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Effect of salinity induced pH changes on benthic foraminifera: a laboratory culture experiment

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Abstract

The coastal water pH varies with salinity. Therefore, to study the effect of salinity induced pH variations on benthic foraminifera, live specimens of *Rosalina globularis* were subjected to different salinities (10, 15, 20, 25, 30, 35 and 40 ‰) with pH varying from 7.2 to 8.2. A total of 210 specimens were used and the experiment was conducted in replicates. It was observed that the salinity induced pH changes affect the calcification of foraminifera. However the response is not linear. The maximum growth is reported in the specimens kept at 35 ‰ salinity (pH 8.0) while the rest of the specimens maintained at salinity higher or lower than 35 ‰, showed comparatively lesser growth. A significant drop in pH severely hampers the calcification capability of benthic foraminifera. Specimens kept at 10 and 15 ‰ (pH 7.2 and 7.5, respectively) became opaque within two days of lowering the salinity and later on their tests dissolved within 24 and 43 days, respectively. Besides calcification capability, pH also affects reproduction. No specimen reproduced at 10 and 15 ‰ salinity while only a few specimens (3 %) reproduced at 20 ‰. As compared to 10–20 ‰ salinity, ~ 60 % reproduction was observed in specimens subjected to 25–40 ‰ salinity. The drop in pH also decreased the calcification rate as specimens at 20 ‰ salinity took twice the time to reach maturity than normal range (25–40 ‰). We conclude that salinity induced drop in pH adversely affects the calcification capability and reproduction in benthic foraminifera. It is inferred that the time required to reach reproductive maturity increases at the extreme salinity tolerance limits. However, beyond a certain limit, a further increase in pH does not affect benthic foraminifera; rather they respond to salinity as per their salinity tolerance range.

1 Introduction

While the increasing open ocean acidification (OA) is the result of enormous input of CO_2 in the atmosphere by various anthropogenic activities (Caldeira and Wickett, 2003), local factors like fresh water runoff, coastal erosion, fertilizer input have also

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lead to the development of regions of increased ocean acidification (Doney et al., 2007, 2009; Cheung et al., 2009; Cooley and Doney, 2009; Kelly et al., 2011). Such shallow water OA zones are responsible for the declining fish catch, changing marine diversity and ocean ecosystem (Cheung et al., 2009; Doney et al., 2009). However, the capability of marine organisms to adapt to the increasing OA is not well known. Laboratory culture studies have shown that marine calcifiers will be adversely affected under increasing ocean acidification as decline in seawater pH hampers the capability of marine calcareous organisms to secrete calcite (Gattuso et al., 1998; Bijma, 1999; Marubini and Atkinson, 1999; Leclercq et al., 2000; Riebesell et al., 2000; Langdon et al., 2000). The response, however is not uniform as increased calcification under projected OA has been reported in case of a few taxa (Iglesias-Rodrıguez et al., 2008; Ries et al., 2009). The growth of a few non calcifying organisms also increases under high CO_2 conditions (Palacios and Zimmerman, 2007). Therefore the effect of OA on marine organisms has to be studied in detail. The benthic foraminifers, unicellular preferentially marine microorganisms are amongst the dominant group of organisms with calcareous exoskeleton (test), living in the shallow water regions of the world oceans. The foraminifers sequester huge amount of CO_2 and help in its removal from the atmosphere, thus helping in mitigating the effect of anthropogenic CO_2. It is expected that calcareous benthic foraminifers will also be adversely affected due to the increasing ocean acidification. However benthic foraminifera are capable of increasing the pH of vacuolized seawater at the site of calcification by one unit above seawater pH to secret calcite (Nooijer et al., 2009). The capability of benthic foraminifers to modulate the pH of vacuolized seawater under different seawater pH conditions is not known. Even the lowest pH that calcareous benthic foraminifera can tolerate is not clear (ter Kuile et al., 1989; Le Cadre et al., 2003; Kuroyanagi et al., 2009; Kurtarkar et al., 2011). It is expected that the pH tolerance range will vary from species to species as different benthic foraminiferal species are adapted to different habitats. Under such a scenario it is difficult to predict the response of benthic foraminifers to increasing ocean acidification. Controlled laboratory culture studies can help understand the response of benthic foraminifera to OA. Since riverine influx is one of the major factors for the development of localized zones of increased ocean acidification (Padmavati and Goswami, 1996; Kurtarkar et al., 2011), here we have studied the response of benthic foraminifera to salinity induced seawater pH changes, under controlled conditions in laboratory.

2 Materials and method

To understand the relationship between seawater salinity and pH, water samples were collected from the Mandovi and Zuari estuaries which bring freshwater to the shallow water coastal regions off Goa during the southwest monsoon season. The water was collected from the mouth of the estuaries till the inner reaches (to cover a wide range of salinity) by using Niskin water sampler. The water was transferred into narrow mouth 1 l bottles and kept in dark and its salinity and pH was measured on the same day by using autocell and pH meter.

To get live benthic foraminifera specimens, material including the top 1 cm of the sediments as well as sea grass attached to the perennially submerged rocky cliffs was collected from the waters off Dias beach, Goa coast. The sea water was also collected for further use in laboratory as media. The sea grass was transferred to a plastic tub containing sea water and shaked vigorously to detach foraminifera attached to it. The entire material was sieved through 1000 and 63 µm sieves to remove extraneous material. After thoroughly cleaning the sample, 63–1000 µm fraction was transferred to glass beakers with sea water.

In the laboratory, the material was scanned under stereo-zoom microscope (Olympus SZX16) to separate live benthic foraminifera. Probable live specimens were picked using a micropipette or a very fine tipped brush and transferred to multi-well (6-wells) culture petri-dishes (Axygen). The live specimens separated under binocular microscope were subsequently scanned under inverted microscope (Nikon ECLIPSE TE2000-U) for pseudopodial activity, movement, food collection, etc., to confirm their live status. Once confirmed to be live, the specimens were divided into several batches and kept at different ecological conditions (salinity varying from 20 to 40 ‰ and

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temperature varying from 15 to 30 °C) to facilitate reproduction. The offsprings of the reproduced specimens, that is the juveniles with two-three chambers, were subjected to different salinities. All the juveniles were taken from specimens kept at 35 ‰ salinity as maximum reproduction was observed at this salinity. The advantage of conducting experiments on juveniles is that there is ample time for the specimens to respond to physico-chemical conditions, before they mature and reproduce.

