Deciphering the life span of a few species

5.1 Introduction
Foraminifera first appeared in the Cambrian and are found in all marine environments from the intertidal regions to the deepest trenches of the ocean and from the tropics to the poles. Although unicellular, they perform all the functions performed by the multi-cellular organisms (Boltovskoy and Wright, 1976). Many studies have been conducted on live foraminifera wherein biological processes such as locomotion, growth, chamber development, calcification, reproduction, life cycles, pollution effects etc. were observed (see Lee and Anderson, 1991; Goldstein, 1999). In view of its widespread application for paleoclimatic studies, it is important to know their life-span. The species with a life-span of a few days to a few weeks will incorporate seasonal climatic signals, while the species with life spans of a several months to years will indicate annual climatic changes. It will help in selecting species to infer seasonal and annual climatic changes. The knowledge of various stages in the life cycle of foraminifera is important to measure its life span. The precise documentation of the stages in the life cycle of a species further helps in its application for paleoclimatic studies.

5.2 Life cycle of Foraminifera
Life cycle in foraminifera is a complex one with alternation of generations (Fig. 5.1). D’Archiac & Haime, (1853) noticed that foraminiferal specimen belonging to the same species were morphologically different from each other. This observation was confirmed by Parker and Jones (1861). This phenomenon is known as dimorphism. This concept was confirmed by Lister (1895) and Schaudin (1895), who simultaneously and independently, explained the cause of dimorphism to be alternation of generation. Lister (1895), (1903), Schaudin (1895) and Jepps (1942) observed the complete life cycle of *Elphidium crispum* and further confirmed the presence of dimorphism in foraminifera. Figure 5.1 gives an idea of foraminiferal life cycle which is characterized by alternation of sexual and asexual generations.
(Goldstein, 1999). Later on, the alternate generations were defined as microspheric and megalospheric, based on its morphological features.

Figure 5.1: Various stages in the life cycle of foraminifera (Goldstein, 1999).

5.2 A. Microspheric Forms
The adult agamont, or the Schizont form, which produces numerous young ones by multiple fission is multinucleate with tiny proloculus and a large test, is termed as microspheric. Each of these nuclei gathers small amount of protoplasm from the mother cell and forms the initial chambers of the new individual. Later these individuals are released from the microsphereic test. The new individuals formed are usually megalospheric. The reproduction is thus asexual (schizogony) (Boltovskoy and Wright, 1976).
5.2 B. Megalospheric Forms

The adult gamont, or the sporozont form which produces gametes, has a single nucleus and a test characterized by a relatively large proloculus and small diameter. Such forms are called as megalospheric forms. It has only one nucleus but, when the specimen matures this nucleus divides into many small nuclei which carry a small quantity of cytoplasm with them when they leave the test as flagellated zoospores (gametes). When two such gametes join, a zygote is formed. This process of sexual reproduction is called gamogony wherein microspheric generation is formed (Boltovskoy and Wright, 1976).

The life cycle of foraminifera, however, may or may not have alternate generations. Observations by Arnold (1955), Phleger (1960), Frankel (1975), Kitazato (1992), Gross (2000) etc. have shed light on the complicated life cycles and biology of foraminifera. Some stages of the life cycle of *Ammonia tepida* have been studied by Schnitker (1974), Goldstein and Moodley (1993), Goldstein (1997). Though there are more than 10,000 extant species of foraminifera, complete life cycle has been documented for less than 30 species (Sen Gupta, 1999). These studies show that the life span of benthic foraminifera may vary from a few weeks to more than a year (Boltovskoy & Wright, 1976). Unfortunately, of late, not much attention has been paid to understand the life-span of benthic foraminiferal species. Therefore, it was decided to document the life span of a few benthic foraminiferal species.

5.3 Experimental Set-up

Individuals of *Cymbaloporetta plana* (Cushman), *Discorbina concinna* (Brady) and *Spiroloculina* sp. were isolated from the sediment and sea-grass samples collected from the coastal areas off Goa. Initially when the specimens were isolated they were kept in the conditions same as that at the time of sample collection. This was done in order to avoid the sudden stress which the specimens would experience if transferred to different conditions. After a few days, in order to observe the optimum temperature and salinity for each species, the specimens were transferred to media with different combinations of salinity and temperature. The salinity as well as the temperature was reduced or increased slowly to avoid the salinity or temperature shock which the specimen would experience if directly subjected to different
conditions. The illumination of 12 h light and 12 h dark was provided. The specimens of *C. plana* and *D. concinna* were subjected to 16 combinations of temperatures (25°C, 27°C, 30°C and 35°C) and salinities (25%, 30%, 35% and 40%) whereas *Spiroloculina* sp. was subjected to 12 combinations of temperature (25°C, 27°C, 30°C and 35°C) and salinities (25%, 30% and 35%). 30 specimens were subjected at each combination. The above temperature and salinity range was selected as it was the same as reported from the study area in literature as well as during continuous monitoring of the physico-chemical parameters as part of this study.

