

## **Freeze-Drying Partially Affects Dead Benthic Foraminiferal Shells In Estuarine Sediments**

**Rajeev Saraswat\***, **Sujata R. Kurtarkar**, **S.M. Saalim**, **S.R. Bhadra**, **A.M. Gawas**  
Geological Oceanography Division, National Institute Of Oceanography, Dona Paula, Goa  
For Correspondence: \* rsaraswat@nio.org

### **Abstract**

Foraminiferal proxies are often used to understand both the pollution and past climatic changes. The freeze-drying is a viable alternative to commonly used method of oven-drying to process marine sediments for foraminiferal studies. The freeze-drying has, however, not yet been tested to process estuarine sediments to study living benthic foraminifera. Here, we assess the difference, if any, in benthic foraminiferal population after processing the estuarine sediments by both direct wet sieving, and freeze-drying followed by wet sieving. The surface sediments were collected from the Zuari estuary, Goa, India. Both the living and dead benthic foraminifera were picked. The sediments contain a large number of reworked foraminifera. The absolute abundance of both the calcareous and agglutinated dead benthic foraminifera was consistently lower in the sediments that were freeze-dried before wet sieving, as compared to the samples that were directly sieved, without any pre-treatment. Interestingly, the absolute abundance of living benthic foraminifera, as well as that of calcareous and agglutinated foraminifera was similar in both the freeze-dried and directly processed samples. The lower benthic foraminiferal abundance in the freeze-dried sediments suggests, breakage of comparatively fragile, thin-walled foraminifera tests. Therefore, estuarine sediments can be freeze-drying before wet-sieving to study living benthic foraminifera. The freeze-drying may, however, reduce the dead benthic foraminiferal count in the estuarine sediments.

**Keywords:** Benthic, foraminifera, processing, freeze-drying, calcareous, agglutinated, living

## Introduction

Foraminifera are extensively used to reconstruct the past climate (Saraswat *et al.*, 2017a) as well as for environmental monitoring (Nigam *et al.*, 2006; Bouchet *et al.*, 2012; 2017). The widespread applicability of benthic foraminifera for paleoclimatic reconstruction and pollution monitoring stems from their extreme sensitivity to even the short term changes in the ambient environment (Goineau *et al.*, 2012). Foraminiferal proxies for paleoclimatic studies are developed by comparing their modern distribution with the ambient environmental parameters (Manasa *et al.*, 2016; Saraswat *et al.*, 2017b; Dubey *et al.*, 2018a; 2018b). In such studies, the living benthic foraminifera, usually abundant in the top several centimeters of the sediments (Caulle *et al.*, 2015; Singh *et al.*, 2017), are preferred than their dead counterparts (Schönfeld *et al.*, 2012; Barras *et al.*, 2014; Saalim *et al.*, 2017).

Marine sediment samples are usually directly wet sieved to study living benthic foraminifera. By following this commonly followed methodology, the living benthic foraminiferal distribution is expressed either in unit area (Mojtahid *et al.*, 2009; Goineau *et al.*, 2012; Caulle *et al.*, 2015) or volume (Schumacher *et al.*, 2007; Mojtahid *et al.*, 2016) of surface samples. The absolute abundance of benthic foraminifera in dry sediments by weight, a reliable indicator of the total population, cannot be estimated after directly wet sieving the sediments. The difference in estimating living benthic foraminiferal abundance either in unit volume or area makes the inter-comparison of results difficult. The abundance in unit volume or area of sediments is also marred by the fact that the pore-water content of the top several centimeters of the marine sediments is very different. This approach further complicates the comparison of absolute abundance of living benthic foraminifera in vertical section, as the pore-water content as well as the density of the sediments, significantly changes with increasing depth. The varying water content in different sediment intervals significantly biases the living foraminifera abundance by volume. In such cases, only the relative abundance of benthic foraminifera can be compared. The usually followed procedure of oven-drying the sediments before wet sieving, cannot be applied to document living benthic foraminifera. The oven-drying causes hard lumps in organic matter rich fine-grained sediments. Such lumps are treated with hydrogen peroxide to oxidize the organic matter and sodium hexametaphosphate to dissociate the clay. The twin chemical treatment severely damages foraminiferal tests (Oda *et al.*, 1975, Hodgkinson, 1991). The H<sub>2</sub>O<sub>2</sub>, also oxidizes the stained organic matter of the living benthic foraminifera, and thus severely reduces the living benthic foraminiferal count.