The experimental specimens were subjected to seven different salinities (10, 15, 20, 25, 30, 35, 40 ‰) with pH varying from 7.2 to 8.2. This salinity range was selected based on continuous monitoring of physico-chemical parameters in the field from where live specimens were collected (Rodrigues, 1984; Nigam et al., 2008), and expected possible change in salinity in future. Temperature (27 °C) was kept constant throughout the experiment. Five juvenile specimens were kept in each well of the 6-well culture petri-dish and thus each dish contained 30 specimens. Each well has a diameter of 35 mm, height 20 mm and the working volume of each well was 10 ml. One culture petri-dish was designated for a single salinity making a total of 7 dishes for the experiment. The experiment was carried out in replicate.

Initially, all the dishes were maintained at 35 ‰ salinity as the juveniles were taken from specimens subjected to this salinity. Subsequently, the salinity was changed gradually (in steps of 2/3 ‰) to reach desired salinity. The salinity was changed gradually in order to avoid experimental shock which specimen could experience if transferred directly to the required salinity (Fig. 1). On second day, salinity in one culture dish was maintained as 35 ‰ and other one was increased to 37 ‰. The specimens subjected to 35 ‰ were treated as the control set. On the same day the salinity in rest of the trays was lowered to 33 ‰. On 4th day, salinity of the media in the dish kept at 37 ‰ was increased to 40 ‰ and that of the remaining 5 sets was lowered to 30 ‰. On 6th day, salinity of the four out of five sets kept at 30 ‰ salinity was further lowered to 27 ‰. Likewise, the salinity was gradually lowered every alternate day until all the sets had desired salinity of 25, 20, 15 and 10 ‰. All the culture trays were covered with polythene cling-film to avoid evaporation.

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The media of different salinity was prepared by mixing water of salinity varying from 5 ‰ to 35 %, which was collected from the Mandovi-Zuari estuaries. Seawater of salinity > 35 ‰ was prepared by natural evaporation of 35 ‰ salinity seawater. The salinity of the media was measured by auto-cell as well as ATAGO hand-held salinity refractometer. The pH was measured by Labindia PHAN microprocessor controlled pH analyzer. Live diatom *Navicula* (100 ml solution, ~ 20 cells) was added as food, every time the medium was changed. This diatom species was chosen for food since foraminifers feed on diatoms and *Navicula* species has been reported from the area from where material for live specimens was collected. Media was changed every alternate day whereas pseudopodial activity, growth and maximum diameter was measured every fourth day. The experiment was stopped after 75 days when either the specimens had reproduced or died, or stopped growing.

During the course of the experiment, a few specimen developed abnormal tests. The growth of such abnormal specimens was not considered to calculate average growth. But, these abnormal specimens were monitored throughout the experiment, till they reproduced or died or stopped responding. Additionally, the chambers of a few specimens also broke in between. The growth of such specimens was also not considered to calculate the average growth. Since it was difficult to identify and track individual specimens kept in a well of the culture tray, average of the growth of all the specimens kept in each well was considered. Five specimens were kept in each well to facilitate sexual reproduction which requires pairing of specimens.

3 Results

3.1 Growth

The seawater pH decreases with decreasing salinity (Fig. 2). The relationship between salinity and pH of the seawater samples collected from the field matches with those prepared in laboratory. The slope of the best fit lines for the field and laboratory samples

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varies. The small difference can be attributed to the preparation of high salinity water by controlled evaporation as well as the mixing of waters of different salinity to prepare seawater with intermediate desired salinity intervals, in the laboratory.

A considerable growth occurred in all the sets during the initial 15–20 days (Fig. 3). It should however be noted that during this period salinity of the various sets was gradually changed to bring it to the desired salinity levels. The desired salinity levels in all sets were achieved after 20 days from the beginning of the experiment. A few of the specimens kept at > 20 ‰ salinity were alive till ~ 75 days. The specimens subjected to 10 ‰ salinity responded till only 45 days whereas those kept at 15 ‰ salinity responded till 63 days from the beginning of the experiment.

The average growth was highest (167 ± 10 µm) in specimens kept at 35 ‰ salinity (Fig. 3). The average growth of specimens subjected to 25 and 40 ‰ salinity (166 ± 16 and 164 ± 1 µm, respectively) was nearly same as that of the specimens subjected to 35 ‰ salinity (Fig. 3). Average growth of the specimens subjected to 30 ‰ salinity (151 ± 25 µm) was lower than that of the 25 ‰ salinity. It should however be noted that the during the later stages of the experiment, many of the specimens kept at 30 salinity reproduced. Such specimens were than not considered to calculate the average growth. As compared to this, less number of specimens kept at 25 salinity reproduced and most of the specimens were still growing, leading to higher average growth. The minimum growth (129 ± 10 µm) was observed in specimens subjected to 20 ‰ salinity. The specimens at 10 and 15 ‰ grew only when the salinity was still lowered to bring it to the desired levels. The growth in these specimens stopped immediately after attaining the desired salinity of 10 and 15 ‰.

The maximum diameter of any specimen at different salinity was also noted since the average growth was calculated from the live specimens and not all the specimens lived till the end of the experiment. The maximum diameter of the specimen subjected to 40 ‰ salinity was the largest (359 ± 40 µm), followed by that of 35 ‰ salinity (324 ± 24 µm) (Fig. 4). The maximum diameter of the specimen subjected to 10 and 15 ‰ salinity is irrelevant as these specimens grew only when the salinity was still lowered

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to reach desired salinity levels. Except 25 ‰ salinity, the maximum diameter varied linearly with the salinity.

3.2 Dissolution

Specimens at 10 and 15 ‰ became opaque and began to dissolve within 2 days of lowering the salinity. After a few days it was observed that the number of pores in the last chamber increased. This resulted due to dissolution which rendered chambers completely transparent. Those chambers which became transparent began to dissolve. Dissolution progressed from last chamber to initial chamber (Plate 1). Initially 4–5 chambers were dissolving one after another but later the whole test started to dissolve at a time. Dissolution was more prominent in specimen subjected to 10 ‰ salinity and within 24 days from lowering the salinity 60 % specimen died. As compared to this, out of all the specimen kept at 15 ‰ salinity, the tests of ∼ 23 % specimens dissolved with 39 days of lowering the salinity. However later on the rate of dissolution increased and the tests of ∼ 93 % specimen dissolved within next 11 days. The tests of all the specimen at 10 ‰ salinity dissolved by this time.