The *Navicula* sp. of diatoms was used as food for these foraminiferal species and was also maintained in laboratory by sub-culturing after every seven days. Every time the media was changed, 100 µl of food (~20 cells/ml) in the form of diatoms was added to the culture media. The species were kept in six welled culture dish and each well had 5 specimens. The experiment was carried out in replicate. The reproduced specimens were kept undisturbed after each time the reproduction took place. This was done to monitor the life span and different growth phases for each specimen. The media conditions, at which the reproduction took place, were maintained and finally more than 8-10 generations for each species were monitored. It was observed that not all the specimens belonging to the same species reproduce at the same time; a few of them reproduced after two days while others reproduced after four days. It was noted for most of the specimens. Further, it was also observed that during reproduction, neither all the juveniles come out of the mother specimens at one stretch, nor do all the specimens have three to four chambers. A few of the juveniles had two chambers at the time of reproduction. The life-cycle of various species is discussed individually in the next section.

5.4 *Cymbaloporetta plana* (Cushman)

It has a calcareous test which is planoconvex, trochospiral, subcircular in contour, with rounded periphery, and lobulate margins (Plate 5.1). The test wall is prominently perforate on its dorsal side which is convex. The initial chambers are off center, small somewhat inflated brownish in colour followed by larger irregular more inflated chambers. The last formed whorl comprise of four shallow, broad chambers. The ventral side is flat to concave. The central wall of four visible chambers is
perforate and is separated by rather broad radial channels at sutural area. The test size is 0.442 x 0.221 mm (McCulloch, 1977).

Plate 5.1: Micrograph of *Cymbaloporetta plana* (Cushman) (A) Ventral view and (B) Dorsal view.

### 5.4.1 Systematic Classification

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### 5.4.2 Reproduction

*Cymbaloporetta plana* (Cushman) reproduced asexually in laboratory. Reproduction and chamber formation in this species occurs within a cyst (Plate. 5.2). The specimens build a cyst of food material and debris when it is about to reproduce or to add a new chamber. The food gathered by the mother specimen to form the cyst at the time of reproduction, is then utilised by the offspring to build its test before these
offspring's are released by breaking open the cysts. The specimens reproduced asexually at 25°C to 30°C temperature and 30% to 35% salinity in laboratory.

Plate 5.2: Chamber formation within the cyst formed of food material by *Cymbaloporetta plana* (Cushman) (the arrow indicates the cyst and the newly formed chamber).

The test size of the adult specimens varies but a fully formed adult consist of 20-25 chambers. The cytoplasm in the initial chambers is dark in colour compared to the later chambers, whereas the newly formed chambers are almost transparent (Plate 5.2). The time taken for reproduction varies from specimen to specimen. *Cymbaloporetta plana* took almost three to four days from the time of cyst formation to the final dispersal of the juveniles. Number of offspring's depends on the
maximum diameter of the mother specimen. The relationship is highly significant ($R=0.88$) (Fig. 5.2). Since this specimen reproduced asexually, the juveniles are formed within the mother cell and are released with two, three or four chambers. Just after reproduction, once the juveniles come out of the mother test, it takes almost two days to add a new chamber depending on the availability of food. The time taken to add subsequent chambers however, increases slowly. Finally when the specimens mature, it again forms the cyst and remains dormant for one or two days. During this dormant phase the cytoplasm within the parent cell starts dividing into new specimens and then after two or more days the test of the mother specimen bursts open thus releasing the juveniles. The released juveniles are very active with an extensive pseudopodial activity. These reproduced juveniles were then again subjected to identical conditions to observe the next generation. A total of 10 such generations of this specimen were observed in laboratory. It was found that *C. plana* reproduces within 45-55 days. The different stages in the life cycle of *C. plana* including the time taken to form chambers are shown in Plate 5.3.

![Graph showing the relationship between maximum diameter of the parent cell of *Cymbaloporetta plana* (Cushman) and number of juveniles. The relationship is highly significant with a correlation coefficient of $R=0.88$.](image)
Plate 5.3: Chamber formation and reproduction in *Cymbaloporetta plana* (Cushman) Scale bar = 50μm (0 day to day 4) and 100 μm (day 6 to day 49).
5.5 *Discorbina concinna* (Brady)

The tests of *Discorbina concinna* (Brady) are circular in outline, with convex superior face (Plate 5.4). The interior of the tests is somewhat concave. The peripheral edge is angular, composed of more than two convolutions, of which the latest consists of three to four segments. On the inferior side, the final segment occupies nearly half the entire surface. The umbilical flaps are distinct but not greatly developed. The tests walls very thin and conspicuously perforate. It has sutures marked by fine lines, neither depressed nor limbate externally. The diameter of the tests is ~0.25 mm.

