JOURNAL GEOLOGICAL SOCIETY OF INDIA Vol.67, January 2006, pp.41-46

Laboratory Experiment to Study the Effect of Salinity Variations on Benthic Foraminiferal Species - *Pararotalia nipponica* (Asano)

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Abstract: Culture experiment has been carried out to observe the response of *Pararotalia nipponica* (Asano) to different salinities and its salinity tolerance limits. The specimens of *P. nipponica* kept in 33‰ saline water achieved optimum growth, while rest of the specimens maintained under either higher or lower salinities showed comparatively lower growth. *Pararotalia nipponica* specimens kept at 10‰ and 15‰ salinity started becoming opaque and later their test dissolved, within a span of 25 days. Towards the end of experiment in order to find the maximum salinity tolerance limit, the salinity was gradually increased to 100‰, in the culture dishes having specimens at 40‰ saline water and it was noted that *P. nipponica* specimens were alive even at 100‰ salinity. It is concluded that comparatively lower salinities are much more detrimental to the foraminiferal tests than higher salinities. Results are significant as the salinity response of benthic foraminifera is being used for environmental assessment.

Keywords: Benthic foraminifera, Salinity tolerance, *Pararotalia nipponica*, Culture Experiment, Retarded growth, Dissolution, pH.

INTRODUCTION

Application of foraminifers for paleoclimatic reconstruction as well as environmental assessment work relies on the state of preservation of foraminifers after death. Factors that are responsible for the complete preservation of the foraminiferal population include, composition of test, 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, 1999). Among the numerous changes brought by the fresh water influx, change in salinity is the most prominent (Liu et al. 2001). Thus by looking at the foraminiferal assemblage found in an area and its down core variation, one can infer salinity variations in the past. 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 about the specific response of the species or the assemblage of species, which are to be used to infer past salinity variations.

Various workers have investigated the effects of salinity on growth and overall size of foraminifers (see Boltovskoy et al. 1991, for review). Even though a vast amount of literature, based on field observations, is available on the effect of salinity on distribution of foraminifers 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. Not much work has been done in this direction (Bradshaw, 1961; Murray, 1963).

Here an attempt has been made to characterize the specific response of a widely occurring benthic foraminiferal species, *Pararotalia nipponica* (Asano, 1936), to different salinities and to find its salinity tolerance limits.

P. nipponica has been widely reported from the inner shelf region of world oceans. Matoba (1970) reported it from Matsushima Bay, northeast Japan, while Murray (1991) compiled its presence from western margin of Pacific Ocean with a salinity tolerance range of 33.1‰ to 34.9‰. P. nipponica was reported from the continental shelf and slope off West Africa by Debenay and Basov (1993). From Indian waters, Bhalla (1970) recorded this species from Marina Beach sands on the east coast. From the west coast of India,

P. nipponica was reported by Bhatia and Kumar (1976) at 5 to 13 m depth from Anjidiv Island near Karwar, in 28.5 to 30.1°C temperature and 33.49‰ to 35.01‰ salinity. Venkatachalapathy and Shareef (1976) recorded it from shelf area near Mangalore and Nigam et al. (1979) from shelf area off Ratnagiri, western margin of India within 50 m water depth. Rao (1998) reported it from the inner shelf sediments of the Bay of Bengal with the hypotype of 540 μm dimensions.

MATERIALS AND METHOD

Field Collection

Material for obtaining live specimens was collected from the waters off Goa, where the salinity shows large seasonal fluctuation varying from 11% to 36% (Rodrigues, 1984). The floating as well as attached (to rocks submerged in seawater) algal material was collected and transferred into plastic tubs having filtered seawater, and shaken vigorously to detach foraminifers from the algal substrate. Whole of the material was transferred on to the sieves of size $1000~\mu m$ to get rid of extraneous material and then over to 63 μm sieve. The plus 63 μm material was collected in beakers along with seawater and brought to the laboratory.

Laboratory Methods

Once the material was brought to the laboratory, live specimens of Pararotalia nipponica species were picked under reflected light microscope. The living nature of specimens was confirmed by observing their response such as movement, collection of food material, extended pseudopodia etc. under inverted microscope. Initially the specimens were collectively kept in 'Nunclan' multiwell culture dishes. Once they showed visible signs of being alive, they were individually transferred to different wells for the experiment. Single specimen was kept in each well of the six-welled culture dish. All the specimens kept in single tray, consisting six separate wells, were subjected to same salinity. Since the specimens collected from the field were directly subjected to the experiment therefore care was taken to select specimens of comparable size for all salinities, as far as possible (Table 1).

In the beginning all the specimens were subjected to seawater having salinity the same as at the time of collection from the field (33‰). Later, in order to avoid the salinity shock, which may be, if the specimens are directly transferred to different salinities, salinity was increased or decreased gradually to reach desired salinities. *P. nipponica* specimens were subjected to seawater of salinities varying from 10‰ to 40‰, at an interval of 5‰, keeping one set at the salinity as it was at the time of collection. All the culture trays were maintained at room temperature. Towards the end of the experiment in order to find the maximum salinity tolerance limit, the salinity was gradually increased to 100‰, in the dishes having specimens at 40‰ saline water.

