ORIGINAL RESEARCH

BULLETIN OF ENVIRONMENTAL STUDIES

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Living behaviors of benthic foraminifera from eastern Bahrain and the Saudi coastline

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Abstract

The benthic foraminifera are among the major carbonate producers in modern Arabian Gulf waters and are found living in all marine habitats. They have been recognized as proxies to assess paleoenvironmental changes, however, their biological behavior in modern environments needs to be further studied. The current study attempts to explain the biology of benthic foraminifera in terms of their living behaviors (locomotion, reproductive strategy, survival, response to external stimuli), from different regions of the western side of the Arabian Gulf. Accordingly, two major groups of benthic foraminifera, namely rotaliids and miliolids, are examined under laboratory conditions. Results illustrate that the rotaliids are more resistant to environmental changes than miliolids, as their granular reticulopodial network is stronger than among the miliolids, with high cytoplasmic streaming. The pseudopodia extend out from both primary and secondary apertures, and aid the organism in locomotion by attaching to the wall of hard substrate. As a result, they drag their whole bodies toward the direction of motion. In rotaliids, the movement rate is high and is attributed to the extension of pseudopodia through all apertures, compared with miliolids in which pseudopodia extend out from the primary aperture only. The innate behavior of both groups was observed as a function of external stimulus, i.e., light, nutrients, and availability of substrate. Lastly, the observation on the average life span indicates that the rotaliids are able to survive longer than miliolids. It is suggested that further studies on living behaviors should be conducted for different species towards better understanding of foraminiferal responses.

Article Information

Edited by:
M. Tahir, KFUPM, Saudi Arabia

Reviewed by:
F. Frontalini, DiSPeA, Italy
Inaam Ullah, SBBU, Pakistan

Article History:
Received; September 12, 2015
Received in revised form; December 28, 2015
Accepted; January 2, 2016
Published online; January 31, 2016

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Keywords: Arabian Gulf, Benthic Foraminifera, Living Behaviors, Pseudopodia

Introduction

Benthic foraminifera are bottom-dwelling unicellular eukaryotes whose living behaviors are still a subject of debate. Amazingly, the majority of foraminiferal species have never been observed alive and among those that were studied, the authors do not provide sufficient information on their biology (Pawlowski and Holzmann, 2008). These few studies further present scattered information on their living behaviors in terms of survival, locomotion, and reproduction (Kitazato, 1988; Lipps and Erskian, 1969). It has been reported that the individuals of some species can live only for a few weeks, whereas some others can live for years. Similarly, some benthic foraminifera burrow actively, at burrowing rate of 82 µm per minute (Kitazato, 1988), whereas others mostly attach or hide themselves to the surface of rocks or marine plants (Karleskint et al., 2009). Moreover, their average locomotion rates were found to vary among different species, with high movement rates in epifaunal species (Kitazato, 1988). The study on living behaviors of benthic foraminifera was initiated by Dujardin who first reported the locomotion patterns of *Elphidium* sp., in natural settings (Dujardin, 1835). Afterwards, several authors reported detailed investigation of movements in different foraminiferal species (Kitazato, 1988; Lee, 1980; Lee and Anderson, 1991; Wright and Boltovskoy, 1976). However, most of their observations were made on the glass surface of petri plates and, hence, their behaviors were subjected to laboratory environments (Arnold, 1953; Banner et al., 1973). Later on, Severin and coworkers measured the vertical velocity of *Quinqueloculina impressa* in a natural environment (i.e., sand particles), in order to obtain the relationship between time of emergence and burial depth (Buzas and Severin, 1982). Their principle objective was to measure the escape behavior of living individuals, attempting to avoid burial in the sediments. Besides locomotion and burying, other living behaviors were also studied by different authors. For instance, Lipps and Erskian reported plastogamy in *Glabra-*

tella ornatissima during sexual reproduction (Lipps and Erskian, 1969). Similarly, Kitazato and coworkers described the breeding behaviors in four species of the genus *Glabratella* and reclassified three morphogroups from the four morphospecies using morphological characters and interbreeding experiments (Kitazato et al., 2000). The current study attempts to document the biological behaviors of living benthic foraminifera under laboratory conditions collected from two regions, i.e., eastern Bahrain, and the Saudi coastline. More specifically, we investigated and compared the locomotion patterns, relationship to substrate, survival response, preservation of pseudopodia, presence of symbionts, and plastogamy of selected benthic foraminiferal taxa.

Materials and Methods

Sample Collection

Sediments containing living benthic foraminifera were collected from two areas, i.e., Eastern Bahrain in the fishing village of Askar and the Saudi Coastline in Khobar. Water depths of the sampling stations range from 40 cm to 100 cm. Sample processing was carried out at the Research Institute and Environmental Sciences labs at King Fahd University of Petroleum and Minerals (Saudi Arabia) (Arslan et al., 2016).