3.3 Abnormality

Abnormalities developed in several specimens subjected to various salinities (Plate 2) maintained in the experimental set up from the 6th to 11th day onward. The number of abnormal specimens was very low at 25 to 40 ‰ salinity, with only 2–3 specimens having abnormal chambers. The maximum number of abnormal specimens was noticed at 20 ‰ salinity, wherein a total of 10 specimens had abnormal chambers. After lowering the salinity, abnormal chambers were observed in 4–5 specimens at 10 and 15 ‰ salinity as well, before calcification stopped and shells started to dissolve. Specimens maintained at 15 ‰ salinity developed abnormalities after 17 days of lowering the salinity while those kept at 10 ‰ salinity had abnormal chambers within 2 days. The abnormalities included exceptionally large or small chamber and addition of chambers in a plane other than the normal plane of addition of chambers.

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3.4 Reproduction

Many of the specimens reproduced during the course of the experiment. Though paired specimens (prerequisite for sexual reproduction) were also observed in a few wells, none of such pairs reproduced, probably because not all the requirements for sexual reproduction were met. Thus all the specimens reproduced asexually. The specimens formed a cyst (of food material) prior to reproduction. The juveniles with three-four chambers came out by breaking the parent test (Plate 3). The percentage of specimens reproduced was comparable at 30, 35 and 40 ‰ salinity (60 ± 9, 60 ± 9 and 63 ± 5 %, respectively) (Fig. 5). Although the percentage of specimen reproduced at 40 ‰ salinity was same as that at 30 and 35 ‰, a few of the specimens reproduced abnormally. The number of juvenile in such abnormally reproducing specimens was less (only 5–8) as compared to other specimens (59 ± 9). As compared to this, only 40 ± 19 % of the specimens subjected to 25 ‰ salinity reproduced. The least reproduction was noted at 20 ‰ salinity (∼ 3 %). None of the specimen reproduced at 10 and 15 ‰ salinity. Out of all the juveniles, those at 35 ‰ have shown good growth.

The commencement of reproduction varied with salinity (17 to 66 days). The earliest reproduction (17 days) occurred at 35 ‰ salinity, while the specimens kept at 20 ‰ salinity were the last to reproduce (66 days). Average time taken for reproduction was comparable for both 30 and 35 ‰ salinity (24 and 25 days, respectively), whereas that for 40 ‰ salinity, it was more (29 days). The specimens kept at 25 ‰ salinity took still more time to reproduce (35 days). The majority of the specimens have reproduced by ∼ 38 days. Time taken for reproduction at 20 ‰ was twice that of specimens reproduced at higher salinities; by this time second generation was observed at higher salinities. All the specimens have reproduced asexually. There was no apparent relationship between average size of the specimen at the time of reproduction and salinity.

The average number of juveniles produced per specimen was highest (62 ± 8) at 35 ‰ salinity, followed by those at 40 ‰ salinity (59 ± 9) (Fig. 6). The average number of juveniles produced by the specimens maintained at 25 and 30 ‰ salinity was less

(35 ± 9 and 49 ± 7, respectively) than those at 35 and 40 ‰ salinity. The least number of juveniles per specimen were observed at 20 ‰ salinity.

3.5 Mortality

Out of all the specimens, 1 specimen each at 30 and 35 ‰ did not respond well. The one at 30 ‰ grew by 17 µm before it died after 31 days while the other at 35 ‰ died within 24 days after a growth of 3 µm only. Though initially all specimen showed good growth, the growth rate declined at all salinities after attaining a certain growth (Fig. 3). Further progress of experiment resulted in the death of 20 % specimen at 30 and 35 ‰ while ~ 13 % specimens died at 40 and 25 ‰ salinity (Fig. 7). It was observed that, after 40 days of attaining the desired salinity of 20 ‰, the specimen began to become opaque in appearance and ~ 7 % specimen died at 20 ‰. All the specimens (100 %) kept at 10 ‰ salinity died within 45 days while all those at 15 ‰ died within 63 days. The death of the specimens kept at 10 and 15 ‰ salinity was preceded by dissolution of the entire test.

4 Discussion

The freshwater influx from the land during monsoon season decreases the seawater salinity and pH in the shallow water coastal regions. This salinity induced pH change is one of the most important ecological factors that affect foraminiferal population especially in the coastal areas (SenGupta, 1999). The fact that *R. globularis* was alive at salinity ranging from 20 to 40 ‰ (pH 7.7 to 8.2) shows that it can tolerate wide range of salinity (pH). A similar response was also observed in corals which survived well when subjected to a range of aragonite saturation levels, which depends on the seawater pH (Gattuso et al., 1998). Immediate dissolution of the test of specimens subjected to 10 and 15 ‰ salinity (pH 7.2 and 7.5, respectively) puts the lower limit of salinity tolerance for this species at 15 ‰ (pH 7.5). The dissolution of the shells below 20 ‰ salinity

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was also observed in another species belonging to the same genus, i.e. *Rosalina leei* (Kurtarkar et al., 2011). Nigam et al. (2006) also reported dissolution and death of *Pararotalia nipponica* specimens below 15 ‰ salinity. In a field study from the seas around the island of Ischia (Italy) where volcanic gas vents emit carbon dioxide from the sea floor lowering the pH, no calcareous forms were reported in the region with seawater pH below 7.6 (Dias et al., 2010). Even the large benthic foraminifera, *Amphisorus hemprichii* and *Amphistegina lobifera* which are usually reported from coral reefs, show a significant calcification only up to pH 7.6 (ter Kuile et al., 1989). However, this limit is well above than that reported by Le Cadre et al. (2003) who observed decalcification of benthic foraminifer *Ammonia beccarii* at pH 7.0. A similar preferential syndepositional dissolution of calcareous tests as compared to the agglutinated forms has also been reported from shallow water regions of several places and was attributed to the fresh water influx, metabolization of organic matter and bacterial destruction (Murray and Alve, 1999).

