# **RESPONSE OF BENTHIC FORAMINIFERA** *ROSALINA LEEI* TO DIFFERENT TEMPERATURE AND SALINITY, UNDER LABORATORY CULTURE EXPERIMENT

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#### ABSTRACT

Live specimens of benthic foraminiferal species Rosalina leei were subjected to a combination of temperature (25° C, 30° C and 35° C) and salinity (25‰, 30‰ and 35‰) to assess its differential response to the annual range of seawater temperature and salinity reported at the sampling site. A total of 216 specimens were used for the experiment. Within the range of temperature and salinity, to which R. leei specimens were subjected as part of the present experiment, growth increased with increasing salinity, whereas increase in seawater temperature resulted in retarded growth. Maximum growth was reported in the specimens kept at 25° C temperature and 35‰ salinity while rest of the specimens maintained in 30‰ and 25‰ saline water, showed comparatively less growth. 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. However, none of the R. leei specimens subjected to the present experiment reproduced during the course of experiment. The absence of reproduction under the present set of conditions, probably indicates that R. leei reproduces at a very narrow range of temperature and salinity which is different than the temperature and salinity conditions in the present experiment. It is further inferred that under the present set of temperature-salinity conditions,  $25^{\circ}$  C temperature and  $35_{\infty}$  saline water is most suitable for the growth of R. leei. Results are significant as the response of benthic foraminifera to different temperature and salinity are being used for paleoclimatic reconstruction.

### **INTRODUCTION**

Temporal variation in foraminiferal population and species assemblage are among the most extensively applied foraminiferal proxies for paleoclimatic and paleoceanographic reconstruction (Gooday, 2003). The applicability of such for a proxies for paleoclimatic reconstruction arises from the studies suggesting that under natural field conditions, reproduction in foraminifera is influenced by a number of ecological parameters, including food, temperature, salinity, pH, dissolved oxygen, etc (Boltovskoy & Wright, 1976; Murray, 1991; Gooday & Rathburn, 1997; Gooday, 2003). Thus, changes in the mode and rate of reproduction of foraminiferal species under different environmental conditions, lead to the variation in foraminiferal assemblages and population. Such changes in planktic foraminiferal population and species diversity have mainly been attributed to productivity and hydrographic changes (Pujol & Grazzini, 1995; Brunner & Biscaye, 2003). Additionally, planktic foraminiferal abundance and species assemblage is altered significantly during the sinking of planktic foraminiferal tests towards the sea-bottom, after the death of the organisms (Berger & Piper, 1972). The lunar cycle has also been shown to affect the reproductive cycle of certain planktic foraminiferal species (Bijma et al., 1990; Carstens & Wefer, 1992; Schiebel et al., 1995).

However, the changes in 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 bottom water oxygenation (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 affect the distribution, growth, and reproduction of foraminifera along the coastal areas (Boltovskoy & Wright, 1976). Pollution has also been reported as an important factor that significantly influences the benthic foraminiferal population, species assemblage and morphology, in the near-shore shallow water regions (Debenay et al., 2000; Chatelet et al., 2004; Nigam et al., 2006).

However, according to Bradshaw (1961), in area with imperceptible pollution, 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, 1999).

Increased information of the factors affecting the foraminifera can increase the reliability of paleoclimatic proxies that are based on temporal changes in foraminiferal abundance and species diversity. 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 (Bradshaw 1955; 1957; 1961; Nigam et al., 2006). However, laboratory culture studies to understand the effect of different temperature and salinity on different benthic foraminiferal species are limited.

Therefore, in the present study an attempt has been made to study the combined effect of temperature and salinity on the growth as well as mode and rate of reproduction of shallow water benthic foraminiferal species *Rosalina leei*. Since the reported range of seawater temperature over a period of one year was found to be between  $24.4^{\circ}$  C ( $24.8 \pm 0.4$ ) and  $31.7^{\circ}$  C ( $30.6 \pm 1.1$ ) (Table 1, this study; Rodrigues, 1984)) it was decided to subject the *R. leei* specimens to seawater temperatures within this limit.

#### **MATERIALS AND METHODS**

In order to pick live specimens, material (sediment and sea-grass) was collected from the coastal areas off Goa (Fig. 1), by using the methods suggested by Myers (1935) and Arnold (1954). Material for live specimens was collected from the shallow water regions off Goa. Both, floating as well as attached (to rocks submerged in seawater) seaweeds and algal material was collected and transferred to plastic tubs having filtered seawater. The seaweeds and algal material was shaken vigorously to detach foraminifera from the substrate. The complete material was then put over the sieves of size 1000  $\mu$ m and 63  $\mu$ m, in order to get rid of extraneous material and concentrate the foraminifera. The >63  $\mu$ m and <1000  $\mu$ m material was collected in glass beakers, along with seawater and brought to the laboratory for further processing. The foraminifera were isolated from the samples with the help of Stereozoom microscope, and transferred to multi-well culture dishes. When the isolated specimens responded through movement, collection of food material or extended pseudopodia, as observed under the inverted microscope, the specimens were confirmed to be live.