Isolation of Foraminifera

At least 10 living individuals from every group were picked from raw sediment samples of 5 ml using a GENEX beta variable pipette (fixed volume at 200 µl) under a reflected-light, binocular microscope. Isolated individuals were transferred to Petri plates containing natural sea water where their living behaviors were observed every 8 hours. Furthermore, the study of average life span was conducted for four selected species in glass jars (diameter of ¼ inches) containing filtered sea water.

Living Behaviors Study

The laboratory observations on living behaviors particularly locomotion, attachment with substrates, and reproduction begun after 24 hours, as most of the living individuals migrate to their natural positions after a day (Kitazato, 1984). The observations were made on the glass surface of the Petri plates under a phase-contrast stereo inverted microscope. An automatic photographic system (Nikon) attached to the microscope was used to observe and record their behavior. Measurements on velocity were made by photographing living individuals after 20 second intervals. Moreover, their movement rates were calculated using the following formula,

$$S = \frac{\Delta D}{\Delta t} = \frac{P_N - P_{N-1}}{t_N - t_{N-1}}$$

where, P_N is the recent position at time t_N and P_{N-1} is previous position at time t_{N-1}.

Survival Response

The survival response of four species (*Quinqueloculina seminula, Quinqueloculina poeyana, Glabratellina* sp. and *Ammonia* sp.) was calculated using the Kaplan-Meier procedure and Log rank test in order to study their survival in glass jars having diameter of ¼ inches.

Identification and Classification

Selected species were photographed using scanning electron microscope (SEM), at the University of Geneva (Switzerland). The photographs were edited and compiled with Adobe Photoshop (Ps) CS7.

Several taxonomic guides were used for their identification, particularly Loeblich and Tappan (1987), Boltovskoy (1980), Colom (1974), Jones (1994) and the Ellis and Messina (1942–2012) online catalogue. Most of the foraminifera were classified according to the generic classification proposed by Loeblich and Tappan (1987). For the higher levels of taxonomy, other than genus and species, the Worm's classification was followed (World Register of Marine Species – www.marinespecies.org).

Results

Locomotion

Emergence of the pseudopodal extensions was observed in different species of foraminifera. In miliolids, initially, the specimen was lying horizontally on the glass surface and then, after 24 hours, a single strand of pseudopodia was observed to extend out from the primary aperture along with the cytoplasmic streaming. After some time, the extension became elongated leading to further branching, hence, resulting in locomotion. Initially, the individual was moving in a straight line at slow speed but later it adopted a speedy curved path. In miliolids, the individuals were found to move in the direction of the apertural opening. The average speed in *Quinqueloculina seminula* and *Quinqueloculina poeyana* was recorded between 0.32 to 0.41 centimeters per hour. The movement patterns recorded for both species are presented in Figure 4.

Locomotion in species with supplementary apertures is different from locomotion in miliolids. More specifically, in rotaliids, the individual extends its pseudopodia earlier than in the miliolids, i.e., after 8 hours staying in Petri plates. The individual attaches its pseudopodial extensions to the hard substrate and then the distally streaming protoplasm help drags the body forward. Furthermore, a clear cytoplasmic streaming of protoplasm showed bidirectional movements of viscous granules between the aperture and the tip of pseudopodia. However, the direction of movement was directly related to the apertural position and orientation of the foraminiferal test.

In rotaliids, individuals move in various directions depending upon the external stimuli, i.e., light and nutrients. The average speed in *Glabratellina* was recorded between 0.43 and 0.49 centimeters per hour. In the case of *Elphidium*, pseudopodia were observed in