The freeze-drying is a viable alternative to process marine sediments to recover calcareous microfossils (Kennedy and Coe, 2014). The freeze-drying can help to determine the absolute

abundance of living benthic foraminifera in unit weight, an age-old method to study changes in foraminiferal abundance (Murray, 2006). The advantage of freeze-drying is that it does not lead to hard lumps, even in organic matter rich fine-grained sediments. Thus, there is no need to treat the freeze-dried sediments with H<sub>2</sub>O<sub>2</sub> or sodium hexametaphosphate. A concern in the freeze-drying method is, however, the possible test breakage. The freeze-drying method has not been tested yet, to process sediment samples to study living (rose-Bengal stained) benthic foraminifera. Here we assess the suitability of freeze-drying followed by wet sieving method, as compared to direct wet sieving, to study living benthic foraminifera from estuarine samples.

### **Environmental Setting**

The Zuari is a tropical estuary in the Western Ghats of India. It is ~50 km long with the catchment area mainly in the lateritic terrain (Fig. 1). A large seasonal change in seawater temperature, salinity, flow pattern, dissolved oxygen, and nutrient concentration in the estuary is observed. The seasonal changes are mainly due to precipitation and subsequent land runoff from June to September, resulting in a huge freshwater influx into the estuary (Qasim and Gupta, 1981). The tides control the flow in the estuary throughout the year, except for the monsoon season. The tides uniformly influence the entire estuary, except the last 10 km stretch towards the upstream end. The mean water level at the upstream end increases by 1.6 m during the wet season (Shetye *et al.*, 1995). The dissolved oxygen concentration varies from 2.95 ml/l to 6.97 ml/l and is inversely related to the waxing and waning tide (Qasim and Gupta, 1981). The salinity during the sampling period varied from 32.01 psu to 34.24 psu (Figure 2). A corresponding change in pH of the ambient water was also observed with a range of 7.798 to 7.990 (Fig. 2). The salinity was high towards the seaward end of the estuary and decreased upstream. The drop in salinity was concomitant with a drop in pH (Fig. 2). The suspended particulate matter is the highest during the monsoon season (Rao *et al.*, 2011). The particulate organic carbon towards the seaward side of the Zuari estuary is low (5.2-8.7%) during the dry season as compared to that during the wet season (7.3 to 10.6%). The organic carbon content of the sediments is lower than that of the overlying suspended matter. The organic matter is dominated by the terrestrial soil-derived fraction during the wet season (Shynu *et al.*, 2015).

### **Materials And Methodology**

A total of 12 surface sediment samples were collected from the mouth of the Zuari estuary in the Goa state of India (Fig. 1). The sediments were collected by a Van Veen grab sampler, onboard a trawler, during the pre-monsoon season of the year 2016. The water depth varied from 4.5 m to 8.5 m (Table

1). The top two centimeter sediments were transferred to storage vials and immediately stained with ethanol rose-Bengal solution (2 g/L) (Walton, 1952). The ambient seawater parameters (salinity, pH) were measured onboard, by using a portable hand-held multi-parameter meter (Thermo Orion Star A329). The precision of the multi-parameter was 0.01 psu for salinity and 0.001 for pH. After three weeks, the stained sediment samples were split into two aliquots. One half was directly wet sieved by using a 63  $\mu\text{m}$  sieve. The other half of sediments was first freeze-dried, weighed and then wet sieved by using a 63  $\mu\text{m}$  sieve. The dried coarse fraction ( $>63 \mu\text{m}$ ) was weighed and stored in plastic vials. The living and dead benthic foraminifera were picked from the weighed representative coarse fraction obtained from both the directly wet sieved as well as freeze-dried followed by wet sieved samples. The dead benthic foraminifera were separated into calcareous and agglutinated forms. The dead foraminifera also contained reworked tests. The abundance of living dead, calcareous, agglutinated and reworked foraminifera was calculated in a unit weight of dry sediment. The absolute abundance of living benthic foraminifera in directly wet-sieved samples was calculated by using the coarse fraction per gram dry sediment data of the freeze-dried samples.