![Plate 5.4: Micrographs of Discorbina concinna (Brady). A. Ventral view and B. Dorsal view.](image)

### 5.5.1 Systematic Classification

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5.5.2 Reproduction

*Discorbina concinna* (Brady) specimens are found along the coastal areas off Goa. After keeping them at 16 different combinations of salinity and temperature, it was noted that it reproduces asexually at a temperature range of 25°C - 27°C and salinity range of 30%o - 35%o. When the specimen is about to reproduce or to form a new chamber, it also forms a cyst of food particles and debris. When the specimen is about to add a new chamber, an extensive pseudopodial network is first formed outside the last chamber spreading to a distance corresponding with the size of the newly added chamber. When the specimen is about to reproduce it forms a cyst of food material around itself. This food material in the cyst helps the juveniles to feed on, while they are within the cyst (see Plate 5.5).

Plate 5.5: Cyst formed around the specimen during chamber formation (A&B) and cyst formed around the specimen during reproduction (C&D) in *Discorbina concinna* (Brady).
Plate 5.6: Chamber formation and reproduction in *Discorbina concinna* (Brady). Scale bar=50μm (0 day to day 2) and 100μm (day 3 to day 26).
Immediately after reproduction, the proloculus of the juveniles has a reddish ting. With the progress of addition of new chambers, the cytoplasm colour in the proloculus becomes lighter, while the newly added chambers are transparent as seen in Plate 5.6. The lifespan of this species is ~22-25 days. The life span estimates are based on a total of 10 generations as also in case of *C. plana*. The juveniles are released with three to four chambers and the total number of chambers in the adult test is around 13 to 15. Number of juveniles released during reproduction again depends on the size of the specimen, i.e. the larger the size of the parent, more the number of juveniles. The relationship is highly significant (R=0.97) (Fig. 5.3).

Twinned specimens were also noted in *D. concinna* (Plate 5.8). However, the twinned specimens in laboratory culture were noticed only in this species. It was evident that initially the two specimens grew separate as two different individuals. But later on both the specimens formed a common chamber and thus the two different individuals joined into one test. The twinning was not seen under stressed conditions as it was noted at the same conditions, at which reproduction in this specimen took place.
Plate 5.8: Twin specimens of *Discorbina concinna* (Brady).

### 5.6 *Spiroloculina* sp.

*Spiroloculina* specimens are found abundantly along the coastal areas off Goa. It is procellaneous in nature with chambers added at 180°. The test is elongated, more or less oval in shape and shiny. The periphery of the chambers is smooth and curved. It has a single aperture occupying terminal position. It was noted that *Spiroloculina* sp. tests remain in upright position spreading its pseudopodia through the terminal aperture.

### 5.6.1 Systematic Classification

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5.6.2 Reproduction

The specimens of *Spiroloculina* sp. were subjected to 12 combinations of salinity and temperature. From all these temperature-salinity combinations, it was observed that these specimens reproduced at a temperature range of 25°C - 30°C and salinity range of 30%o - 35%o. Asexual reproduction was seen in this species too. It was found that when the specimen is about to reproduce it forms a cyst with pseudopodial network slightly away from the mother test whereas in case of *C. Plana* and *D. concinna* the cyst is formed very near to the mother test (Plate 5.9).

![Plate 5.9: Formation of pseudopodial network (↔️) with a cyst of food material (→️) at the time of reproduction and addition of new chamber in *Spiroloculina* sp.](image)

Scale bar = 100μm
Plate 5.10: Formation of cyst before reproduction (1), cytoplasm from the parent cell moving in the cyst (2-7), formation of juveniles (8-11), fully formed juveniles in the cyst (12) in *Spiroloculina* *sp.*
As part of reproduction, once the specimens complete the cyst around the test by pseudopodial network, all the cytoplasm comes out of the parent cell in the cyst area (Plate 5.10). Unlike, previous specimens, reproduction in *Spiroloculina* sp. take place outside the test (Plate 5.10). No pseudopodial activity is noted for several days once the cytoplasm comes out and starts dividing into offsprings. Then each of the nuclei, along with a small amount of cytoplasm forms the new offspring. The juveniles form the initial few chambers within the cyst formed outside the parent test.