The shells of *P. nipponica* are robust and thick walled as compared to that of both the *R. globularis* and *R. leei*. But all three of these are epifaunal species. It indicates that large freshwater influx will create localized zones of low seawater pH which will result in significant dissolution of all types of epifaunal benthic foraminiferal shells. Such large scale dissolution of benthic foraminiferal shells will however occur only if the low pH condition persists for longer time. Otherwise the benthic foraminifer are capable of recovering and rebuilding the shells after short-term exposure to low saline (low pH) conditions (Kurtarkar et al., 2011). Such short-term low pH events lead to abnormality in benthic foraminifera. The dissolution of the benthic foraminiferal shells, after prolonged exposure to 20 ‰ salinity indicates that hyposaline waters (low pH) are detrimental to the survival of *R. globularis*. However the response time vary from species to species. While the complete dissolution and death of all the specimens of both *P. nipponica* and *R. globularis* occurred within 25 days of lowering the salinity to 10 ‰, the *P. nipponica* specimens survived longer (∼ 100 days) than *R. globularis* (63 days) at 15 ‰ salinity. This difference in survival time at 15 ‰ salinity is probably due to the

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more robust shells of *P. nipponica*. The lower pH tolerance limit of benthic foraminifera (7.5) is comparable with that of the corals as reef building also stops at pH below 7.7 (Fabricius et al., 2011). The response of benthic foraminifera is also similar to that of pteropods which also start dissolving under low pH conditions (Orr et al., 2005). The calcite production at increased CO_2 concentrations (low pH) also declined in two dominant marine calcifying phytoplankton species, the coccolithophorids *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Riebesell et al., 2000).

The maximum growth was observed in the specimens maintained at 35 ‰ while growth was low in specimens kept at both the higher and lower than 35 ‰ salinity. Non-linear response to salinity induced pH change indicates that within a certain range of seawater pH, other factors (here salinity), affect the growth and reproduction (Nigam et al., 2008). A similar non-linear response of this species to a physical parameter was also observed when subjected to different temperature (Saraswat et al., 2011). A few other benthic foraminiferal species also show a non-linear response to seawater pH (Kuroyanagi et al., 2009). Bradshaw (1961) also reported that the highest growth rate was observed in cultures of benthic foraminifera *Ammonia beccarii tepida* at normal salinity (34 ‰) and that the growth rate decreased at lower salinity. Less growth at high salinity is in agreement with the results of Stouff et al. (1999) who found that *Ammonia tepida* specimens showed less growth when subjected to salinity higher than normal. A similar decrease in shell growth was also noted in modern planktic foraminifera collected in sediment traps deployed in the Southern Ocean as compared with the Holocene counterparts of the same species (Moy et al., 2009). The reduced planktic foraminiferal shell growth during modern times was attributed to the high CO_2 concentration as compared to the preindustrial levels. Even during older times, the foraminiferal shell weight varied with changing atmospheric CO_2 concentration (Gonzalez-Mora et al., 2008). Laboratory culture experiments on planktic foraminifera (*Orbulina universa* and *Globigerinoides sacculifer*) also show significant drop in calcification under high CO_2 (low pH) condition (Lombard et al., 2010).

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The maximum size attained by any specimen was the largest at the highest salinity, again showing effect of seawater salinity induced pH change on the benthic foraminifera. The highest saline water (40 ‰) has the most alkaline pH (8.2). It is easier for the benthic foraminifera to secrete shells under alkaline conditions. A similar response was also observed in another large benthic foraminifera *Marginopora kudakajimensis*, wherein maximum size was noted at highest pH (Kuroyanagi et al., 2009). The larger size, however, may also reflect growth of specimens without undergoing reproduction. Since reproduction occurs under narrow range of physico-chemical conditions, the specimens continue to grow without reproducing at conditions other than those favorable for reproduction, leading to a larger size. This explanation is supported by the larger size of the specimens subjected to 25 ‰ salinity as compared to those at 30 and 35 ‰ salinity. Here also, the specimens took longer time to reproduce as compared to the specimens subjected to 30 and 35 ‰ salinity.

The salinity induced pH change affected the normal growth of the specimens. The number of abnormal specimens increased with decreasing pH. A similar response has been observed in several benthic foraminiferal species collected from ecologically stressed environments (Boltovskoy et al., 1991). A similar response was observed in coccoliths wherein an increased proportion of malformed coccoliths and incomplete coccospheres developed under low pH condition (Riebesell et al., 2000).

The salinity induced pH also affects the reproduction in benthic foraminifera. Difficulty in calcification at salinity below 20 ‰ (pH $>$ 7.7), resulted in no reproduction. Reproduction first started in specimens subjected to 35 ‰ salinity followed by those at 30 and 40 ‰ salinity. Continuation of reproduction in specimens subjected to 25 ‰ salinity for longer time as compared to those at 30 ‰ or higher salinity was probably the effect of larger time taken to reach maturity by the specimens at 25 ‰ salinity. Specimens at 20 ‰ required an average of 75 days to reproduce. It shows that a critical minimum size has to be reached before the specimens could reproduce. This experimental result is supported by Bradshaw (1957), who reported that the time required to reach reproductive maturity by *Streblus beccarii (Linné) var. tepida* increased as the

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extreme tolerance limits were approached so much so that it required twice as long for each generation at lower salinity (13 ‰) than normal range (20–40 ‰). Comparable instances of reproduction in specimens subjected to 30 ‰ or higher salinity indicates that beyond a certain critical limit, salinity induced pH change does not affect the reproduction in benthic foraminifera. However, it affects the number of juveniles produced by each specimen, which was largest at 35 ‰ salinity. It was clear that decreased rate of calcification was responsible for less number of juveniles per reproduction, as only a few juveniles were reported in specimens that reproduced at 20 ‰ salinity. However, like the growth, beyond a certain critical limit, the number of juveniles per specimens does not depend on the pH but the salinity as evident from more number of juveniles per specimen at 35 ‰ salinity as compared to those subjected to 40 ‰ salinity. The adverse effect of hypersaline (40 ‰) as well as hyposaline (25 ‰) conditions on reproduction was also evident from the abnormal reproduction in a few specimens at these salinities. It was observed that at both 25 and 40 ‰ salinity a few specimens had some amount of protoplasm still left in the parent test even after reproduction. Such specimens also showed pseudopodial activity before decomposing. Bradshaw (1957) also reported that although, normally, reproduction terminates the life of the parent, occasionally some protoplasm showing pseudopodia activity may remain behind in the test for several days before decomposing. Additionally, although the percentage of reproduction in the specimens at 30 ‰ salinity was same as that at 35 and 40 ‰ salinity, the growth of new born juvenile was less as compared to the ones at 35 ‰ salinity. Death of a few specimens without reproduction is supported by the previous studies wherein it was found that foraminifera would not reproduce if all the conditions are not favorable, even though they may have reached maturity (Bradshaw, 1955, 1957).