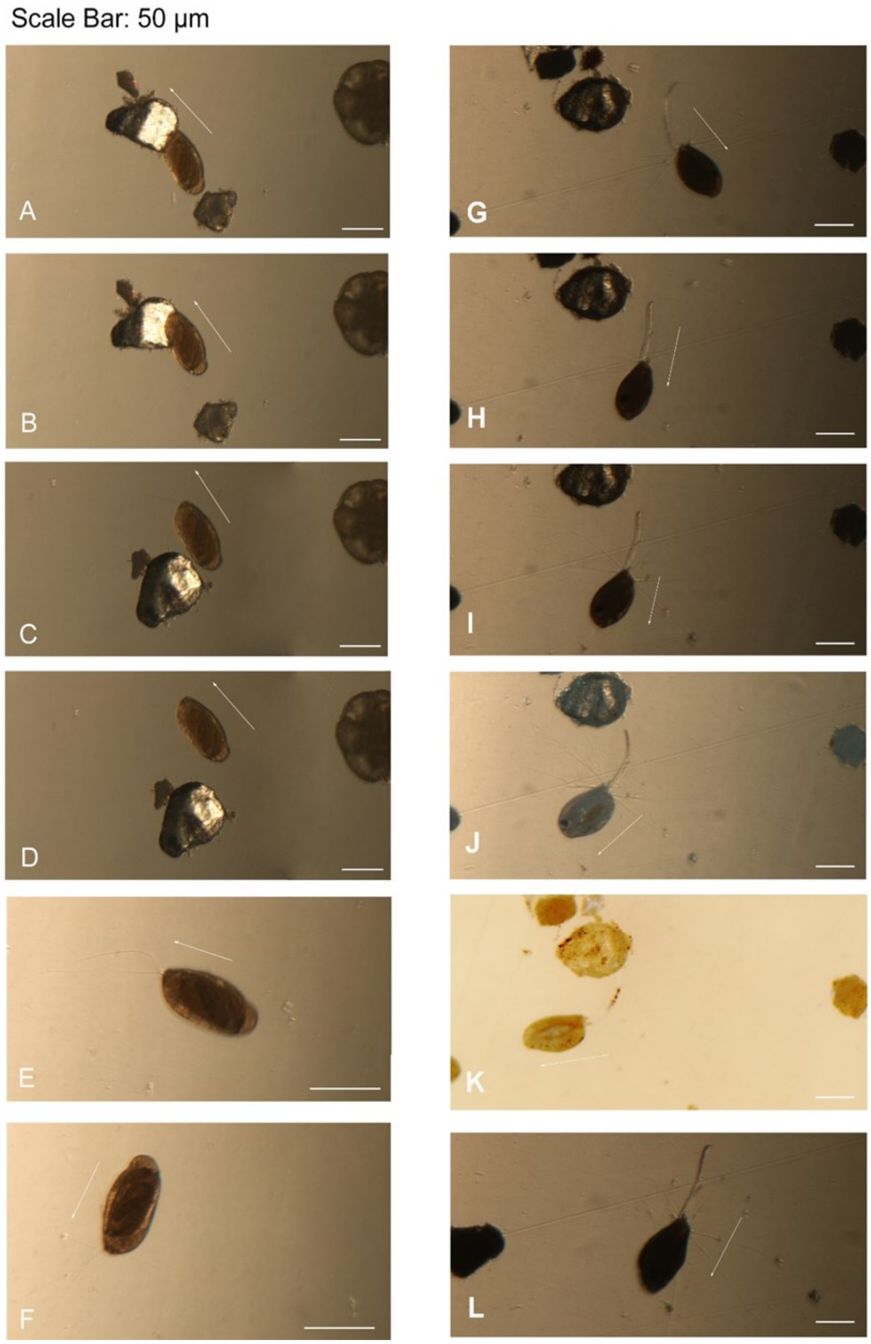

Figure 4: Locomotion in two species of miliolids; (A-F) *Quinqueloculina seminula* (G-L) *Quinqueloculina poeyana* (Scale bar: 50µm)

juveniles only with very little movement in hours. Their movement is presented in Figure 5.

Relationship to Substrate

As it is mentioned earlier, extended pseudopodia in rotaliids anchor the living individual to the wall of hard substrate for locomotion; similarly, many species have shown a number of other living behaviors, such as hiding themselves beneath the substrate for protection and nutrition, in the presence of external/internal stimuli. For instance, miliolids move into the dark by hiding themselves under the sand particles due to light stimuli while examined under the light microscope (Figure 6).

Similarly, rotaliids prefer to attach their bodies onto biogenic hard substrates in order to get nutrition and protection. The observation was stronger for juveniles compared to adults who were able to survive independently. Furthermore, most of the living individuals were found to gather small sand particles around their bodies using their pseudopodia. The attachment was not limited to non-living substrate only as some of the living individuals also stick to the other living foraminifera. The overall response of benthic foraminifera towards biogenic substrate is shown in Figure 7.