## Results

The absolute abundance of dead benthic foraminifera varied from 1 individual/g sediment to 423 individuals/g sediment in freeze-dried samples and 6 individuals/g sediment to 692 individuals/g sediment in samples that were directly wet sieved (Table 1). The absolute abundance of dead benthic foraminifera was higher in directly wet sieved samples as compared to the samples that were freeze-dried before wet sieving (Fig. 3A). The absolute abundance in both the samples was comparable, only at a couple of stations (Station Number 3, 8). A similar consistent pattern was not observed in the absolute abundance of living benthic foraminifera (Fig. 3B). The absolute abundance of living benthic foraminifera (varying from being absent to 103 individuals/g sediment in freeze-dried samples and 1 individuals/g sediment to 129 individuals/g sediment in directly wet sieved samples) was low as compared to the dead benthic foraminifera. The absolute abundance of living benthic foraminifera was higher in directly wet sieved samples at station number 1, 4-8, 10 and 11, and lower than the freeze-dried samples at station number 2, 3, 9, 11 (Fig. 3B). The difference in absolute abundance of living benthic foraminifera, between directly wet sieved and samples that were freeze-dried before wet sieving, was lower than that in the dead benthic foraminifera. The absolute abundance of dead calcareous benthic foraminifera was higher in all but a couple of (Station Number 1, 3), directly wet sieved samples (Fig. 4A). As compared to the dead calcareous benthic foraminifera, the absolute abundance of living calcareous benthic foraminifera was higher in directly

wet sieved samples, only at station number 1, 4-6, 10, 12. The abundance in directly wet sieved samples was lower than the samples that were freeze-dried before wet sieving, at station number 2-3 and 9. At station number 7 and 11, the absolute abundance of living calcareous benthic foraminifera was similar in both the samples (Fig. 4B).

The absolute abundance of dead agglutinated benthic foraminifera was also much higher in directly wet sieved samples at all the stations (Fig. 5A). At three stations (station number 4, 7 and 12), dead agglutinated benthic foraminifera were only found in directly wet sieved samples. The absolute abundance of living agglutinated benthic foraminifera was an order of magnitude lower than their dead counterparts. However, like living calcareous benthic foraminifera, we do not observe a consistent difference in absolute abundance of living agglutinated benthic foraminifera in samples that were directly wet sieved as compared to the samples that were freeze-dried before wet sieving (Fig. 5B). Like the dead agglutinated benthic foraminifera, at a couple of stations (Station Number 5, 6), even the living agglutinated benthic foraminifera were found only in directly wet sieved samples. The absolute abundance of living agglutinated benthic foraminifera at these stations was, however, very low. The dead benthic foraminiferal population also included reworked benthic foraminifera. Interestingly, the absolute abundance of reworked benthic foraminifera was higher in freeze-dried samples at 8 out of the 12 stations (Fig. 6). At four stations (station number 8-10, 12), even though the absolute abundance of reworked benthic foraminifera in directly wet sieved samples was higher than the samples that were freeze-dried before wet sieving, the difference in the two, was small.

## **Discussion**

The pronounced decrease in absolute abundance of dead benthic foraminifera in samples that were freeze-dried before wet sieving is attributed to the breakage of thin-walled, fragile and physically weaker tests due to freeze-thawing (Hodgkinson, 1991). Marine sediment samples are either directly wet sieved or oven-dried before wet sieving to concentrate living (rose-Bengal stained) benthic foraminifera, (Lee *et al.*, 2015). The oven-drying causes hard lumps in fine-grained organic matter rich sediments (Thomas and Murney, 1985). The sediments have to be chemically treated to dissociate the lumps. The chemical treatment affects the foraminiferal population as well as the isotopic and elemental composition of the calcareous tests (Serrano *et al.*, 2008; Feldmeijer *et al.*, 2013). Therefore, there is a need to develop an alternative method to process marine sediments to study living (rose-Bengal stained) benthic foraminifera. A few other workers have suggested freeze-

drying the coarse fraction obtained after directly wet-sieving the samples, as it helps in long term storage of living benthic foraminifera (Duijnste *et al.*, 2003).

A large seasonal fluctuation in ambient physico-chemical parameters, especially the salinity and pH also adversely affects the calcareous tests in the shallow marine environments (Berkeley *et al.*, 2007; Milker *et al.*, 2015). The low pH in estuaries that are influenced by fresh-water influx is detrimental to benthic foraminiferal tests (Saraswat *et al.*, 2015). The low pH-induced diagenetic changes in the sediments, dissolve the dead calcareous tests (Murray and Alve, 1999), thus weakening it to withstand thawing during the freeze-drying process. Additionally, the transport of dead tests during tidal flushing causes large wear and tear of the tests (Murray, 1983; Barbieri, 2001). The organic cement of agglutinated benthic foraminifera in the shallow waters quickly oxidizes, thus weakening the tests. At the same time, the under-saturation of pore-waters with respect to calcium carbonate results in partial to complete dissolution of calcareous benthic foraminifera (Berkeley *et al.*, 2007).

