased. The vertical lines are the standard deviation of growth as calculated from the growth between two consecutive readings.



specimens) of living *R. leei* were used for the experiment. Out of the total six sets, two sets were maintained at 35 psu salinity, the same salinity 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 psu) throughout the experiment. Remaining four sets of specimens, considered as experiment set (ES-1 to 4), were subjected to salinity varying from 10 to 35 psu. In each of the four experiment sets, salinity was gradually decreased from 35 psu to 10 psu, in steps of 3 and 2 psu, every second or third day. Once the effects of lower than normal saline water became evident, the salinity was increased gradually again, till it reached to the initial level (35 psu, same as at the time of collection 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 ex-

periment. In order to avoid evaporation, culture trays were wrapped in thin polythene film, immediately after changing the culture media. 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, with the help of LABINDIA  $\mu$ p controlled pH analyser. The seawater of different salinity was prepared either by diluting the seawater with distilled water (to get seawater with salinity lower than that of the field) or by evaporating the seawater at 40° C temperature (to get seawater with salinity higher than that of the field). The salinity was measured with ATAGO Hand Refractometer. Growth, abnormality of test, if any, and pseudopodial activity were observed every alternate day. Growth was estimated by measuring the average maximum diameter

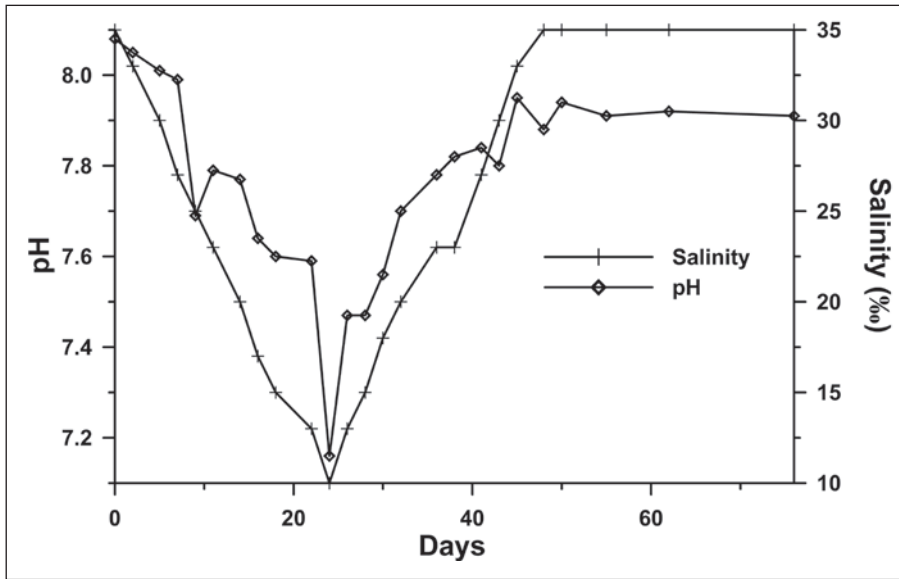


Fig. 4 - Relationship between seawater salinity and pH as observed for the media prepared in the laboratory. The pH decreased with the lowering of salinity.

of the specimen under inverted microscope (Nikon Eclipse TE 2000-U) connected to computer by using ACT 2U (Auto Camera Tame to you / utility) software. Though, occasionally, the tests were enclosed in a cyst made up of food provided to the specimens, the outline of the test was still visible. Therefore, even in case of specimen with test enclosed in cyst, it was possible to measure the diameter and thus the growth of the specimen.