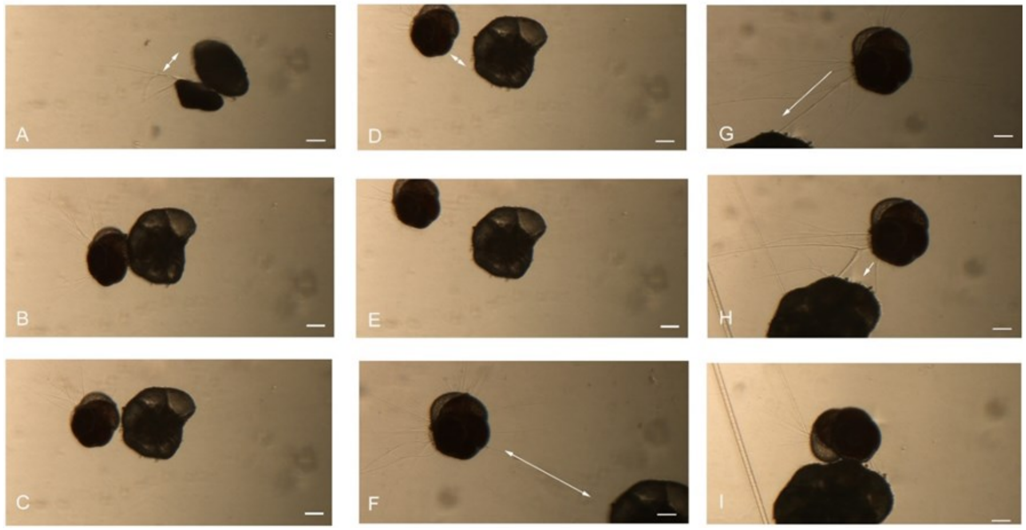

Figure 5: (A-F) *Glabratellina* **sp.** pushing dead *Ammonia* away with its pseudopodial network (G-I) *G. margaritaceus* anchoring its pseudopodia with a hard biogenic substrate (Scale bar: 50μm)

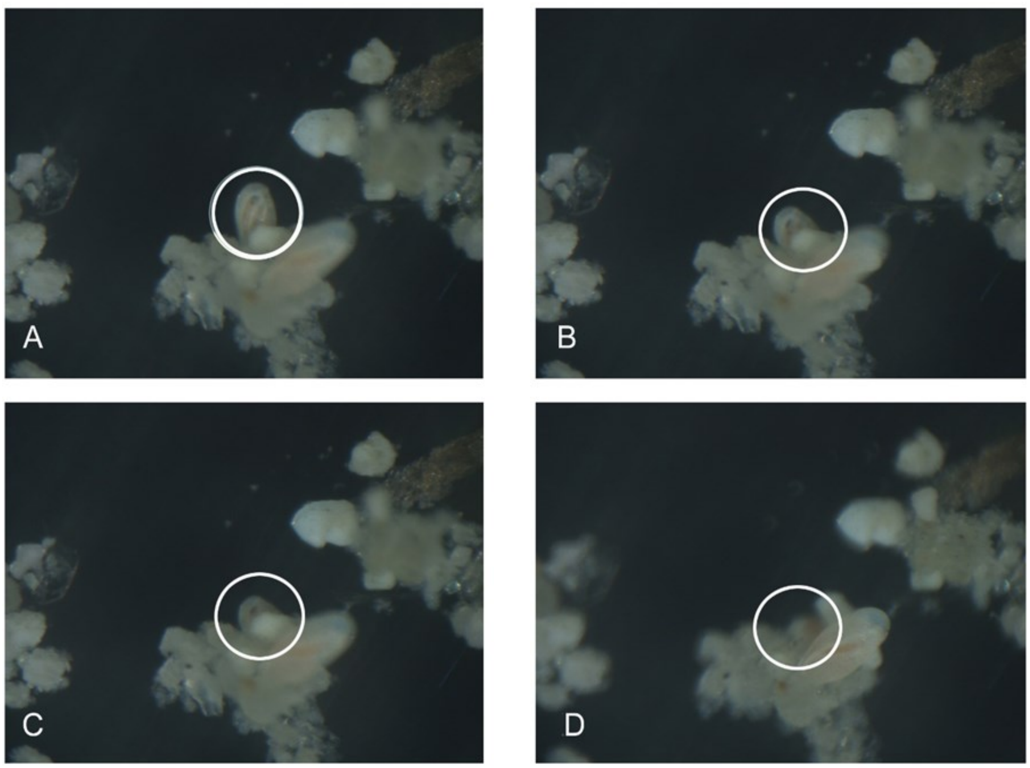

Figure 6: *Quinqueloculina seminula* hiding under the sediment substrate due to external light stimulus: (A) The individual is half hidden trying to move underneath the sediment clump (B-C) The individual is partially visible (D) The individual is completely hidden under the sediments. (Scale bar: 50μm)

Preservation of Pseudopodia

The visible pseudopodial extensions were preserved in an accidental observation in which live specimens were placed in the Petri plates containing seawater for more than a week. Resultantly, the sea water evaporated and their pseudopodia were preserved in the cubic crystals of salt that precipitated from the seawater (Figure 8).