The severity of the detrimental effect of freeze-drying in agglutinated benthic foraminifera is attributed to the minimal cementing material, thus rendering them inherently weak for thawing (de Rijk and Troelstra, 1999). The higher abundance of reworked foraminifera in freeze-dried samples is intriguing. The surface sediments contain benthic foraminiferal tests, pale in colour, with a dull luster, abraded surface, occasionally pyritized, suggesting their old age and transport from the pre-existing formation on the banks of the estuaries. Such, reworked foraminifera are common in both the Zuari and Mandovi estuaries (Nigam *et al.*, 2005). The appearance of such benthic foraminifera suggests that these specimens are recently reworked. The minimal adverse effect of freeze-drying on reworked foraminifera is attributed to their entirely filled tests. A majority of the reworked benthic foraminiferal tests are entirely filled with secondary material. The infilling reduces the cracks/gaps that act as potential breakage points due to thawing during freeze-drying.

The robust nature of the living benthic foraminiferal tests and the presence of organic matter inside it, reduces the adverse effect of freeze-drying induced thawing. Additionally, the living benthic foraminifera can sustain extreme pH, detrimental enough to completely wipe off the dead tests, without any substantial damage (Engel *et al.*, 2015). The presence of organic matter ensures that both the calcareous and agglutinated living benthic foraminifera can withstand freeze-drying, without any significant preferential damage. Therefore, freeze-drying the sediments before wet sieving is a viable alternative to study living benthic foraminifera. The response to both the methods, however, has to be checked for sediments collected from different oceanic setting.

## Conclusion

We report that the living (rose-Bengal stained) benthic foraminiferal population is not significantly affected, even if the sediments are freeze-dried before washing. The freeze-drying of sediments before wet-sieving helps in estimating the absolute abundance of benthic foraminifera in sediments by weight. The freeze-drying, however, partially affects the dead benthic foraminiferal population in the estuarine setting. Both the calcareous and agglutinated benthic foraminifera are affected by freeze-drying, the effect being more pronounced in agglutinated foraminifera.

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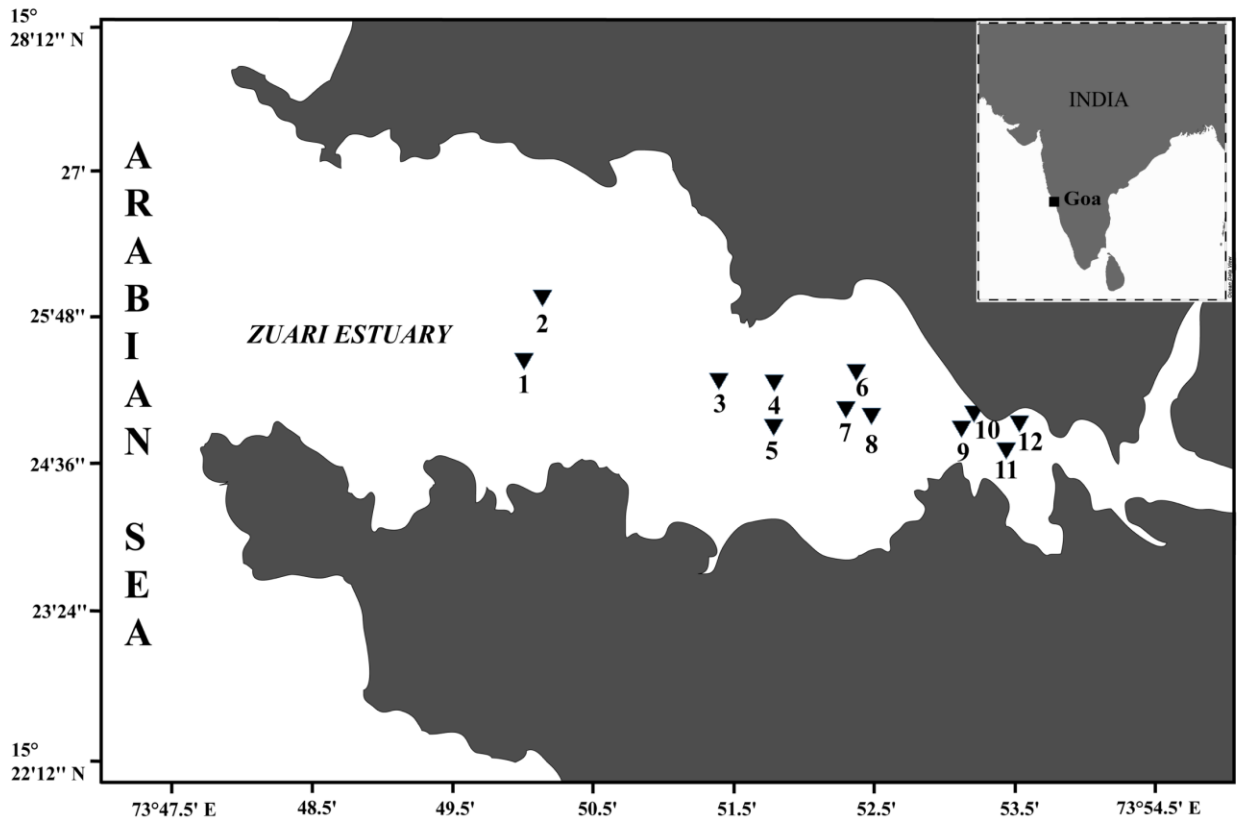
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### Legend to Figures and Table