## Results and Discussion

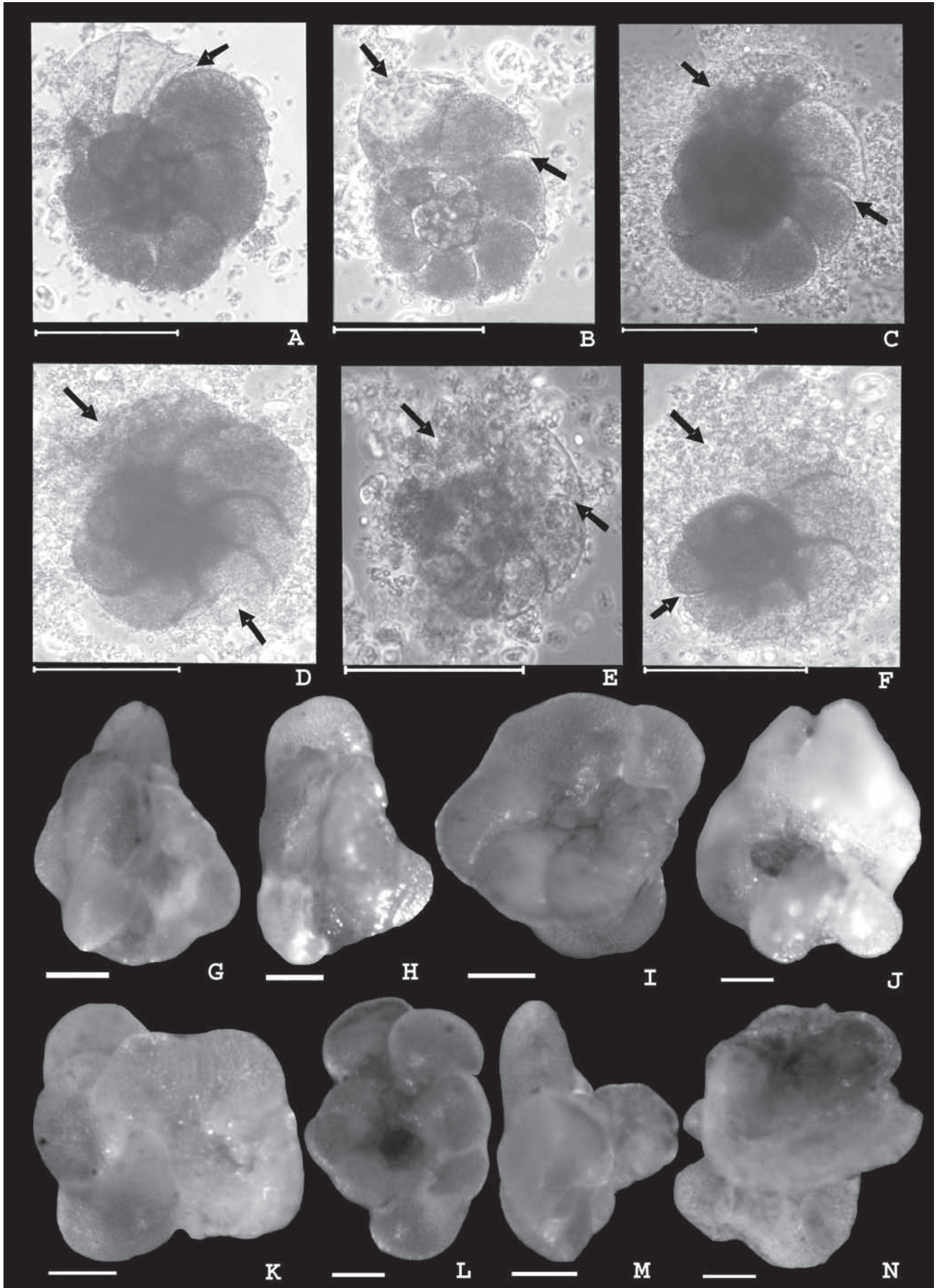
From the beginning of experiment till the salinity was lowered to 23 psu, all the specimens were very active showing visible signs of being alive. Growth was observed in all the specimens of both controls as well as treatment sets till 23 psu salinity (Fig. 3). Once the salinity was lowered below 23 psu, it resulted in decreased pseudopodial activity and dissolution of foraminiferal tests (Fig. 3). However, the effect was not uniform on all the specimens. Test of 8 specimens (2 in ES-1, 3 in ES-2, 1 in ES-3 and 2 in ES-4) started dissolving once the salinity was decreased to 20 psu. A further decrease in salinity to 17 psu resulted in dissolution in 9 more specimens (1 each in ES-1 and 2, 5 in ES-3 and 2 in ES-4). Test of additional 6 specimens (3 in ES-1, 2 in ES-2 and 1 in ES-3) started dissolving once the salinity was further lowered to 15 psu. One specimen showed visible signs of dissolution only when the salinity was decreased to 13 psu. All the specimens continued to dissolve till they were subjected to seawater of 10 psu salinity. The specimens were alive as evident from the collection of food but the pseudopodial activity was very limited. The dissolution started from the last chamber, however, only partial dissolution of chambers was noted. Outline of almost all the dissolved chambers remained visible (Pl. 1).