Symbiont-Bearing Foraminifera

Similar to the attachment of living individuals with other benthic fauna, some benthic foraminifera were found to host symbiotic algae in their protoplasm. The samples obtained from eastern Bahrain had a high number of symbiont-bearing individuals, whereas no specimens were found in the samples obtained from the Saudi

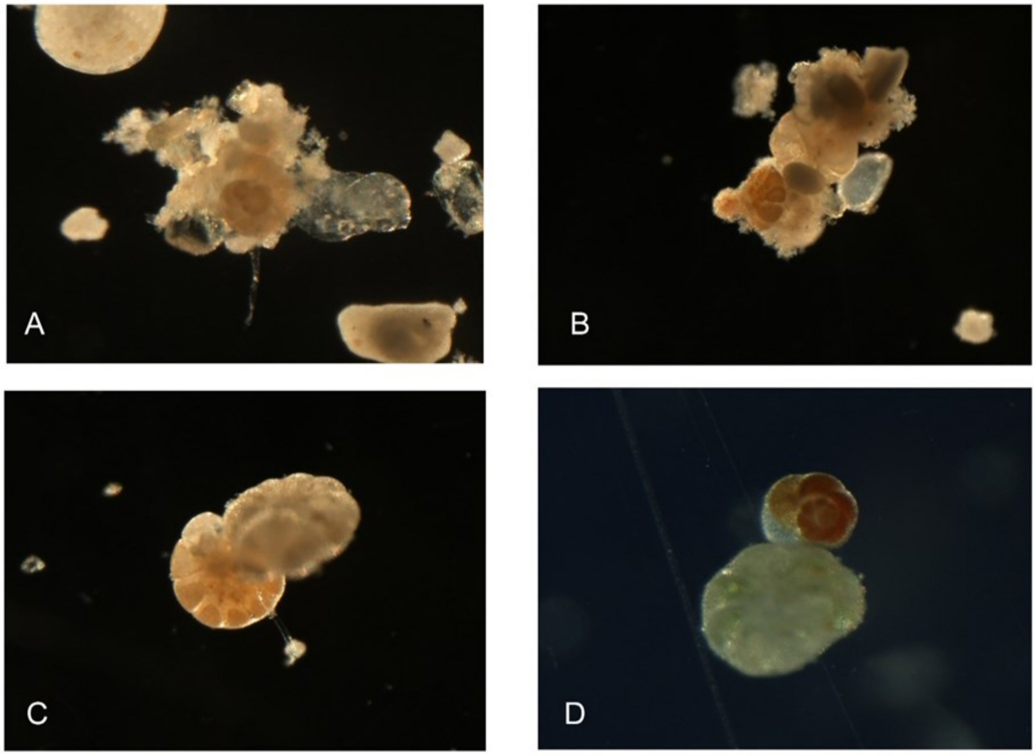

Figure 7: Living foraminifera adhering to hard biogenic substrates for protection and/or nutrition, (A) *Ammonia tepida* attracting sand particles (B) *Ammonia tepida* attaching to biogenic substrates and sand particles (C) *Ammonia* sp. adhering to a dead *Ammonia* (D) *Glabratella margaritaceus* attaching to a dead *Ammonia* with pseudopodial extensions.