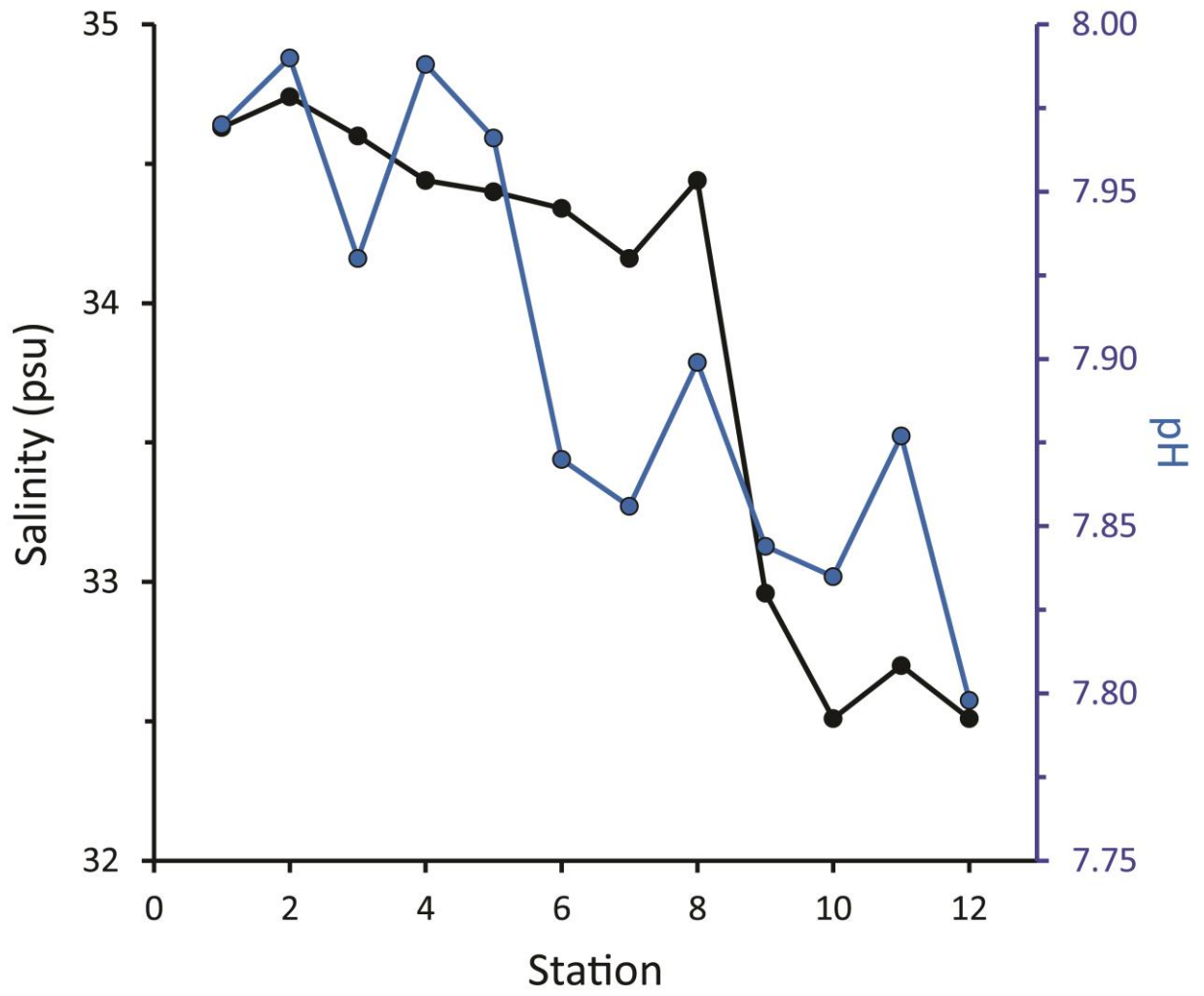
- Figure 1:** Location of surface sediment samples in the Zuari estuary in the Goa state of India. The inset map shows the sample location on the western margin of India.
- Figure 2:** The ambient seawater salinity and pH at all the stations.
- Figure 3:** Absolute abundance of the dead (A) and living (B) benthic foraminifera in the directly wet sieved as well as the samples that were freeze-dried before wet sieving.
- Figure 4:** Absolute abundance of dead (A) and living (B) calcareous benthic foraminifera in the directly wet sieved as well as the freeze-dried followed by wet sieving samples.
- Figure 5:** Absolute abundance of dead (A) and living (B) agglutinated benthic foraminifera in the directly wet sieved as well as the freeze-dried followed by wet sieving samples.
- Figure 6:** Absolute abundance of reworked benthic foraminifera in the directly wet sieved as well as the freeze-dried followed by wet sieving samples.
- Table 1:** Details of surface sediment samples as well as the characteristics of benthic foraminifera at each station. DO- dissolved oxygen (mL/L), FD- freeze-dried, DW- directly wet-sieved.

