CHAPTER 4

GROWTH AND REPRODUCTION IN BENTHIC FORAMINIFERA
Chapter 4

Growth and reproduction in benthic foraminifera

"The moment of enlightenment is when a person's dreams of possibilities become images of probabilities."

- Vic Braden

4.1. Introduction

Boltovskoy & Wright (1976) while listing the “unresolved problems” in their classical book “Recent foraminifera” stated “one of the urgent tasks in the future study of foraminifera is the investigation of living specimens, their reproductive cycles, their physiology, ontogeny and ecology. These studies must be conducted in laboratory cultures...” Even after one and a half decade, Lee & Anderson (1991) while writing introduction to book on foraminifera expressed their opinion that “...it has become clear to us, from our perspective as editors, that we really know very little about most aspects of foraminiferal biology and much further research is required on the life cycles of foraminifera to provide background information essential for classical and molecular genetic research.” Status has not changed much since then. The present chapter is designated to the laboratory studies on the growth and development of benthic foraminifera under laboratory conditions with special emphasis on Strebloides advena and Rosalina leei. The very basics of the foraminiferal biology and ecology are discussed in brief in the beginning of the chapter in order to make the terminologies and parameters used in the whole thesis/entire work on culturing understood.

4.2. The living organism

Although foraminifera are often thought of as simply being amoebas possessing an outer shell, there is more to them than that. The shell is commonly known as ‘test’ (hard part) which act as support and protection for the majority of the protoplasm that constitute the ‘living’ (soft part) part of foraminifera. The protoplasm of foraminifera contains all the organelles and inclusions typical of other animal cells as well as some of which are unique to foraminifera (Boltovskoy & Wright, 1976). The protoplasm renders a typical colour to the living organism (Fig. 4.1), which helps primarily distinguish the living from the dead foraminifera. If the wall is transparent the most frequent colours encountered are green, yellow, orange, brown and all intermediate shades.
This colour is due to the combined effect of protoplasm and symbiotic algae or the algae trapped for food. Colour variation may be caused due to nutrition variation and symbionts.

**Fig. 4.1: A bunch of living specimens of *Ammonia tepida* illustrating the typical protoplasmic coloration**

In multi chambered forms, the protoplasm can be differentiated in to a vegetative segment, which contains the food particles and food residues and comprises the last chambers and a chromatic segment, which contain the nucleus and other cell inclusions and occupy the initial chambers. The foraminiferal protoplasm can be divided in to internal protoplasm (endoplasm) occurring within the test and external protoplasm (ectoplasm) located outside the test in contact with the environment. The endoplasm is responsible for the general metabolic processes of the organism whereas the ectoplasm is intimately involved in the secretion of the test and most of the interchange between the organism and the environment occurs across it. It has the ability to simultaneously dissolve the calcareous material on its external surface and secrete calcareous test on its inner layer (Banner, 1971). The ectoplasm forms elongate pseudopodia, which give the organism the ability to move and attach itself. The length attained by the pseudopodia is variable.
These cytoplasmic threads, the thinnest of which are <1μm across, may reach a distance of several times to their test diameter. If a foraminifer is disturbed, the pseudopodia retract but re-emitted from the test within a relatively short time.

Fig. 4.2: A living 'Miliolid' foraminifera with extended pseudopodial network: Pseudopodia is visibly emitted from the apertural opening only

The imperforate foraminifera extend their pseudopodia from their aperture (Fig. 4.2). In the perforate forms, aperture is the primary orifice through which pseudopodia come out and pores may also serve this function (Fig. 4.3). The type of pseudopodia in foraminifera is not only branching, but the branches might also fuse. Such connections between branches are called anastomoses. This branching pattern creates a pseudopodial network. Granules occur within the pseudopodia and are transported along them. They can simultaneously move outwards and inwards in the same pseudopodium. The pseudopodia looks like a much branched network with numerous small granules, and is called granuloreticulopodia.

The pseudopodia not only provide surface for respiration, but also perform functions like feeding, locomotion, test building, metabolite release, adhering etc. The pseudopodia act as sensory devices warning the organism of nearby objects and changes in the chemical environment.
When comes in contact with food particles, the pseudopodia surrounds it and transport it to the ectoplasm. Within the pseudopod there are 2 currents simultaneously moving towards and away from the test (Boltovskoy & Wright, 1976).

Fig. 4.3: A living foraminifera 'Trochamina' with extended pseudopodial network: Pseudopodia is visibly emitted from all the pores

These currents carry the nutritive material to the endoplasmic layer and then in to the test either through the aperture or the other test perforations. The digestion of the nutrient particles may occur even as they travel toward the final assimilation in the cytoplasm. The indigested food particles are carried by the cytoplasmic currents and expelled from the test.