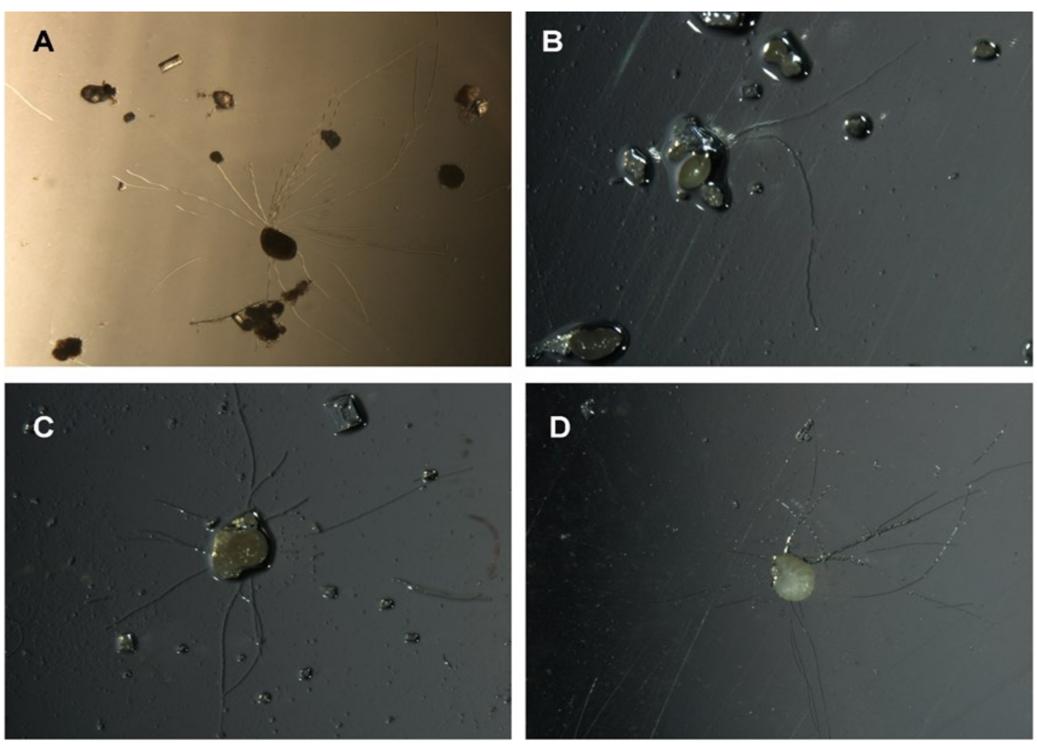

Figure 8: Preservation of pseudopodia in seawater salt crystals: (A) *Quinqueloculina* sp. 1; (B) *Quinqueloculina* sp. 2; (C) *Ammonia* sp. (D) *Elphidium advenum*

coastline. Furthermore, there were more symbiont-bearing rotaliids than miliolids, i.e., 5-10 individuals vs. 1-3 individuals per 5cc of sediments. Different specimens of symbiont-bearing foraminifera are shown in Figure 9.

Symbionts-Bearing Foraminifera

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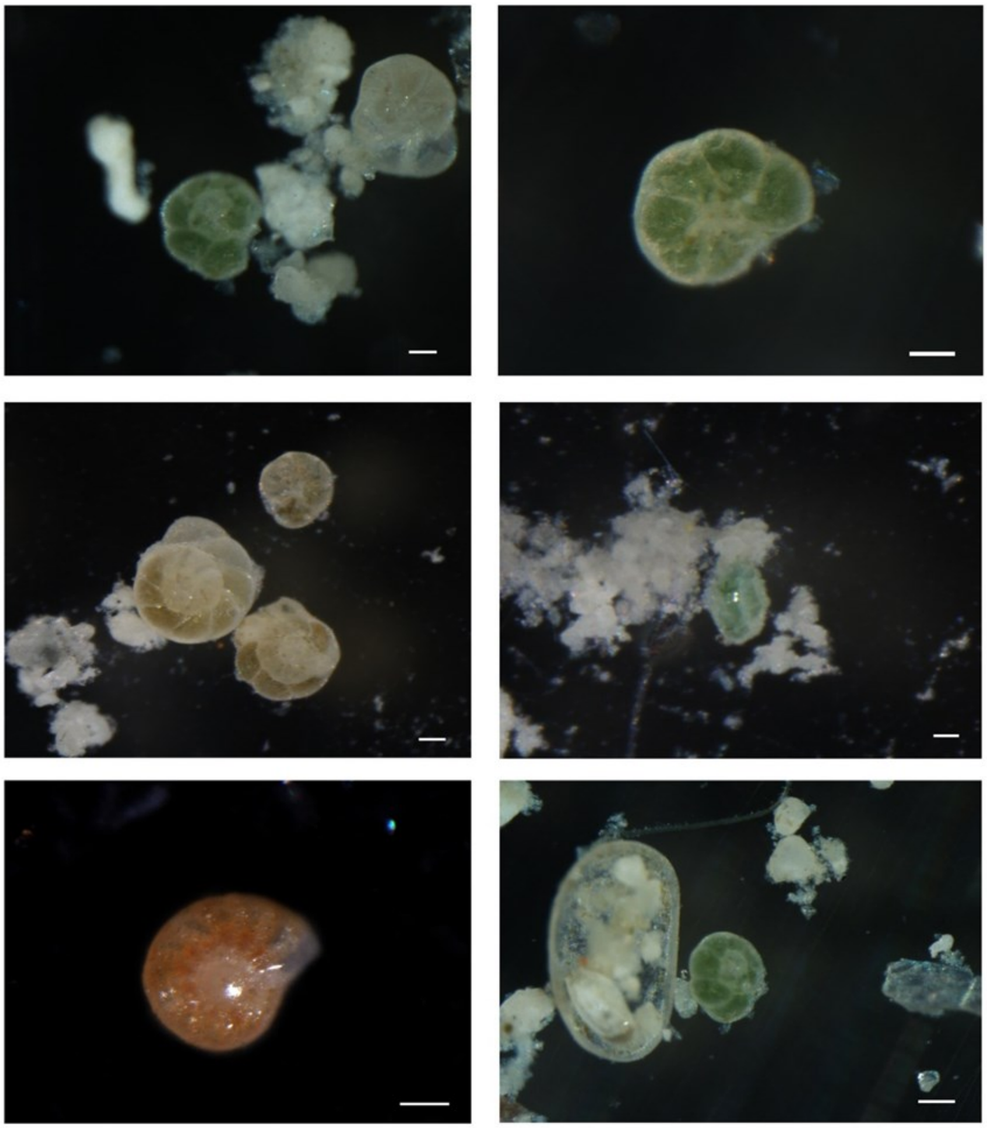