Culture media of all sets was changed every alternate day. Food was added in the form of diatom *Navicula sp.* growth, abnormality of test, if any, pseudopodial activities etc. were observed every alternate day with the help of Leica inverted microscope. Table 1 exhibits the number of specimens used in each experimental set up.

RESULTS

Till 3-4 weeks after the start of experiment all the specimens including those kept at the low salinity of 10‰, and also those at a high salinity of 40%, were alive as evident from their pseudopodial activity. The differential response to salinity was reflected in different growth rates of specimens maintained at different salinities (Fig.1). The maximum growth attained was found to be strongly influenced by the salinity as the specimens kept at 33% salinity showed more growth than the specimens kept at both comparatively lower and higher salinities (Fig. 1). The period for which the growth took place was also affected by the salinity to which the specimens were subjected. Growth in case of specimens kept at 33% salinity continued for the maximum of 249 days, while in rest of the specimens growth ceased well before 200 days (Fig.2). The specimens maintained at 15% and 10% salinity grew only during the period of lowering of salinity from 33% to 15% and 10%. Growth of specimens almost ceased once the salinity of the media was lowered below 15%.

Table 1. Average initial and final size of the specimens of *Pararotalia nipponica* along with number of specimens subjected to experiment

Salinity	40‰	35‰	33‰	30‰	25‰	20‰	15‰	10‰
Average Initial Size (µm)	377	440	396	375	347	387	390	330
Average Final Size (µm)	403	480	452	405	373	413	407	340
Growth (µm)	26	40	56	30	26	26	17	10
Specimens kept under observation	6	6	6	6	6	6	6	6
Number of specimens responded	6	4	5	4	6	4	6	6

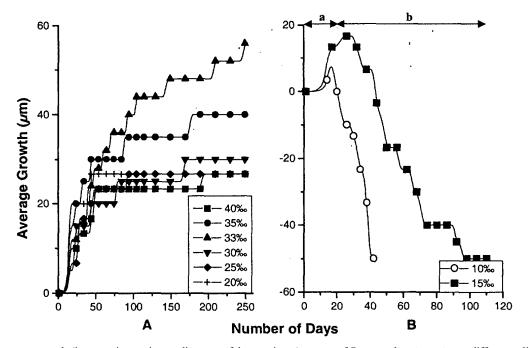


Fig.1. A. Average growth (increase in maximum diameter of the specimen) curves of *Pararotalia nipponica* at different salinities. The curves are stepped because of the limit of accuracy of readings. The symbols are put to distinguish growth curves at different salinities. Because of dissolution of tests, the curves for 10% and 15% salinities are given separately. B. The negative values indicate reduction in size of test with time due to dissolution. Here 'a' shows the increase in size from the start of experiment till the specimens were gradually transferred to 10% and 15% salinities, while 'b' shows the decrease in size (dissolution) after being transferred to 10% and 15% salinities.

The specimens whose salinity was lowered to 15% became opaque in appearance within three days of lowering of salinity to 15%. This phenomenon became more prominent in the specimens kept at 10% salinity. A few days later a peculiar feature, in the specimens that turned opaque, was noticed in the form of increased pore spaces in the last chamber. This resulted from the dissolution and rendered the chambers almost transparent. The chambers that became transparent started dissolving (Fig. 3). The dissolution initially progressed with one after the other chamber, starting from the last chamber, becoming transparent first and ultimately dissolving completely. But later, after the dissolution of last 4-5 chambers the whole specimen started dissolving at the same time. The dissolution was very fast in case of the specimens kept at 10% salinity, than those kept at 15% salinity.

Further progress of the experiment resulted in the death of specimens kept at 15% salinity after an average of 102 days from the start of experiment and within an average 91 days after bringing to 15% salinity, and that of specimens kept in 10% saline water after 54 days since commencement of experiment. Rest of the specimens were alive even after twelve months; they were showing pseudopodial activity

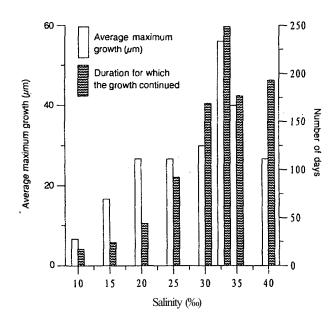


Fig.2. Graphs show maximum growth (increase in' maximum diameter of the specimen) attained and number of days taken to achieve maximum growth by *Pararotalia nipponica* at different salinities. The maximum growth attained is strongly correlated with the time taken to achieve this maximum growth.