Once live specimens required to carry out the experiment were available, they were subjected to different combinations of temperature ( $25^{\circ}$  C,  $30^{\circ}$  C and  $35^{\circ}$  C) and salinity ( $25^{\circ}$ ,  $30^{\circ}$  and  $35^{\circ}$ ). Though, the *R. leei* has previously been reported to reproduce at 17-20° C (Hedley & Wakefield, 1967), seawater temperature in the present study area throughout the year, remains well above the reported range of seawater temperature for the reproduction of *R. leei* (Table. 1) (Rodrigues, 1984).

A total of 216 specimens were used for the experiment. Initially, all the specimens were kept at 25° C temperature and 35‰ salinity, as this temperature and salinity was reported at the time of collection of material for picking of live specimens. 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 desired combination. The schematic diagram of the experimental set-up is illustrated in Fig 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.

The number of chambers and maximum diameter for each specimen subjected to the experiment were noted in the beginning, and subsequently at a regular interval of eight days during the course of the experiment. Seawater with salinity lower than that from the field was prepared by diluting the seawater with distilled water. Higher saline water was prepared by controlled evaporation at 45° C temperature. Food in the form of mixed diatom culture was added every time the media was changed, i.e. every fourth day. All the observations regarding the growth, number of chambers and general physiological changes, including pseudopodial activity etc, were made with the help of Leica inverted microscope. The experiment was carried out in duplicate.

### **RESULT AND DISCUSSION**

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. 3, Table 1). Growth was observed even till 189 days and the average growth was ~55  $\mu$ m. The specimens maintained at 30‰ salinity showed growth only for 157 days with an average growth of 31  $\mu$ m and in case of the specimens kept at 25‰ salinity the specimens showed growth for 92 days with an average growth of 25  $\mu$ m. 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.

Out of the three sets of specimens kept at  $30^{\circ}$  C temperature and different salinities, specimens kept at 35‰ salinity showed the maximum growth (Fig. 3, Table 1). The growth was reported for 122 days and the average growth was noted to be around 18  $\mu$ m. Likewise the specimens maintained at 30‰ salinity showed growth only for 96 days with an average growth of 15  $\mu$ m and in case of the specimens subjected to 25‰ salinity, the growth was reported for 96 days with an average of 13  $\mu$ m.

In case of set of specimens maintained at  $35^{\circ}$  C temperature, specimens kept at 30‰ salinity showed maximum growth (Figure 3, Table 1). Growth was reported for 135 days and the average growth was noted to be around 17 µm. Similarly, the specimens at 35‰ salinity showed growth only for 159 days with an average growth of 12 µm and in case of the specimens kept at 25‰ salinity the specimens grew for 95 days with an average growth of 15 µm. 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 during the course of the experiment.

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 CaCO<sub>3</sub> is proportional to salinity of the seawater. Therefore, as the salinity decreases, the concentration of CaCO<sub>3</sub> also decreases which in turn lowers the growth of the foraminifera. In the present experiment maximum growth 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. The lack of reproduction under the present set of experimental conditions probably indicates that R. *leei* prefers a narrow range of seawater temperature and salinity for reproduction, which is different than the seawater temperature and salinity conditions in the present study. However, in another experiment, R. leei shown dissolution when subjected to 25 ° C and 15 to 10 ‰ (Kurtarkar, In preparation). Such response is significant for the application of benthic foraminiferal characteristics for paleoclimatic studies. Comparatively cooler as well as extremely warmer 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.

# CONCLUSION

With this laboratory culture experiment wherein live specimens of *Rosalina leei* were subjected to different combinations of temperature (25°C, 30°C and 35°C) and salinity (25‰, 30‰ and 35‰), it is inferred that:

- Out of the set of temperature-salinity conditions, 25°C temperature and 35‰ salinity appeared to be the best suited for the growth of *R. leei*.
- 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* prefers a narrow range of temperature and salinity for reproduction, which is different than the set of conditions in the present study. However, the specimens survived for longer time span, and significant growth was also noticed. The findings are significant as the response of benthic foraminifera to different temperature and salinity is often used for paleoclimatic reconstruction.

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### FIGURE AND TABLE CAPTIONS

Figure 1: Location of sampling station.

- Figure 2: The experimental set-up as and how the salinity and temperature was changed.
- Figure 3: Graph showing observed average growth in response to varying temperaturesalinity combinations.
- **Table 1:** The temperature salinity and pH during different times of the year, at the sampling station.
- **Table 2:** Average Initial and final size of the specimens of *Rosalina leei* along with the number of specimens subjected to experiment.