4.2.1. The test
The soft part of living foraminifera is enclosed within an external covering commonly referred to as test. The foraminifers differ from many other exoskeletal invertebrates (Boltovskoy and Wright, 1976) in that the soft parts of the organisms sometimes extend beyond the exoskeleton i.e. the test. The test is composed of one or more chambers. When more than one chamber is present the initial (first formed) one is generally the
smallest and is known as the proloculus. The chambers are separated by septa whose intersection with the test wall produces a line of contact or suture and the adjacent chambers are interconnected by an opening called the foramen. Hence the organisms are named “Foraminifera” meaning “foramen-bearing”. The primary orifice through which the protoplasm enters or leaves the test is known as aperture. The morphological characters of the test are used to distinguish one species from another. The three different views of a foraminiferal test with the main morphological features are given below (Fig. 4.4).

4.2.2. Composition of the test

Composition and construction of test walls are of great systematic importance in foraminifera. There are 4 distinctive types of walls:

- Chitinous: These walls are the most primitive. Fossil examples are not well known due to the ease with which erosive and transportive agents destroy this delicate material. It is actually not true chitin but a tectinous substance (combination of protein and various hydrates of carbon) with different characteristics. These walls are usually transparent and lack pores.

- Agglutinated: They are commonly composed of a layer of chitinous material capped by cemented detrital sediments. Both the cementing agent and detrital material constituting the agglutinated tests are highly variable. The agglutinating grains usually include quartz, mica plates, coal, volcanic glass, rutile, ilmenite, feldspar, tourmaline, zircon, amphibole, sponge spicules, diatoms, mollusk fragments, coccoliths and even foraminiferal fragments, which form a tightly interlocking mosaic with spaces between them filled with finer grains and cement.
Calcareous: Both calcite (hexagonal) and aragonite (orthorhombic) forms of calcium carbonate is used for construction of calcareous walls. Whether a foraminifera will possess calcite or aragonite wall will entirely depend on the phylogenetic origin of the species rather than ecology.

4.2.3 Factors affecting the life process of benthic foraminifera:
A large number of interrelated factors influence the vital activity of foraminifera and their distribution; a brief description is given below.

- Temperature: This is the primary factor which controls the geographic distribution of species, and is an important factor affecting vertical distribution. The range of temperature fluctuation is probably as important as the absolute temperature. It has a great deal of control over the vital activities of foraminifera. Each species has certain tolerance limits as well as a temperature optimum. A species can only tolerate temperature extremes for a short period. The sea water temperature influences the test size and morphology of the test. Although the majority of observations indicate that increased size is a result of lowered temperature, this relationship is not perfectly understood.

- Salinity: The sea water salinity also affects the geographic distribution of foraminifera. Because of the relatively uniform distribution of salinity in most of the open ocean, this effect is much more pronounced in areas where there is considerable mixing of fresh water (coastal areas). Not only the amount of dissolved salts but the daily and seasonal variation in salinity affects the distribution and occurrence of foraminifera. It is directly related to the vital activity of foraminifera. Each species has certain critical limits as well as an optimum salinity for various physiological functions. Salinity can affect test morphology, primarily size and ornamentation. Most observations indicate a reduction in size and loss of ornamentation near the salinity tolerance limits.

- Depth: Water depth is an important ecological factor, but its isolated effects are not well understood. It may influence foraminifera only because of depth related changes that occur in other environmental parameters; e.g. as depth increases the pressure increases, the temperature decreases, and carbonate solubility increases. Water depth-related changes have a greater effect on the vertical changes of calcareous species than on those of agglutinated species. Depth can affect the morphology in variety of ways; there is a tendency for many species to become (a)
more rounded with an increase in depth, (b) to increase distribution their ornamentation in deeper water (although many do not) and (c) marked changes in size with an increase in depth.

- **Nutrition:** Nutrition is among the most important factors governing foraminiferal distribution and abundance. The physico-chemical ecologic factors (temperature, salinity, depth, turbidity, illumination, etc) set the gross limiting conditions for distribution, but within these limits it is often difficult to effect correlations between these factors with quantitative distribution of foraminifera. The nutritional requirements, while not well understood, probably determine the finer distributional patterns. It may influence the size and morphology of the tests. Reduced nutrition tends to result in undersized specimens whose chambers are less regular in shape.

- **Substrate:** The substrate seems to have some effect on the foraminifera; there are certain species which are found consistently associated with certain type of substrate. Although the correlations are not very good, the bulk of the observations suggest that fine sand mixed with some shelly fragments and silt or clay support the richest standing crop of benthonic foraminifera. The sedentary forms exhibit the greatest morphological variation as a result of substrate condition. The organic content of the substrate can provide nutritive matter and thus be beneficial to foraminiferal development, but excess of it can result in increased acidity and be detrimental to foraminiferal development.

- **pH:** Low pH creates a stress situation in which calcareous specimens must spend considerable energy recalcifying their tests and is detrimental to the foraminiferal organism and thus restricts its distribution. Low pH values in the substrate may also cause the dissolution of empty tests. Little is known about the effects of highly alkaline (high pH) conditions.

- **Trace Elements:** Some trace elements in very specific amounts may be essential to the existence of foraminifera like other marine organisms. In excess amounts these elements as well as the unwanted elements can cause morphological variants and dwarf faunas.