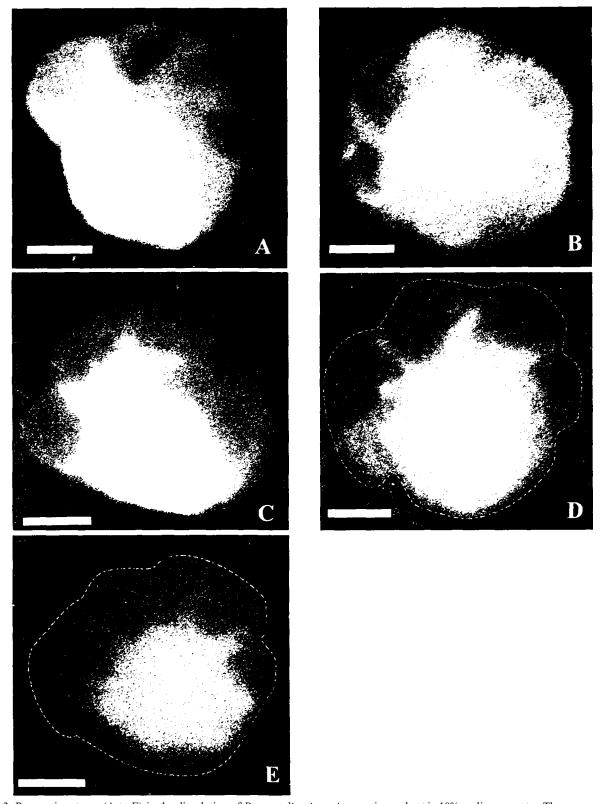


Fig.3. Progressive stages (A to E) in the dissolution of *Pararotalia nipponica* specimens kept in 10‰. saline seawater The progress of dissolution from last chamber towards proloculus is quite evident. The dotted line in figure D and E shows the original predissolution periphery The length of the white bar = $100\mu m$.

and were accumulating food but not growing. The specimens subjected to salinity up to 100‰, also survived but without showing any growth. No significant change was observed in the specimens subjected to up to 100‰ salinity, except for the negligible pseudopodial activity.

DISCUSSION

Salinity is one of the most important ecological factors that affect the foraminiferal population, especially in coastal areas (Sen Gupta, 1999). This has facilitated the use of foraminifers as tool to determine past salinity changes. However sometimes it is difficult to delineate effects of salinity on foraminiferal assemblages from the effects of other ecological parameters especially the temperature (Scott et al. 2001), tidal elevation, nutrients, oxygen, pH etc. The only solution to this problem is to observe the specific response of foraminifers to salinity in controlled laboratory culture conditions.

Therefore, in order to determine effects of varying salinity on the morphology and preservation potential of one of the common benthic foraminiferal species *Pararotalia nipponica* present in tropical world oceans including Indian coastal waters.

Relatively lower growth in the specimens of P. *nipponica* kept under hypersaline conditions in laboratory culture, in the present experiment agrees with the results of Stouff et al. (1999), 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 CaCO₃ 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-bused observations {see Boltovskoy et al. 1991, for review) as well as in laboratory culture experiments (Tappan, 1951;Bradshaw, 1961).

The dissolution and subsequent death of *P. nipponica* 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, 1999). Murray (1991) also noted that the dissolution is more in the water having low CaCO₃: a condition prevalent in low saline water, thus confirming the results of present study. The dissolution progresses through a series of steps in *P. nipponica*, beginning with the test becoming opaque,

followed by the last chamber turning transparent and gradually dissolving. Subsequent chambers follow the same pattern.

The presence of live specimens at 20% salinity, even after 12 months from the commencement of the experiment indicates that *P. nipponica* can tolerate salinities as low as 20% for a considerable duration. 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 *P. nipponica* can withstand- Similarly, the increase in salinity to as much as 100% did not result in any significant adverse effect as far as the morphology of the test is concerned. The capability to tolerate higher salinity for survival is reflected in occurrence of many species in hypersaline environment (Murray, 1991).

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 calcitic layer is added to the earlier formed chambers (Angell, 1967). This means that the wall of chambers keeps on thickening from last formed chambers, towards proloculus (first chamber). Therefore the chambers formed later dissolve earlier in comparison to the earlier formed chambers.

CONCLUSIONS

Based on the laboratory culture experiments, we conclude that one of the inner-shelf benthic foraminiferal species, viz. *Pararotalia nipponica* can tolerate wide variations in salinity ranging from 20% to 100%, under laboratory conditions. At lower salinities (<15%), the test of specimens of *P. nipponica* start dissolving as a consequence of lowering of salinity. The specimens kept at 33% salinity achieved the optimum growth.

Therefore, marked variation in the abundance of these species can be used as indicator of extremely hyposaline conditions arising most probably because of huge fresh water influx in past. Similarly the results can also be applied to use foraminifers to assess the extent and adverse effects of the discharge of water from industries, after being used as coolant.

Acknowledgements: The authors are thankful to Dr. S.R. Shelve. Director. National Institute of Oceanography, for his support and permission to publish this work. Dr. N.H. Hashimi and Mr. Abhijit Mazumder are thanked for review of the manuscript and valuable suggestions. We are thankful to Prof P K. Saraswati. NT Bombay, for helpful discussion on the subject matter of this paper. The help rendered by Mr. Shashank Harithsa in taking photographs is thankfully acknowledged. One of the authors (R.S.) is thankful to Council of Scientific and Industrial Research, New Delhi for granting Senior Research Fellowship.

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(Received: 8 March 2004; Revised form accepted: 11 February 2005)