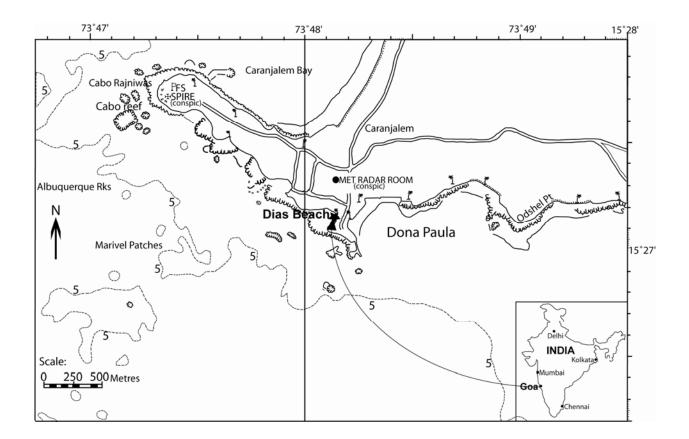


FIGURE: 1

Day 00	Day 03	Day 06	Day 09	Day 12
25°C,	→ 25°C,	25°C,	25°C,	→ 25°C,
35‰	35‰	35‰	35‰	35‰
25°C,	25°C,	25°C, 30‰	25°C,	→ 25°C,
35‰	33‰		30‰	30‰
25°C,	25°C,	→ 25°C,	25°C,	→ 25°C,
35‰	33‰	30‰	27‰	25‰
25°C,	27°C,	30°C,	→ 30°C,	→ 30°C,
35‰	35‰	35‰	35‰	35‰
25°C,	→ 27°C,	→ 30°C,	30°C,	→ 30°C,
35‰	33‰	30‰	30‰	30‰
25°C,	27°C,	→ 30°C,	→ 30°C,	→ 30°C,
35‰	33‰	30‰	27‰	25‰
25°C,	→ 27°C,	30°C,	→ 33°C,	→ 35°C,
35‰	35‰	35‰	35‰	35‰
25°C,	27°C,	30°C,	→ 33°C,	→ 35°C,
35‰	33‰	30‰	30‰	30‰
25°C,	→ 27°C,	→ 30°C,	→ 33°C,	→ 35°C,
35‰	33‰	30‰	27‰	25‰

FIGURE: 2

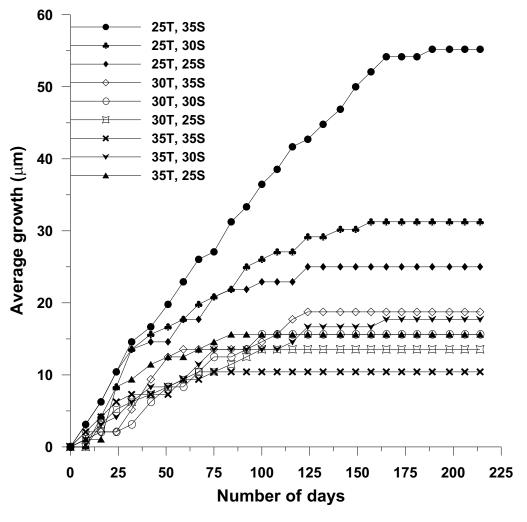


FIGURE: 3

TABLE 1	

	TEMPERATURE				SALINITY		РН			
Period	Pre-	Monsoon Post-		Pre- Monsoon		Post- Pre-		Monsoon	Post-	
	monsoon		monsoon	monsoon		monsoon	monsoon		monsoon	
1981-1983	$26.6\pm0.5$	$26.1\pm0.4$	$24.8\pm0.4$	$30.84 \pm 1.34$	$11.76\pm1.45$	$28.52\pm1.72$	$7.65\pm0.04$	$7.74\pm0.08$	$7.69\pm0.03$	
(Rodrigues,	to	to	to	to	to	to	to	to	to	
1984)	$30.2\pm0.3$	$29.2\pm0.2$	$30.6\pm1.1$	$36.57\pm3.91$	30.53±1.06	$34.00\pm0.77$	$8.14\pm0.09$	$7.89\pm0.07$	7.95±0.02	
2005-2007	27-31	26 (Aug)	26-30	33-37	10-35	32-36	7.90 - 8.22	7.20 - 8.04	7.83 - 8.01	

Premonsoon- February-May; Monsoon –June-September; Postmonsoon – October -January

Temperature and Salinity	25°C & 35‰	25°C & 30‰	25°C & 25‰	30°C & 35‰	30°C & 30‰	30°C & 25‰	35°C & 35‰	35°C & 30‰	35°C & 25‰
Average Initial Size (µm)	299	257	281	224	227	273	303	250	274
Average Final Size (µm)	354	289	306	243	243	286	316	268	290
Average Growth (µm)	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 2