5 Conclusions

– We conclude that the salinity induced pH change affects calcification in benthic foraminifera. However the response is not linear. Lowering the pH below a critical

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limit (7.5) severely hampers the capability of benthic foraminifera to secrete calcite. Specimens kept at 10 and 15 ‰ became opaque within two days and later on their tests dissolved within 24 days.

- Besides calcification capability, pH also influence reproduction. No specimen reproduced at 10 and 15 ‰ salinity while only a few specimens (3 %) reproduced at 20 ‰. As compared to 10–20 ‰ salinity, ~50 % reproduction was observed in specimens subjected to 25–40 ‰ salinity.

- The drop in pH also decreased the calcification rate as specimens at 20 ‰ salinity took twice the time to reach maturity than normal range (25–40 ‰). However, towards the higher side (more alkaline), the calcification does not vary with the increasing seawater pH, but was controlled by the seawater salinity.

- It is inferred that at extreme tolerance limit, time required to reach reproductive maturity increases.

The study shows the pronounced effect of seawater salinity induced pH changes on the growth, survival and reproduction in benthic foraminifera *Rosalina globularis*.

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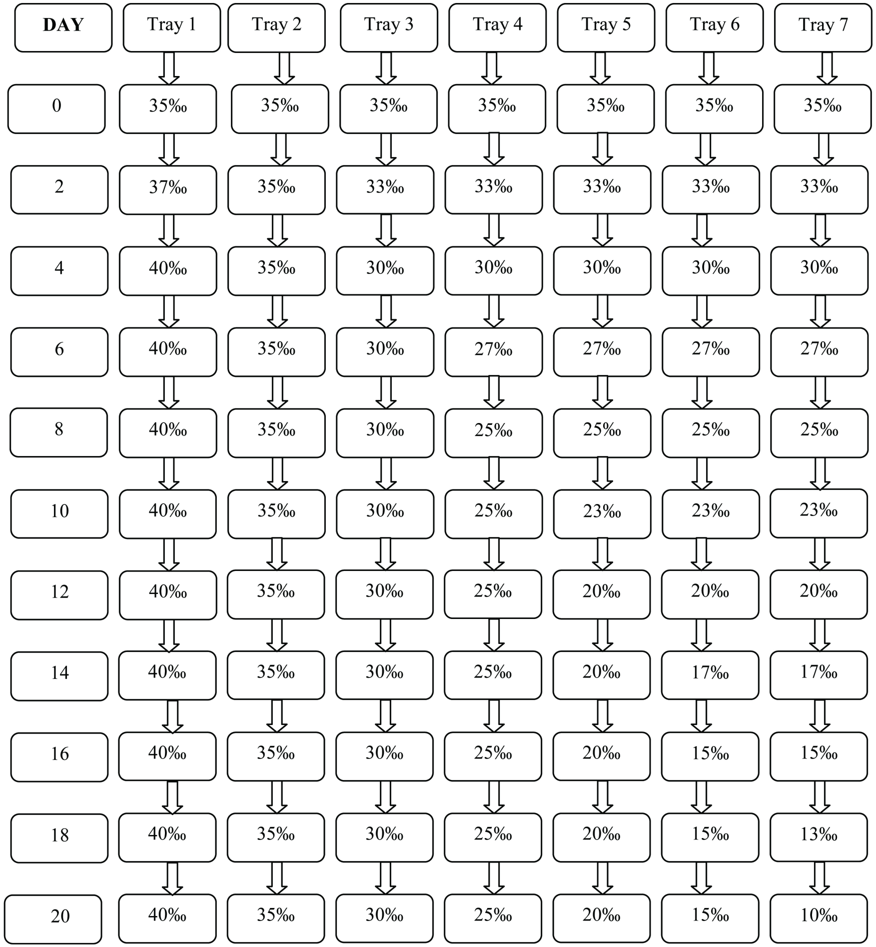

Fig. 1. Schematic diagram of the experimental set-up.

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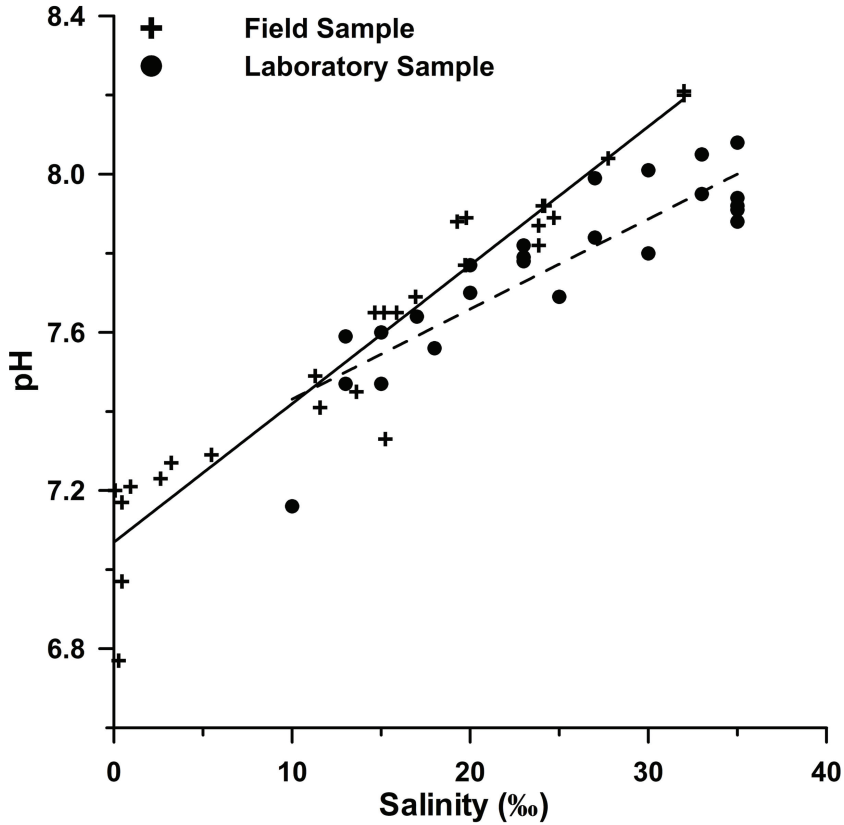

Fig. 2. Relationship between salinity and pH of the seawater collected from the field and that prepared in laboratory. The dotted line is the best fit for the laboratory seawater samples while the continuous line is that for the samples collected from the Mandovi-Zuari estuaries.