Figure 8: Light microscope photographs of symbionts-bearing foraminifera

Survival Response

This experiment was conducted for the project entitled “Forams in Space” under the Student Space Flight Experiment Program (SSEP). The objective was to study the bone loss in humans, especially the calcium for the astronauts who spend a long time in space. Therefore, foraminifera were targeted because of their nature as calcium-carbonate producing organisms. Although the protion, “How long can a foraminiferan remain alive in a closed jar?”

The results of Kaplan Meier analysis illustrated that the survivorship of *Quinqueloculina seminula* was higher than that of *Quinqueloculina poeyana*. Similarly, *Glabratellina* sp. survived longer than the *Ammonia* sp. Comparatively, the survivorship of rotaliids was higher than the miliolids as their pseudopodial network was observed even after the second day of remaining in a glass jar. The survival time for different species is shown in Figure 10.

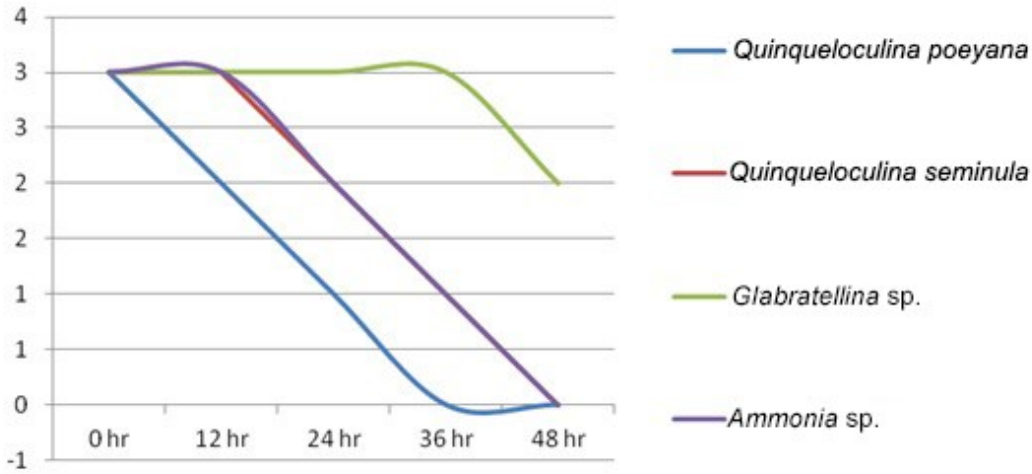

Figure 10: Kaplan-Meier analysis illustrating survival response for different species of rotaliids and miliolids.

Plastogamy

Among all living individuals, *Glabratellina* sp.1 is found to be involved in plastogamy during laboratory experiments. The observations revealed that 2 or more gamonts were joined together (not centered directly on one another) by their apertural sides to mutually exchange gametes. Initially, a cementing membrane bounded both individuals together resulting in the formation of a single chamber. By separating the individuals with needle, opposing apertural sides and internal septa were found to be dissolved (Figure 11-C).

Discussion

The sensitivity of benthic foraminifera towards different environmental parameters makes them a useful tool to understand past conditions. Their use as proxies is subjected to certain limitations, which can be achieved successfully by studying their biology in the modern marine environment as well as in laboratory condition. In this study, the biology of benthic foraminifera from different localities of the Arabian Gulf is investigated in terms of living behaviors and molecular characterization.