- **Oxygen concentration:** It has been reported that low oxygen concentration can reduce the number of species, enhances the abundant development of certain species and also can at times alter the morphology of specimens. Though this is a factor which can affect the foraminiferal populations, it is not a limiting factor for their existence (Boltovskoy & Wright, 1976).
4.3. Life cycle and life span of benthic foraminifera

Both these terms ‘life cycle’ and ‘life span’ sounds similar and are quite often used interchangeably. But it is very much relevant here to specify the difference between the two as with progression, this chapter deals with both. Life cycle is the series of changes in the growth and development of an organism from its beginning as an independent life form to its mature state in which offspring are produced or simply it is the entire sequence of developmental stages of an organism. A life cycle is not a time measure - it describes the progress of the organism through its different stages whereas life span is a time measure - the interval between the birth and death of an organism.

4.3.1. Life cycle studies on benthic foraminifera

In simple organisms, such as bacteria, the life cycle begins when an organism is produced by fission and ends when that organism in turn divides into two new ones. In organisms that reproduce sexually, the life cycle may be thought of as beginning with the fusion of reproductive cells to form a new organism. The cycle ends when that organism produces its own reproductive cells, which then begin the cycle again by undergoing fusion with other reproductive cells. The life cycles of plants, algae, and many protists often involve an alternation between a generation of organisms that reproduces sexually and another that reproduces asexually (www.crsep.org).

The benthic foraminiferal life cycle coincides with the reproductive cycle as reproduction usually terminates the life of the parent specimen in most of the cases leaving some exceptions. The classical life cycle of foraminifera displays an alternation of generations (Fig. 4.5). Gamogamy and agamogamy are the two periods in the life cycle of foraminifera representing the sexually reproductive and asexually reproductive stages respectively. The sexual generation (gamont) alternates with the asexual generation (schizont). The microspheric form with a small proloculus and large test is produced by the union of zoospores; a kind of sexual reproduction and the megalospheric forms with large proloculus and small test is produced by budding. The number of nuclei in the foraminifera is generally variable and depends on the reproductive generation. The individuals that reproduce asexually (megalospheric forms) posses a large number of nuclei while those that reproduce sexually (microspheric forms) have only one nucleus. The micro and megalospheric forms of the
same species are so different that there are instances where they have been assigned to
different genera or species. This morphological difference is called dimorphism.

As a general rule, the number of microspheric specimens is considerably less than the
number of megalospheric forms. But the opposite situation prevails when the specimens
live under unfavorable conditions. Reproduction, like any other physiological trait is
species specific in foraminifera. As of date, reasonably complete life cycles are known
for fewer than 30 of the 40,000 extant species of foraminifera. In spite of this limitation,
these relatively few well documented life cycles illustrate a significant range of
variation in the life cycle and in the corresponding morphological variation imparted to
the test. Table 4.1 summarizes the general information on the life cycles of various
foraminiferal species by the previous workers. Though this table is not covering the
entire information on lifecycle studies, it contains a major amount of information in this regard.