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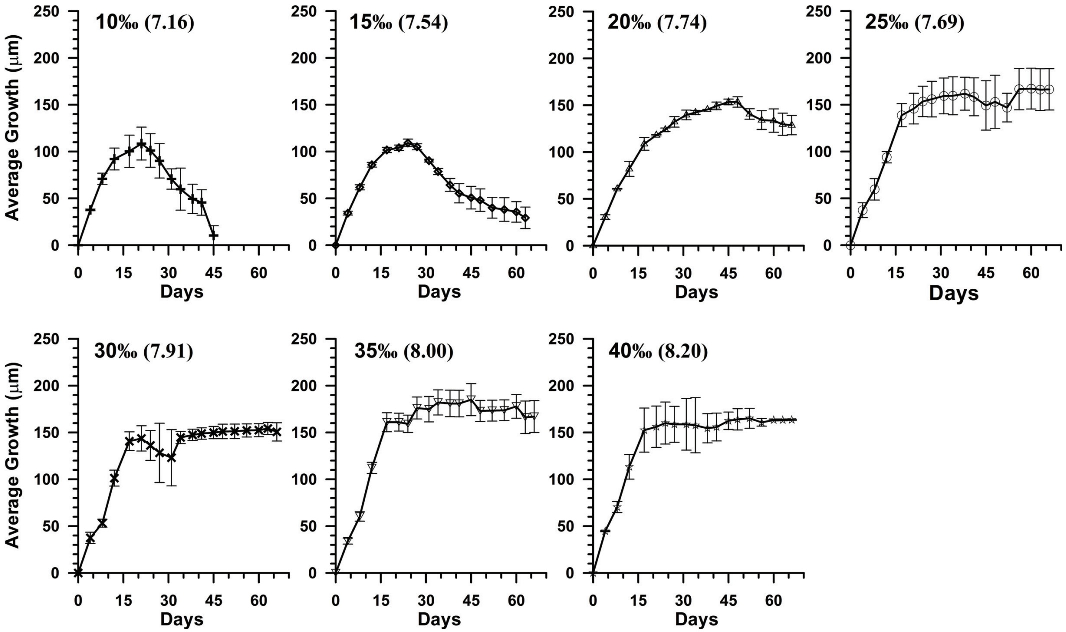

Fig. 3. Average growth at different salinities. The pH at respective salinity is given within brackets.

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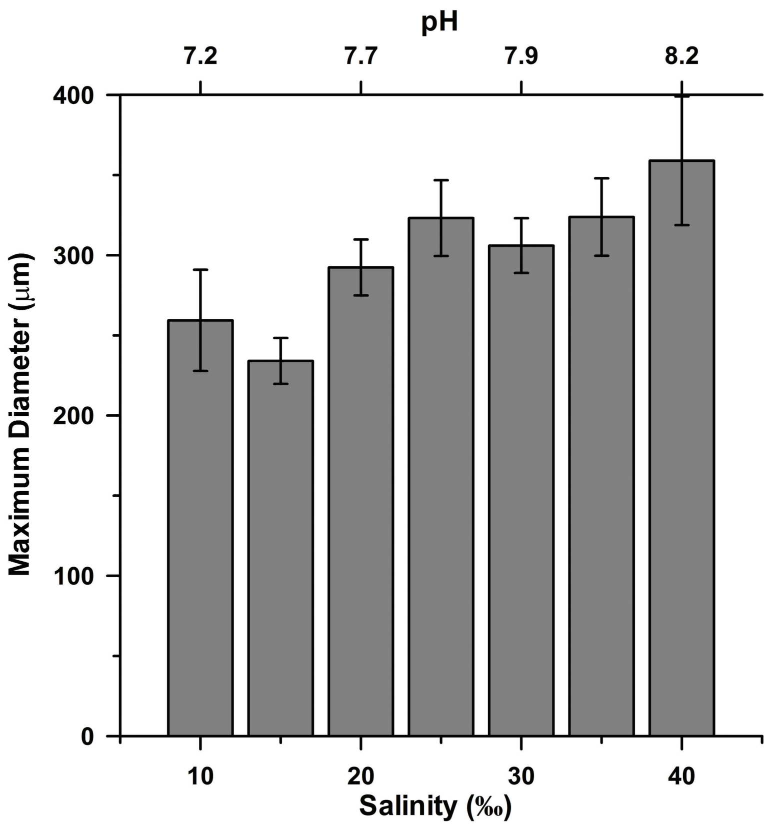

Fig. 4. Maximum size attained by *Rosalina globularis* at various salinities.