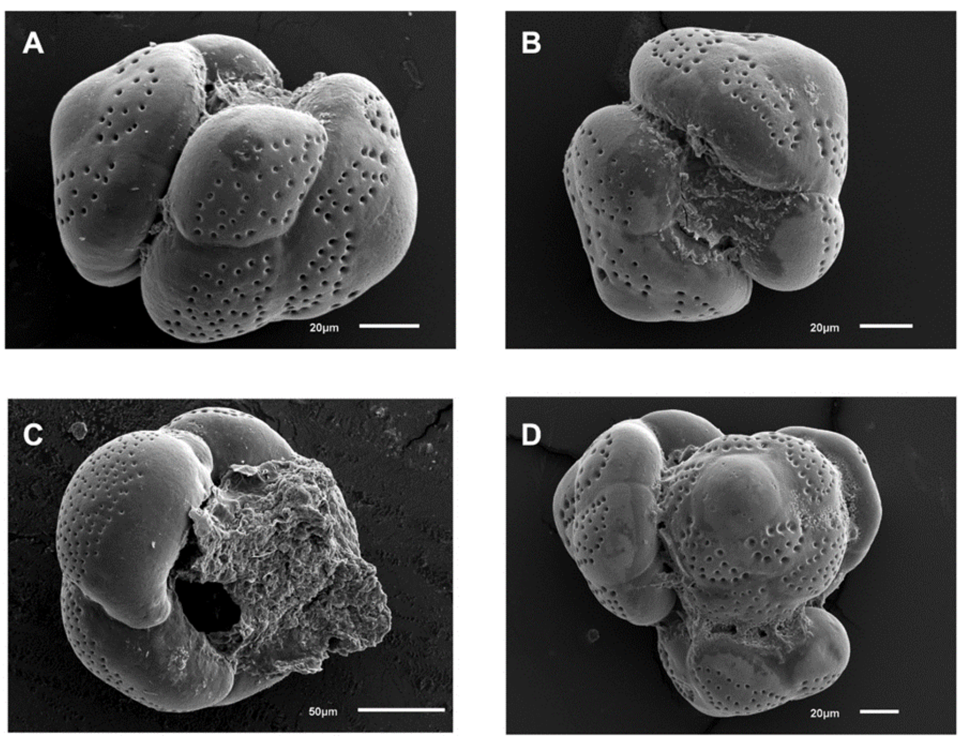

Figure 11: Plastogamy in *Glabratellina* sp.1 , (A-B) Plastogamous pair, attached by their apertural sides (C) Needle separated individual showing internal chamber and cementing material (D) Plastogamous complex of 5 individuals.

Results on living behaviors illustrate that rotaliids, mainly *Ammonia* and *Glabratellina*, were able to develop the pseudopodial network easily, compared to the miliolids (i.e., 8 hours vs. 24 hours respectively). This could be due to the reason that rotaliids are more adaptable to environmental changes than miliolids. Earlier research support this finding in terms of stressed environments where rotaliids were able to withstand unfavorable environs while miliolids were more sensitive to changing conditions (Gooday et al., 2009; Platon et al., 2005). Furthermore, a higher rate of movement was observed in rotaliids compared to miliolids as, in rotaliids, the pseudopodia extend out through all apertures along with the bidirectional movement of cytoplasmic streaming; whereas in miliolids, extension was observed from the primary aperture only. This bidirectional movement of protoplasm has also been discussed by Bowser and coworkers (Bowser and Bloodgood, 1984; Bowser et al., 1985). However, in either case, the individual was anchoring its pseudopodia to the wall of hard substrate resulting into dragging of their bodies in the direction of pseudopodia. The possible reason behind this attachment was to find the hard substrates in order to avoid inhospitable conditions. Gupta and coworkers reported the attachment of numerous foraminifera to vestimentiferan tube worms in cold hydrocarbon seeps in order to avoid the anoxic conditions at the sediment-water interface (Bernhard and Sen Gupta , 1999). Similarly, external light was also resulting in unfavorable conditions, consequently the individual was hiding underneath a substrate clump. It is also reported that light is the main factor controlling the distribution and growth of many species especially the ones bearing symbionts.

In addition to their locomotion, living individuals were observed to gather small sand particles around their bodies using their pseudopodia, which shows their innate behavior of living in a benthic environment. Furthermore, the living individuals were adhering to the hard biogenic substrate which may be providing them nutrition in addition to protection (Kitazato, 1988). The locomotion behavior is often observed for epifaunal species as they colonize the sediment surface or live attached to hard substrates that elevate them above the sediment surface (Linke and Lutze, 1993). In addition to this, during reproduction, organisms are recruited to a hard substrate when larvae settle onto the surface and survive metamorphosis to become adults (Goldstein, 1999). The high juvenile population of *Glabratellina* sp.1 is attributed to plastogamy, which is a most successful way of reproduction compared to asexual methods. Furthermore, it provides protection and ultimately high survival rate to gametes growing into zygotes. Myers (1938) also reported that a plastogamous pair allows zygotes grow to a 2- or 3-chambered stage which later emerge as juveniles from the breeding pair.

Acknowledgements

Funding for this project was provided by the Deanship of Scientific Research, King Fahd University of Petroleum & Minerals, under grant IN121028. We thank Eiichi Setoyama and Abduljamiu Amao for help with sample collection, Khalid Al-Ramadan for advice and logistical support, and Justin Parker for advice on the taxonomy of the benthic foraminifera.

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Citation: Arslan, M., Kaminski, M.A., and Khalil, A.B., 2016. Living Behaviors of Benthic Foraminifera from Eastern Bahrain and the Saudi Coastline. Bulletin of Environmental Studies 1(1), 1-9.