<table>
<thead>
<tr>
<th>Species</th>
<th>Alteration of generations</th>
<th>Morphology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allogromia laticollaris</em></td>
<td>facultative budding, plasmotomy</td>
<td>Variable</td>
<td>Arnold 1954b; McEnery &amp; Lee 1976</td>
</tr>
<tr>
<td><em>Heterotheca lobata</em></td>
<td>obligatory</td>
<td>gamonts generally larger than agamonts</td>
<td>Grell, 1988</td>
</tr>
<tr>
<td><em>Myxotheca arenilega</em></td>
<td>? obligatory</td>
<td>agamont larger than gamont</td>
<td>Grell, 1958c</td>
</tr>
<tr>
<td><em>Iridia lucida</em></td>
<td>? obligatory</td>
<td>no morphological difference between gamont and agamont</td>
<td>LeCalvez, 1936a, 1938</td>
</tr>
<tr>
<td><em>Nemogullmia longevariabilis</em></td>
<td>unknown</td>
<td>no morphological difference between gamont and agamont</td>
<td>Nyholm 1956</td>
</tr>
<tr>
<td><em>Saccammina alba</em></td>
<td>facultative; budding, plasmotomy</td>
<td>gamonts larger than agamonts</td>
<td>Goldstein 1988</td>
</tr>
<tr>
<td><em>Ovammina opaca</em></td>
<td>facultative; budding, plasmotomy</td>
<td>secondary pores form in circumapertural ring in test of gamont prior to release of gametes</td>
<td>Dahlgren, 1962, 1964; Goldstein, unpublished observations</td>
</tr>
<tr>
<td><em>Cribrothalammin a alba</em></td>
<td>facultative; budding,</td>
<td>secondary pores form in test of gamont prior to release of gametes</td>
<td>Goldstein &amp; Barker 1988, 1990; Goldstein, unpublished observations</td>
</tr>
<tr>
<td><em>Patellina corrugate</em></td>
<td>obligatory</td>
<td>reversed test dimorphism</td>
<td>Myers, 1935a; Grell 1958c</td>
</tr>
<tr>
<td><em>Spirillina vivipara</em></td>
<td>obligatory</td>
<td>reversed test dimorphism</td>
<td>Myers, 1936</td>
</tr>
<tr>
<td><em>Spiroloculina hyaline</em></td>
<td>apogamic</td>
<td>not applicable</td>
<td>Arnold, 1964</td>
</tr>
<tr>
<td><em>Fissurina marginata</em></td>
<td>apogamic</td>
<td>not applicable</td>
<td>Le Calvez, 1947</td>
</tr>
<tr>
<td><em>Elphidium crispum</em></td>
<td>obligatory</td>
<td>classically dimorphic</td>
<td>Lister, 1895; Schaudinn, 1895; Jepps, 1942</td>
</tr>
<tr>
<td>Species</td>
<td>Reproductive Cycle</td>
<td>References</td>
<td></td>
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<td>----------------------------</td>
<td>----------------------------------------------</td>
<td>------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Ammonia tepida</em></td>
<td>facultative Variable</td>
<td>Bradshaw, 1957; Schnitker, 1974; Goldstein &amp; Moodley, 1993</td>
<td></td>
</tr>
<tr>
<td>'Tretomphalus' bulloides</td>
<td>obligatory classically dimorphic,</td>
<td>LeCalvez, 1936b; Myers, 1943a</td>
<td></td>
</tr>
<tr>
<td>(=Rosalina globularis)</td>
<td>but a terminal float chamber occurs in mature gamonts</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Discorbis patelliformis</em></td>
<td>obligatory classically dimorphic</td>
<td>Myers, 1940</td>
<td></td>
</tr>
<tr>
<td><em>Discorbis mediterranensis</em></td>
<td>obligatory classically dimorphic</td>
<td>LeCalvez, 1950</td>
<td></td>
</tr>
<tr>
<td><em>Discorbis vilardeboanus</em></td>
<td>?obligatory classically dimorphic</td>
<td>Foyn, 1936; LeCalvez, 1950</td>
<td></td>
</tr>
<tr>
<td><em>Glabratella sulcata</em></td>
<td>obligatory gamont larger than agamont</td>
<td>LeCalvez, 1950, Grell, 1958b, 1979</td>
<td></td>
</tr>
<tr>
<td><em>Rubratella intermedia</em></td>
<td>obligatory not dimorphic</td>
<td>Grell, 1958a, 1979</td>
<td></td>
</tr>
<tr>
<td><em>Metarotaliella simplex</em></td>
<td>obligatory not dimorphic</td>
<td>Grell, 1973, 1979</td>
<td></td>
</tr>
<tr>
<td><em>Metarotaliella parva</em></td>
<td>obligatory not dimorphic</td>
<td>Grell, 1973, 1979</td>
<td></td>
</tr>
<tr>
<td><em>Rotaliella roscaffensis</em></td>
<td>obligatory not dimorphic</td>
<td>Grell, 1957, 1979</td>
<td></td>
</tr>
<tr>
<td><em>Rotaliella heterocaryotica</em></td>
<td>obligatory not dimorphic</td>
<td>Grell, 1954, 1979</td>
<td></td>
</tr>
<tr>
<td><em>Rotaliella elatiana</em></td>
<td>?facultative gamont generally smaller</td>
<td>Pawlowski &amp; Lee, 1992</td>
<td></td>
</tr>
<tr>
<td><em>Heterostegina depressa</em></td>
<td>biologically trimorphic</td>
<td>Rottger et al., 1990a</td>
<td></td>
</tr>
<tr>
<td><em>Amplistegina depressa</em></td>
<td>biologically trimorphic</td>
<td>Harney et al., 1998; Dettmering et al., 1998</td>
<td></td>
</tr>
<tr>
<td>Planktonic taxa</td>
<td>?gametic Unknown</td>
<td>See review in Hemleben et al., 1989</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Table illustrating the record of reproductive cycles in benthic foraminifera
(After Goldstein, 1999)
It is clearly evident from the previous reports that the classic life cycle with alternating sexual and asexual generations is not always observed in many species of benthic foraminifera. Overall the lifecycle of foraminifera is more varied than in any other group of protists.

Every bit of information in this regard is important considering the fact that, studies on the reproductive traits of foraminifera are important in resolving many of the age old taxonomical problems. Almost all studies using foraminiferal proxies are directly or indirectly based very much on the taxonomy of this group, hence proper understanding of the reproductive cycles and the associated morphological variations in different species of foraminifera is very much a relevant topic of research. This is effectively possible in the laboratory cultures owing to the freedom of continuous and clear observation of the living specimens through various generations in response to the conditions provided.

During this research work, a number of different benthic foraminiferal species were cultured in the laboratory where they reproduced to give several generations (Plate 4.1) these include-

- *Rosalina leei*
- *Strebloides advena*
- *Pararotalia nipponica*
- *Discorbina concinna*
- *Cymbaloporetta plana*
- *Spiroloculina* sp.

There were some other species which could only be reared in the laboratory as reproduction was never observed under the given environment in the laboratory during the entire span of this study.

- *Trochammina* sp.
- *Ammonia tepida*
- *Ammonia beccari*
- *Massilina seccans*
- *Elphidium* sp.
- *Bolivina* sp.