**Table 1**

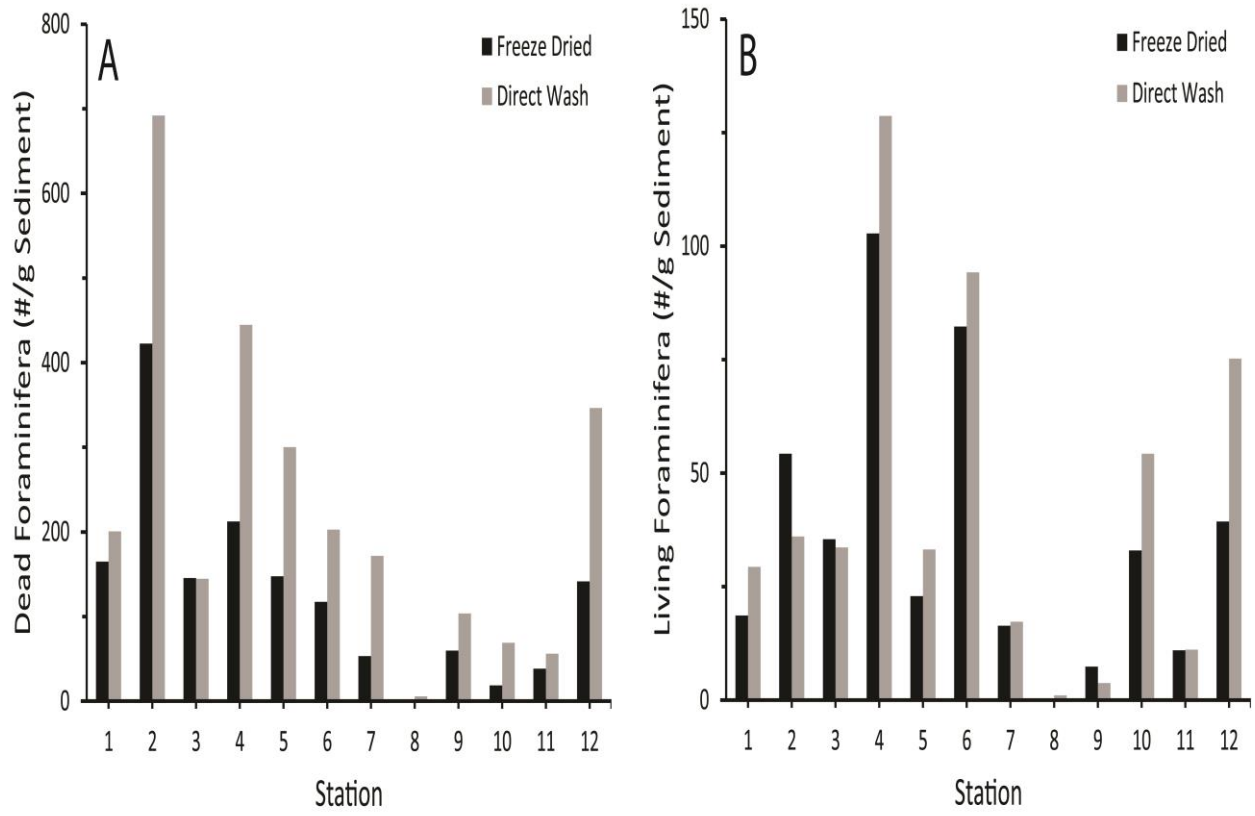
Station	Latitude (°N)	Longitude (°E)	Depth (m)	DO (mL/L)	Salinity (psu)	pH	Dead/g Sediment		Living/g Sediment	
							(FD)	(DW)	(FD)	(DW)
1	15°25.308	73°49.812	6.5	6.2	34.13	7.970	165	201	19	29
2	15°25.835	73°49.981	6.0	6.9	34.24	7.990	423	692	54	36
3	15°25.166	73°51.499	5.5	5.6	34.10	7.930	146	145	35	34
4	15°25.064	73°51.947	6.0	4.8	33.94	7.988	212	445	103	129
5	15°24.733	73°51.953	6.0	5.7	33.90	7.966	148	300	23	33
6	15°24.937	73°52.604	6.0	5.9	33.84	7.870	117	203	82	94
7	15°25.126	73°52.676	4.5	4.9	33.66	7.856	53	172	16	17
8	15°24.844	73°52.799	5.0	5.6	33.94	7.899	1	6	0	1
9	15°24.683	73°53.613	5.0	8.6	32.46	7.844	60	104	7	4
10	15°24.894	73°53.726	6.5	8.4	32.01	7.835	19	69	33	54
11	15°24.624	73°53.986	5.0	8.2	32.20	7.877	38	56	11	11
12	15°24.823	73°54.064	8.5	5.6	32.01	7.798	141	346	39	75