Plate 5.11: Time taken to complete a chamber in *Spiroloculina* sp.
The juveniles come out by bursting open the cyst. After observing several generations of *Spiroloculina* sp., the life span was found to be of ~25 - 30 days. The initial chambers of the specimens were brownish in colour while the last and newly formed chambers were transparent. The juveniles form 2-3 chambers while within the cyst, while additional chambers are added once they come out of the cyst. The parent test dies after reproduction as all the cytoplasm of the parent test is utilized by the offsprings. *Spiroloculina* sp. takes ~6 hours to add a new chamber. Initially when the chamber is in the process of formation, it is transparent. However, later when the chamber has been fully formed, the cytoplasm from the other chambers comes out in to the new chamber giving it a brownish colour (Plate 5.11).

### 5.7 Discussion

All the three species viz. *Cymbaloporetta plana* (Cushman), *Discorbina concinna* (Brady) and *Spiroloculina* sp. reproduced asexually in laboratory. In *C. plana* and in *D. concinna* it was noticed that the juveniles formed within the parent test, whereas in case of *Spiroloculina* sp. the juveniles formed outside the test within the cyst formed by the pseudopodial network. In case of *Heterostegina depressa*, *Amphistegina lobifera* and *A. lessonii* also it was observed that the cytoplasm flows out of the maternal test and then undergoes multiple fission (Rottger, 1974; Hallock et al. 1986). Only asexual reproduction has been recorded in most of the laboratory culture studies, the majority of which were carried out on small shallow water forms (Scott et al. 2001). Haq and Boersma (1978) noted that sexual reproduction is very likely a secondary reproductive mechanism, while asexual reproduction is the basic and the more frequent reproductive mode of the majority of foraminiferal species. Le Calvez (1939) found that megalospheric agamonts of *Planorbulina mediterranensis* can undergo repetitive asexual reproduction before producing sexually reproducing gamonts. Grell (1954, 1957a, b, 1958a, 1979), while studying the tiny *Rotaliella heterocaryotica, R. roscoffensis, Metarotaliella* sp. and *Rubratella intermedia* found no difference in the size of the proloculus of different generations. Microspheric and the megalospheric test differences have been reported only in a few species (Lister, 1895; Schaudin, 1895; Myers, 1935b, 1942; Grell, 1957a, b, 1958a, b; Boltovskoy and Wright, 1976).
The life spans of studied specimens were of the order of several weeks and varied by a few days between different generations. The reason for the slight difference in the life-span of different generations of same species is not known at present. Earlier Myers (1943a) concluded that the life span of *Elphidium crispum* is usually 1 year in temperate regions but may take longer time in deeper waters. He opined that the growth is faster in tropical waters and the life cycle is completed in 1 year. This possibility is ruled out since all the generations of same species were maintained under similar conditions. He also reported that the whole life-cycle that is both sexual and asexual reproduction is completed in 2 years in tide pools. Unfortunately, sexual reproduction was not noted in any of the specimens in this study.

Not much is known about foraminiferal reproduction in relation to environmental dynamics (Bradshaw, 1961; Buzas, 1965). Bradshaw, (1961) reported that foraminifera will reproduce only in favourable conditions. From this study also it is evident that foraminifera prefer certain set of favourable conditions for reproduction. In this study the specimens were subjected to different combinations of salinity and temperature, but reproduction took place at a narrow range of temperature and salinity.

Though, trimorphic forms have been reported in several benthic foraminifera particularly the larger, algal symbiont-bearing foraminifera (Rottger et al. 1986, 1990b; Harney et al. 1998; Dettmering et al. 1998), no such distinction could be made in the juveniles of all three studied species.

Additionally, though the reproduction in *Hastigerina pelagica* and *Globigerinoides sacculifer* is linked to lunar cycle (Spindler et al. 1979; Hemleben et al. 1987). No such lunar link was noted for the benthic foraminiferal species of the present study.

### 5.8 Conclusion

Based on this work, life spans of three species belonging to three different families viz. Spiroloculinidae, Rosalinidae and Cymbaloporidae were noted. All the three specimens reproduced asexually in laboratory. In case of *Cymbaloporetta plana* (Cushman) and *Discorbina concinna* (Brady) the juveniles were formed within the parent cell whereas in case of *Spiroloculina* sp. juveniles were formed in the cyst
built by the pseudopodial network. The life span of *Cymbaloporetta plana* is of 45 to 55 days, *Discorbina concinna* is of 22 to 25 days and *Spiroloculina* sp. is of 25 to 30 days. A slight difference of a few days was noted in the life span of different generations of the same species. In case of *C. plana* and *D. concinna* number of juveniles show significant relationship between number of juveniles and size of parent test. The study shows that these species will incorporate seasonal climatic signatures.