Though the life span of only a few of these species were studied as a part of this work, the information on the viability of growing certain benthic foraminiferal species under laboratory cultures certainly helps to eliminate the misconception about handling the foraminiferal cultures and thus will be a stepping stone for the successors who wish to continue working on similar area of research.
Plate 4.1: Reproduction in various benthic foraminiferal species under laboratory conditions

a) Rosalina leei  b) Strebloides advena  c) Pararotalia nipponica  d) Discorbina concinna  e) Cymbaloporetta plana  f) Spiroloculina sp.
4.3.2. Life span studies on benthic foraminifera

Life span or duration of the reproductive cycle of foraminifera depends on the species and environmental conditions. Small species living in shallow mid-latitude waters may complete their reproductive cycle in several days, whereas some larger species may live for two years. The studies of Myers (1938, 1941, 1943a) on Elphidium crispum are among the most extensive in this regard. The following table (Table 4.2) lists the lifespan/length of reproductive cycles of some of benthic foraminifera reported so far by previous workers (Boltovskoy & Wright, 1976). The list contains information of only 16 out of the thousands of extant benthic foraminiferal species and the list commences with 2 new additions from the present study which itself signifies the present study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Author</th>
<th>Lifespan</th>
</tr>
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<tbody>
<tr>
<td>Spiroloculina hyaline</td>
<td>Arnold, 1964</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Nubecularia lucifuga</td>
<td>Arnold, 1967b</td>
<td>few weeks</td>
</tr>
<tr>
<td>Rosalina sp.</td>
<td>Hedley &amp; Wakefield, 1967</td>
<td>3-5 weeks</td>
</tr>
<tr>
<td>Streblus beccarii tepida</td>
<td>Bradshaw, 1957</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Patellina corrugate</td>
<td>Myers, 1935a</td>
<td>41 days</td>
</tr>
<tr>
<td>Patellina corrugate</td>
<td>Berthold, 1971</td>
<td>40 days</td>
</tr>
<tr>
<td>Bolivina doniezi</td>
<td>Sliter, 1970</td>
<td>9-13 weeks</td>
</tr>
<tr>
<td>Heterostegina depressa</td>
<td>Rottger, 1972</td>
<td>8 months</td>
</tr>
<tr>
<td>Epistominella exigua</td>
<td>Boltovskoy &amp; Lena, 1969</td>
<td>&lt;1 year</td>
</tr>
<tr>
<td>Elphidium gunteri</td>
<td>Boltovskoy &amp; Lena, 1969</td>
<td>&lt;1 year</td>
</tr>
<tr>
<td>E. articulatum</td>
<td>Boltovskoy &amp; Lena, 1969</td>
<td>&lt;1 year</td>
</tr>
<tr>
<td>Quinqueloculina seminulum</td>
<td>Boltovskoy, 1969</td>
<td>1 year</td>
</tr>
<tr>
<td>E. maculum</td>
<td>Boltovskoy, 1969</td>
<td>1 year</td>
</tr>
<tr>
<td>Buccella frigida</td>
<td>Boltovskoy &amp; Lena, 1969</td>
<td>1 year</td>
</tr>
<tr>
<td>Marginipora vertebralis</td>
<td>Ross, 1972</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>Strebloides advena</td>
<td>Present Study</td>
<td>17-19 days</td>
</tr>
<tr>
<td>Rosalina leei</td>
<td>Present Study</td>
<td>14-15 weeks</td>
</tr>
</tbody>
</table>

Table 4.2: Table illustrating the record of length of reproductive cycle/life span in benthic foraminifera
(After Boltovskoy & Wright, 1976)
Increasing growth

sudden stress (pollution/climate extremes)

Fig. 4.6: Schematic diagram showing implication of life span of foraminiferal species in recording sudden/short term changes in environment. A: species with life span ~ years, B: species with life span ~ months, C: species with life span ~ weeks.
4.3.3. Significance of shorter life span of certain species of benthic foraminifera

The species with shorter life span are better suited to investigate the differences between the long-term environmental changes and episodic stress events (Fig. 4.6). In case of species with life spans of the order of years, signatures of short term events will be recorded only in one or two chambers. Therefore abundance may not be affected by such short term changes. Similarly, in case of species with life spans of the order of months, only a few chambers or few specimens may be able to record such events. However the species with life spans of the order of a few weeks and with hard tests are the best suited to register such events. Many specimens will go through the complete life cycle during the span of even short term changes (natural/ anthropogenic). Their test will get preserved in sediments and exhibit the existence of short events in either composition (elemental or isotopic) or morphology of the test and even modification in the abundance may also change. The large number of tests in the sediments deposited in continuous layers therefore holds the key to understand such events in the past.