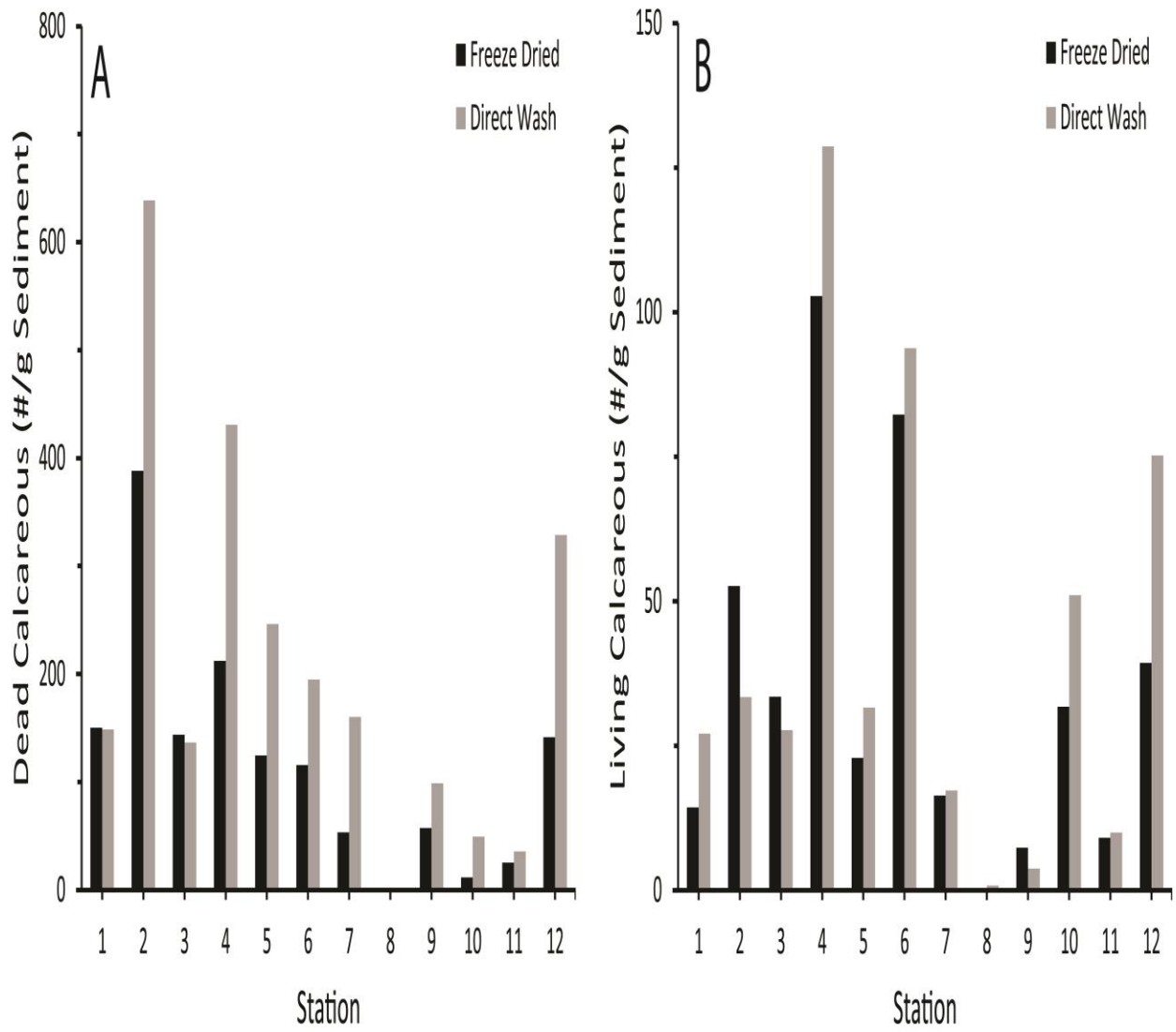
**Figure 1**



**Figure 