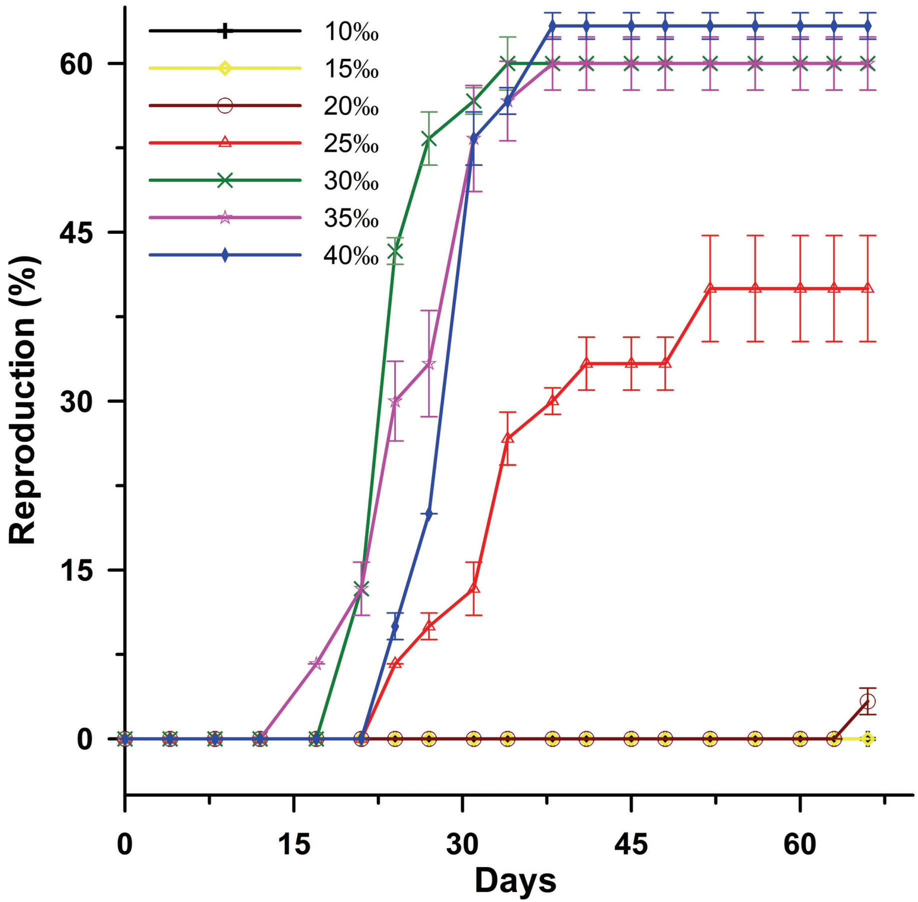

Fig. 5. Percentage of specimens reproduced at different salinities.

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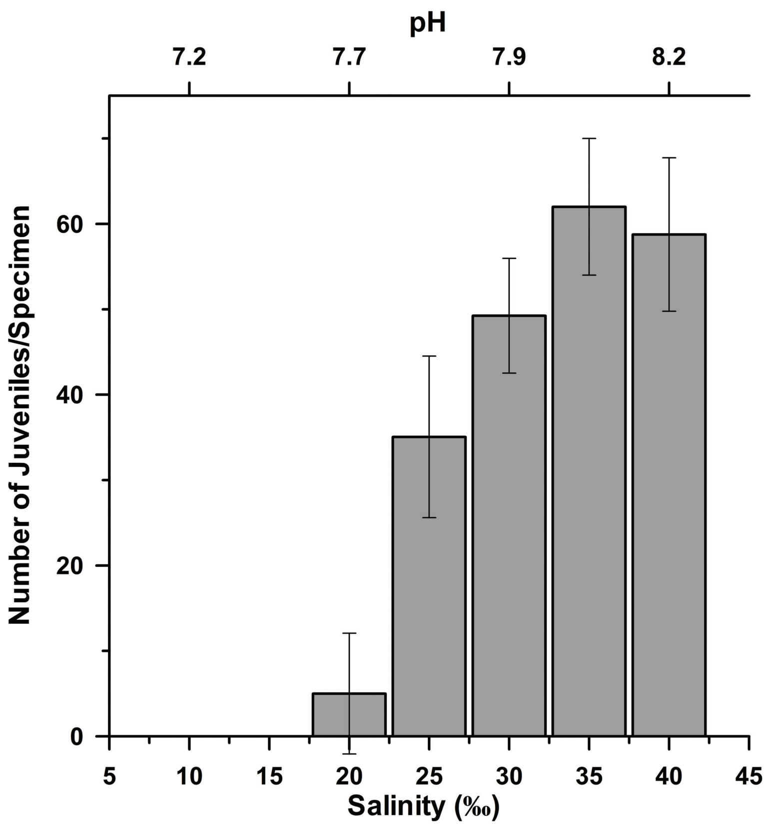

Fig. 6. Average number of juveniles per specimen at different salinities.

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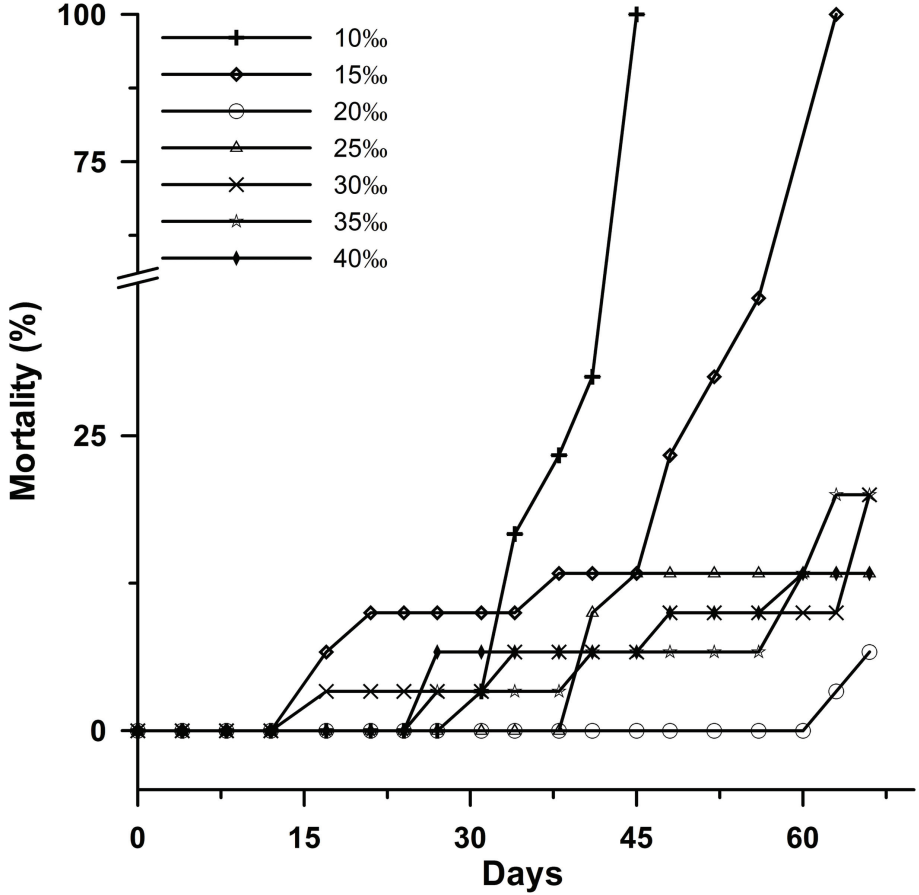

Fig. 7. Percentage of specimens died at different salinities.