4.4 Experimental set-up

In order to study the growth and reproduction in the laboratory, the healthy living specimens of *Strebloides advena* and *Rosalina leei* (Fig. 4.7) were separated (as per the methods discussed in chapter 3) and kept in incubators with 12 hour light 12 hour dark illumination cycle, at several combinations of temperature and salinities. Additional feed was given in the form of living *Navicula* (Diatom) cultures at regular intervals (every third day). The growth pattern was monitored constantly and documented time to time under inverted microscope with the help of real time monitoring system and image analysis software. When the specimens reproduced (*Strebloides advena* as well as *Rosalina leei*), the juveniles along with the mother cell were transferred separately in to new culture dishes after one day of reproduction. The juveniles were maintained in the same conditions under which they were born. Similarly, many generations were monitored continuously to understand their lifecycle as well as life span in the laboratory conditions. The growth of the foraminiferal specimens was recorded in the form of chamber addition.
4.5 Results and discussion

During the study, the dimorphic cycle in both *Rosalina leei* as well as *Strebloides advena* was not observed; only asexual reproduction occurred in our laboratory cultures (Fig. 4.8). Parallel observations on different specimens on *Rosalina leei* and continuous observations through so many generations of *Strebloides advena* revealed the same in case of these species. A diagrammatic representation of the lifecycle of *Rosalina leei* and *Strebloides advena* as observed in our laboratory culture conditions is illustrated in figure 4.8. In this figure, two generations are included so as to exemplify that the asexual reproduction continued with each generation and in between no sexual reproduction was observed in these two species.
4.5.1 Life cycle & Life span of *Rosalina leei*

**Taxonomic status:**

Order: Foraminiferida (Eichwald, 1830)

Suborder: Rotaliina (Delage & herouard 1896)

Family: Discorbidae (Ehrenberg, 1838)

Genus: *Rosalina* d’orbigny, 1826

Species: *Rosalina leei* (Hedley & Wakefield 1967)
4.5.1.1 Life cycle

Asexual reproduction was the only mode of reproduction observed in *Rosalina leei*. During the reproduction, juveniles come out of the mother cells with 2-3 (mostly 3 chambers) chambers partially filled with protoplasm. Pseudopodial activity was observed in the juveniles under high magnification and they were able to move away from the mother cell with the help of pseudopodia. The number of juveniles reproduced from different mother cells varies, but in general it was observed that 40-60 juveniles were formed from a single mother cell (Fig. 4.9). The number of the juveniles depends on the availability of living material i.e. the protoplasm; in that case it is to be assumed that bigger the mother, more number of juveniles produced under favorable conditions.

Fig. 4.9: *Rosalina leei*: Mother specimen along with the juveniles during reproduction in the laboratory culture
The size of the juveniles normally varies from 50-60 μm (Fig. 4.9) and a few of the juveniles at times attain bigger sizes. The juveniles receive protoplasm during their formation within the mother cell and protoplasmic coloration is clearly visible in initial chambers especially the proloculus. The juveniles show extensive pseudopodial activity and the length of the pseudopodia at times may reach several times the size of the individuals (Fig. 4.10).

Fig. 4.10: Juveniles of Rosalina leei showing extensive pseudopodial activity; length of the pseudopodia reaches to 3-5 times the size of the juvenile test

Prior to reproduction, the specimen accumulates food particles around their test to form reproductive cysts (Fig. 4.11a). The juveniles come out of the mother cell, breaking a part of the test and at times the fragments of the mother test were seen in the culture dish. Since the entire protoplasm and at times part of the test material also is used up/utilized by the juveniles, reproduction typically terminates the life of the parent/mother specimen and an empty test remains in place of the mother specimen. Occasionally some protoplasm remains behind in the parent test for several days before decomposing.
Fig. 4.11: *Rosalina leei*: a) Reproductive cyst formed by the specimen prior to reproduction. b) Mother specimen after reproduction, as an empty test devoid of protoplasm

4.5.1.2. Developmental stages of *Rosalina leei* in a complete life cycle as observed in laboratory cultures

In a detailed laboratory culture experiment conducted in our lab (Nigam *et al.*, 2008) 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 was noticed that 25°C temperature and 35‰ salinity is best suited for the growth of *Rosalina leei*. The study revealed that comparatively lower temperature and higher salinity increases the growth rate of *Rosalina leei* whereas the higher temperatures and lower salinity decreases the growth rate in this coastal benthic foraminiferal species. Though the optimum temperature-salinity conditions for the conducive growth was identified as 25°C temperature and 35‰, reproduction was not reported in any of the above mentioned combinations of temperature and salinity.

It was assumed that this species might be reproducing at narrow range which might be different from the combinations used for the study. In a previous report on *R. leei* by Hedley and Wakefield (1967) it is mentioned that this species reproduced at 17-20°C seawater temperatures. But the present study reports successful reproduction in *Rosalina leei* at 27°C temperature and 35‰ salinity.

The growth pattern of *Rosalina leei* was documented with the help of microphotographs taken with the help of a sophisticated inverted microscope which has live imaging facility and image analysis software. The growth rate can be determined either by the number of chambers added or from the linear addition to the maximum test diameter.
The microphotographs are arranged sequentially in order to get a clear understanding of the growth in *R. leei* in terms of chamber addition (Plate 4.2). It is evident from the observations that the growth rate is quite fast in the initial stages of growth. Rate of chamber addition becomes slower as the organism grows larger. Once the specimens attain the maximum size, it remains idle/dormant for some time preparing itself for the reproduction. The total number of chambers formed by *Rosalina leei* during its life varied in different specimens and normally ranged between 10-16, though exceptions were seen (Fig. 4.11b). Similarly the maximum size attained by the specimens varied from 270-340 μm at maturity other than the exceptional cases where specimens either attained much bigger/smaller sizes in the cultures. For this reason, the maximum size attained or the maximum number of chambers formed by this species is expressed as a range rather than a particular value.