Although the response of specimens varied when the salinity was lowered, all the specimens started re-

building the test, once the salinity was increased from 10 psu to 13 psu. 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. 3). 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 (Pl. 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 the 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. Abnormalities in the case of treatment set specimens included bigger than normal chamber, disoriented chambers and differently shaped chambers. Not all the

## PLATE 1

Dissolution (A-F) and abnormalities (G-N) in *Rosalina leei* 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 (Arrows indicate dissolution in the specimens). Almost all of the specimens regenerated the dissolved chambers, but became abnormal (G-N). Abnormalities included addition of larger or smaller chambers, in planes others than the normal plane of addition of chambers.



specimens subjected to hyposaline conditions could recover; three specimens died during the experiment.

The dissolution of tests probably took place because of decreased pH of seawater, associated with the decreased salinity. It was observed that pH decreased with the decreasing salinity, both in case of media prepared in the laboratory (Fig. 4) as well as in case of observations made in the field as part of this study (Fig. 2) as well as previous reports (Brown et al. 1999). Earlier Boltovskoy & 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 psu salinity), different than the earlier observations. The dissolution in the present study started well above the pH value (7.0) reported by Le Cadre et al. (2003) for *Ammonia beccarii*. Though, Le Cadre et al. (2003) postulated that dissolution will start below 7.5, but the specimens were only subjected to seawater pH 7.5 and 7.0. No dissolution was observed in case of specimens subjected to seawater pH 7.5 and even the specimens subjected to pH 7.0 do not show any signs of dissolution till five days. The dissolution in case of *R. leei* at significantly higher pH value than that for *Ammonia beccarii* probably arises due to the comparatively thinner tests of *R. leei*. This study helped to refine the seawater pH value at which dissolution begins in *R. leei*, as against the large range proposed by earlier workers (Bradshaw 1961; Le Cadre et al. 2003). The study shows that the dissolution of calcareous foraminiferal tests can take place at much more 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 & Alve 1999; Kimoto et al. 2003). The change in carbonate ion concentration might have occurred due to the preparation of hyposaline water by adding distilled water. However, unfortunately we don't have any measured values for the calcium carbonate concentration of the differently saline media.

The series of stages followed during dissolution under low salinity in the present experiment (Pl. 1), starting with tests becoming slightly opaque and then dissolution starting from last chamber, has also been reported by Le Cadre et al. (2003), while observing the effects of low pH on *Ammonia beccarii*. Opaque tests have also been reported from field. 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 & Wright (1976) that foraminifera can repair and/or regenerate their tests after damage arising out of either physical injury or chemical effects. Decalcified living specimens, when cultured under favorable conditions, showed pseudopodial emissions and recalcification of chambers leading to morphological abnormalities (Stouff et al. 1999b; Le Cadre et al. 2003). The results are significant in view of the reported presence of abnormal tests near river mouths, where the possibility of breaking and later abnormal regeneration has been expressed (Vilela 2003). Geslin et al. (2002) also reported abnormal tests from areas exposed to periodic salinity changes and acidification. However, the abnormal tests were reported from areas subjected to hypersaline waters, whereas in our study only those specimens which were subjected to hyposaline water developed abnormalities during recalcification after partial dissolution of the tests. 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 significant finding of the present experiment was the abnormality in the chambers added during the regeneration of the dissolved tests. The added chambers were abnormally oriented away from the normal plane of orientation of the earlier chambers formed under normal conditions (Pl. 1). 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, which the *R. leei* specimens are unable to recuperate. Geslin et al. (2002) also suggested that regeneration after damage of tests may also induce high proportion of abnormalities in environments with strong hydrodynamics.

Interestingly, the size of specimens subjected to hyposaline condition was as big as that of control specimens, even after considerable dissolution of the test. It appears that the physiological activity of the specimens increased once they got favorable conditions after severe damage under hyposaline seawater.

Based on the laboratory culture experiment carried out to understand the response of inner shelf benthic foraminifer *Rosalina leei* to short-term decrease in salinity we conclude that lower than normal saline wa-

ter leads to partial dissolution of the tests. We further conclude that although *R. leei* is capable of recovering from short-term low salinity changes, the signatures of hyposaline conditions are retained by the test in the form of visible morphological abnormalities. 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.

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