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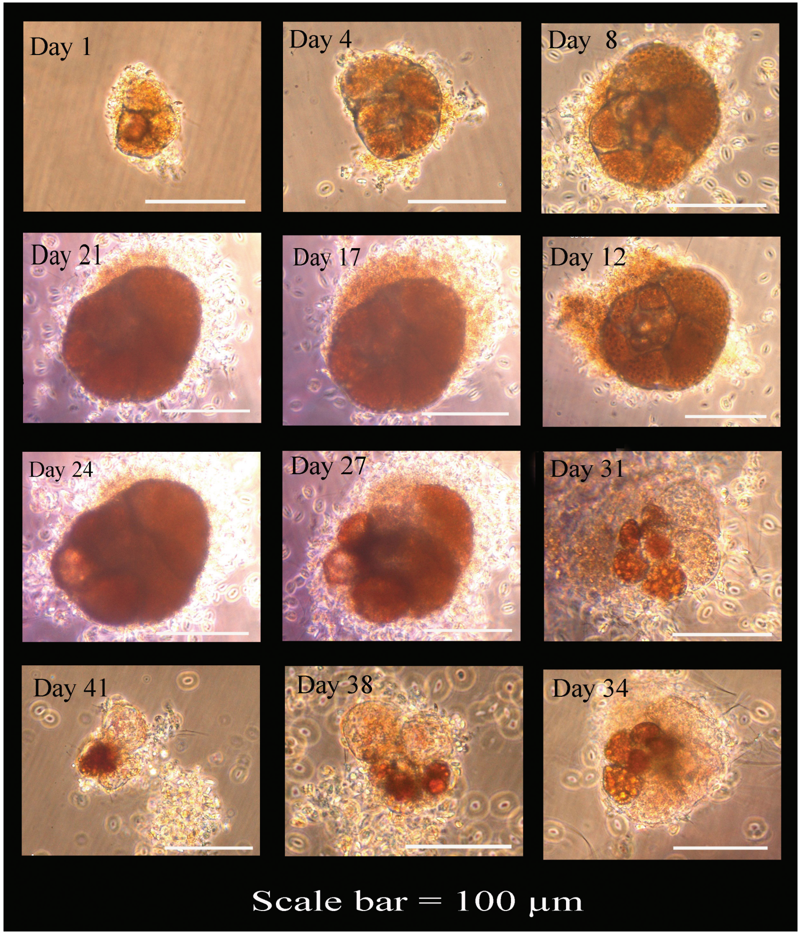

Plate 1. Progressive stages of growth and dissolution in *Rosalina globularis* specimens subjected to 10 ‰ salinity (scale bar = 100 µm).

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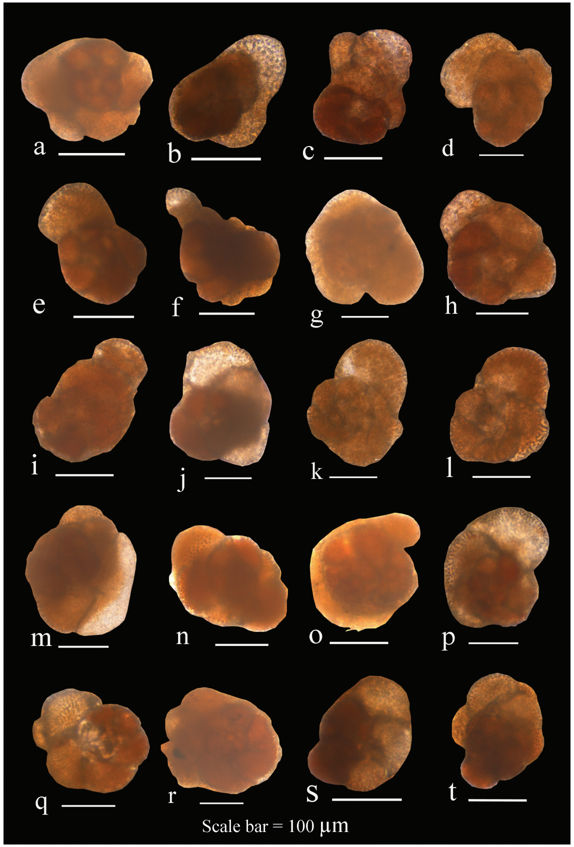

Plate 2. Abnormal specimens observed in *Rosalina globularis* specimens subjected to various salinities. **(a)** at 10‰, **(b–j)** at 20‰, **(k–l)** at 25‰, **(m–p)** at 30‰, **(q–r)** at 35‰ and **(s–t)** at 40‰ (scale bar = 100 µm).

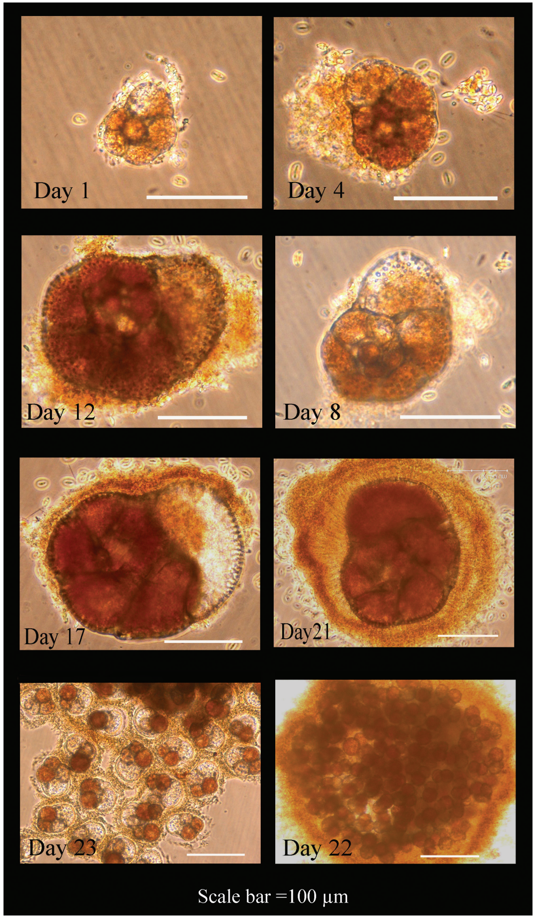

Plate 3. Different stages of growth and reproduction in *Rosalina globularis* specimen subjected at 35 ‰ salinity (scale bar = 100 µm).

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