The chamber formation in *Rosalina leei* revealed that prior to the addition of new chamber; the juvenile specimens use their pseudopodial network to accumulate foreign particles, mainly the food particles to form an outline in the form of the new chamber within which the new chamber is formed. In Fig 4.12 it is clear that this mass of foreign particles does not cover the entire test as it happens during the reproduction, but only forms over the region where the new chamber has to form in this case the 4th chamber. The newly formed chamber is distinct with its transparent colour compared to the previous chambers. Later on the protoplasm from the previous chambers flows in to fill the new chamber.

![Fig. 4.12: (a) Extended pseudopodial network to accumulate the foreign particles (b) New chamber formed within the outline formed of foreign particles](image-url)
Plate 4.2: Growth stages/ chamber formation in *Rosalina leei* from laboratory cultures
4.5.1.3. Life span

From the parallel observations made on several specimens in the laboratory, only asexual mode of reproduction was observed (as described under life cycle of *Rosalina leei*). In all the cases, *Rosalina leei* completed its life cycle within 105-109 days interval ((Fig. 4.13)). Since reproduction terminates the life of the mother specimen, lifespan coincides with the reproductive span of this species. Hence life span can also be described as the length of the reproductive cycle of the benthic foraminiferal species *Rosalina leei* and it is noticed to be 105-109 days under the given laboratory conditions.

Previously a species namely *Rosalina floridana* was studied by Lee *et al.* (1963) who reported that this species has classical dimorphism with alternating sexual and asexual phases during its lifespan. Later on Hedley & Wakefield (1967) modified the taxonomic status of *Rosalina floridana* and proposed it to be *Rosalina leei* and reported the life span of *Rosalina leei* as 3-5 weeks through a series of apogamic reproduction devoid of any dimorphism from the laboratory observations on specimens collected from Plymouth, England and Wellington, New Zealand. However Boltovskoy & Wright (1976) have not agreed with this taxonomic modification and kept it open as *Rosalina* sp. while listing the previous record on the length of reproductive cycles in benthic foraminifera. The specimen studied as a part of this work has most of the characteristics of *Rosalina leei* and from the present study a life span of 14-15 weeks was noted. It appears that this species is cosmopolitan as it is reported from both temperate (by Hedley & Wakefield, 1967) to tropical zones (by Saraswat *et al.*, 2004; Nigam *et al.*, 2008 and also the present study).

The difference in the life span may possibly be attributed to the difference in geographic zones of the samples used for the studies; England as well as New Zealand falls in temperate zones where as the sample for the present study is from a tropical zone. It should also be noted that since the present report of the life span of *Rosalina leei* is based only on two consecutive laboratory generations, more careful observations are required to confirm the life span of the species.
Fig. 4.13: Diagram illustrating the dates of reproduction of *Rosalina leei* in the laboratory cultures. The arrow depicts the successive generations.
4.5.2 Life cycle & life span of *Strebloides advena*

**Taxonomic status:**

Order: Foraminiferida (Eichwald, 1830)

Suborder: Rotaliina (Delage & herouard 1896)

Family: Discorbidae (Ehrenberg, 1838)

Genus: *Strebloids* (Bermudez & Seiglie 1963)

Species: *Strebloids advena* (Cushman 1922)

### 4.5.2.1 Life cycle

Asexual reproduction was the only mode of reproduction observed in the laboratory throughout the number of generations studied. The first signs of reproduction were visible several days earlier than the release of the offsprings. The offsprings per reproducing adult specimens varied from 15-20 in numbers. The pseudopodial activity was observed in many of the offsprings at high magnification, and they were very actively moving around in the media and over the algal matter available in the culture dish. The protoplasm was reddish brown in colour and apart from that fluorescent orange colored vacuoles were seen in all the juveniles and also scattered in the periphery of the mother test. On careful observation it was observed that the juveniles acquired these masses along with the protoplasm from the mother cell as it is visible from the photograph. It is possible that these are the algal symbionts they acquired from the mother cell. Reproductive cyst formed from the food particles accumulated around the test prior to reproduction was not commonly seen for this species though it was noticed for many of the other benthic foraminiferal species in our laboratory.

During reproduction, the juveniles consumed the entire protoplasm of the mother cell, the mother cell became totally empty and died out with the reproduction. The number of juveniles produced from a mother cell thus depends on the availability of the amount of protoplasm.

It was observed that smaller specimens produced less number of juveniles and more number of juveniles was produced from bigger specimens. For e.g. specimens of the order of 65.58 μm size produced less than 10 juveniles whereas mother specimens of the order of 80-81 μm size produced 15-20 juveniles, each of which was 25 μm to 32 μm in size at the time of its birth (Fig. 4.14).
4.5.2.2. Developmental stages of *Strebloides advena* in a complete life cycle as observed in laboratory culture conditions

Out of several combinations of salinity and temperature conditions given to *Strebloides advena* in the laboratory with 12 hour light 12 hour dark illumination cycle and additional feed of mixed diatom culture, best response was observed for the ones maintained at 36% salinity and 30°C temperature.

*Strebloides advena* formed 11-14 chambers at maturity and grew to the maximum size of 90 μm in culture; the lower limit of this range varied between 65 μm-70 μm. The size of the mature specimens remained more or less the same through all the generations. The growth of the *Strebloides advena* during a complete life cycle is
presented with chronologically arranged microphotographs of the specimens taken at the progressing stages of growth in the laboratory (Plate 4.3).

The juveniles were born with 3-4 chambers, further chambers were added after the birth. Chamber addition was faster during the initial 5-6 days when they add 5-6 chambers out of the total 11-13 chambers they have at maturity. Thereafter the chambers were added at a relatively slower rate and by day 12 they have already completed the chamber addition (11-13; vary in individuals). The signs of reproduction were clearly visible through the test; the test was entirely filled with protoplasm and quite a few granular masses were visible in the protoplasm. By day 15, the juveniles were clearly visible within the mother cell. On day 19 (in this particular specimen) the reproduction took place, and it took almost a full day to complete the whole reproduction (till the last baby is out of the mother cell).

Reproduction usually terminated the life of the mother specimens in all the cases. The entire protoplasm of the mother cell was consumed by the juveniles leaving an empty test, totally devoid of protoplasm, either completely broken in to fragments or at times broken only on the ventral side of the last chamber depending how the juveniles came out (Fig. 4.15a,b).

**Fig. 4.15: Strebloides advena: Fate of the mother specimen after reproduction**

a: completely broken  
b: partially broken
Plate 4.3: Growth stages/chamber formation in *Strebloides advena* from laboratory cultures
4.5.2.3. Life span

Several consecutive generations were observed in the laboratory. In all the generations *Strebroides advena* completed its life cycle within 17-19 days (Fig. 4.16). Since reproduction terminates the life of the mother specimen, life span coincides with the reproductive span of this species. Hence life span can also be described as the length of the reproductive cycle of the benthic foraminiferal species *Strebroides advena* and it is noticed to be 17-19 days under the given laboratory conditions. This is the first report on the life span of this particular species and the very short life span of the order of 17-19 days is confirming the assumptions made by previous workers like Myers (1935b, 1937) and Arnold (1948, 1954a) that a smaller species will have shorter life span.

4.6. Conclusions

4.6.1. *Rosalina leei*

- Under the given laboratory conditions, *Rosalina leei* underwent only asexual reproduction in all the three consecutive generations.
- The number of juveniles formed from a single mother cell varies from 40-60 in number and the juveniles were born with 3-4 chambers.
- Prior to reproduction *Rosalina leei* made a reproductive cyst surrounding the test with the pseudopodial network.
- Life span of *Rosalina leei* was observed to be 105-109 days.

4.6.2. *Strebroides advena*

- Under the given laboratory conditions, *Strebroides advena* underwent only asexual reproduction in all the 7 consecutive generations.
- The number of juveniles formed from a single mother cell varied from 15-20 in number and the juveniles are born with 3-4 chambers.
- In *Strebroides advena* the process of making reproductive cyst was not noticed prior to reproduction.
- Life span of *Strebroides advena* was observed to be 17-19 days.
Experiment No: 1

Generation 1

4th August 2005

17 Days
Generation 2

21st August 2005

17 Days
Generation 3

7th September 2005

19 Days
Generation 4

26th September 2005

17 Days
Generation 5

13th October 2005

19 Days
Generation 6

2nd November 2005

Experiment No: 2

Generation 1

13th August 2006

17 Days
Generation 2

30th August 2006

19 Days
Generation 3

18th September 2006

17 Days
Generation 4

05th October 2006

18 Days
Generation 5

23rd October 2006

17 Days
Generation 6

9th November 2006

17 Days
Generation 7

26th November 2006

Fig. 4.16: Diagram illustrating the dates of reproduction of *Strebloides advena* in laboratory cultures. The arrows depict the successive generations.
4.7. Significance of the study

Laboratory studies on various protozoa other than foraminifera have shown that if hundreds of asexually reproduced generations are created uninterrupted by a sexual generation, the broods begin to show signs of decay, the periods between reproductions (length of reproductive cycle) increases, the resistance of the offspring diminishes and the organisms perish. These observations have not held true for those foraminifera which repeatedly reproduce without a sexual generation. Some of the studies have reported a reduction in the size through generations, but the same was attributed to the artificiality of the conditions and the possible lack of sufficient food for the growth in the overcrowded culture (Boltovskoy & Wright, 1976). In the present study, any notable variation in the length of the reproductive cycle or any reduction in the size of the specimens with generations was not observed. It may be possible that the foraminifera possess some mechanism as yet unknown, which maintains their vital processes in equilibrium despite recycling the same genetic material. More studies in this direction may help resolve the existing problems related to the ambiguity on the life processes of the foraminifera in response to various environmental conditions and the related puzzles on the taxonomy based on the test morphology. This in turn will refine the use of both fossil as well as living foraminifera as a proxy for various environmental and paleoenvironmental studies.