2**

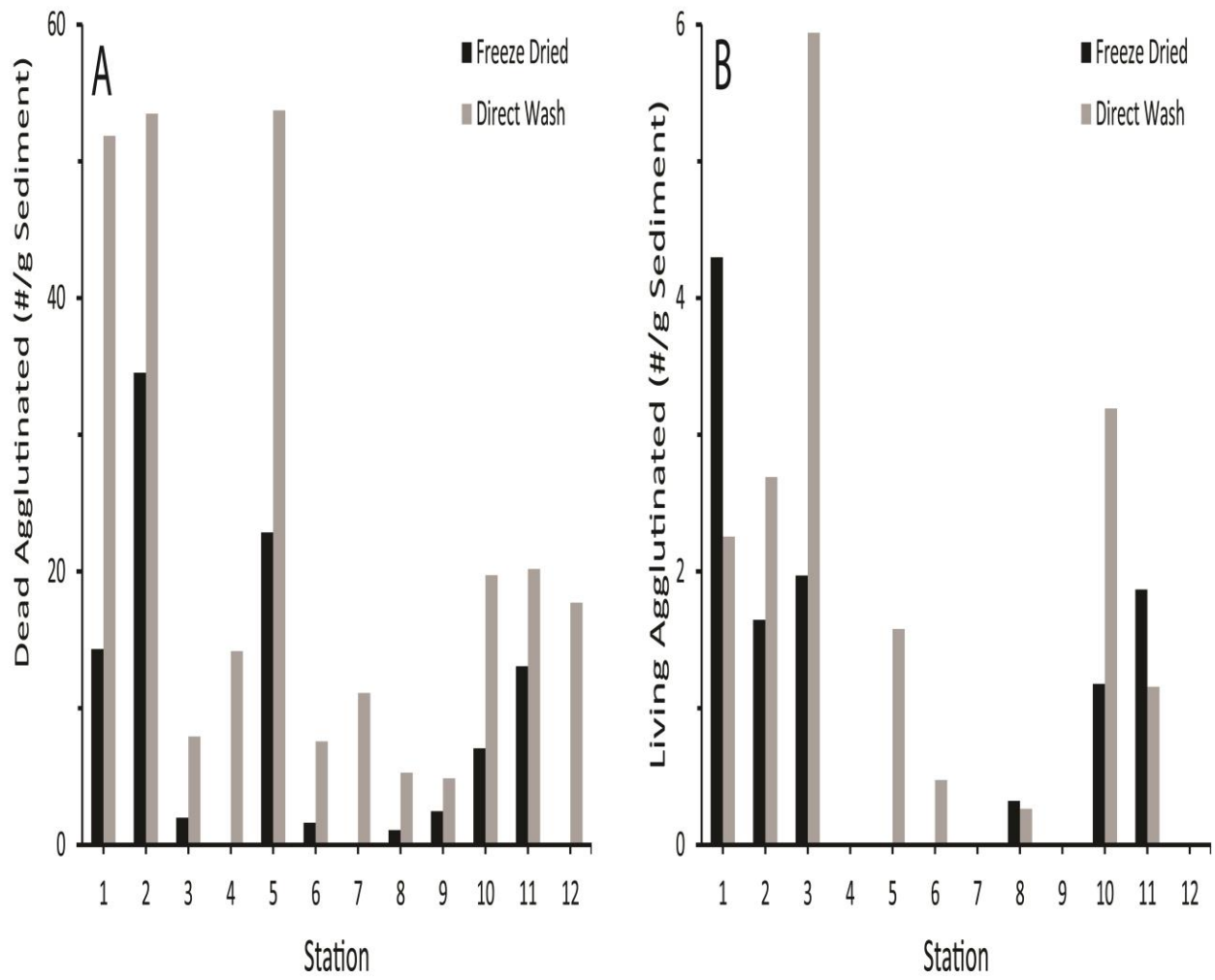


**Figure 3**

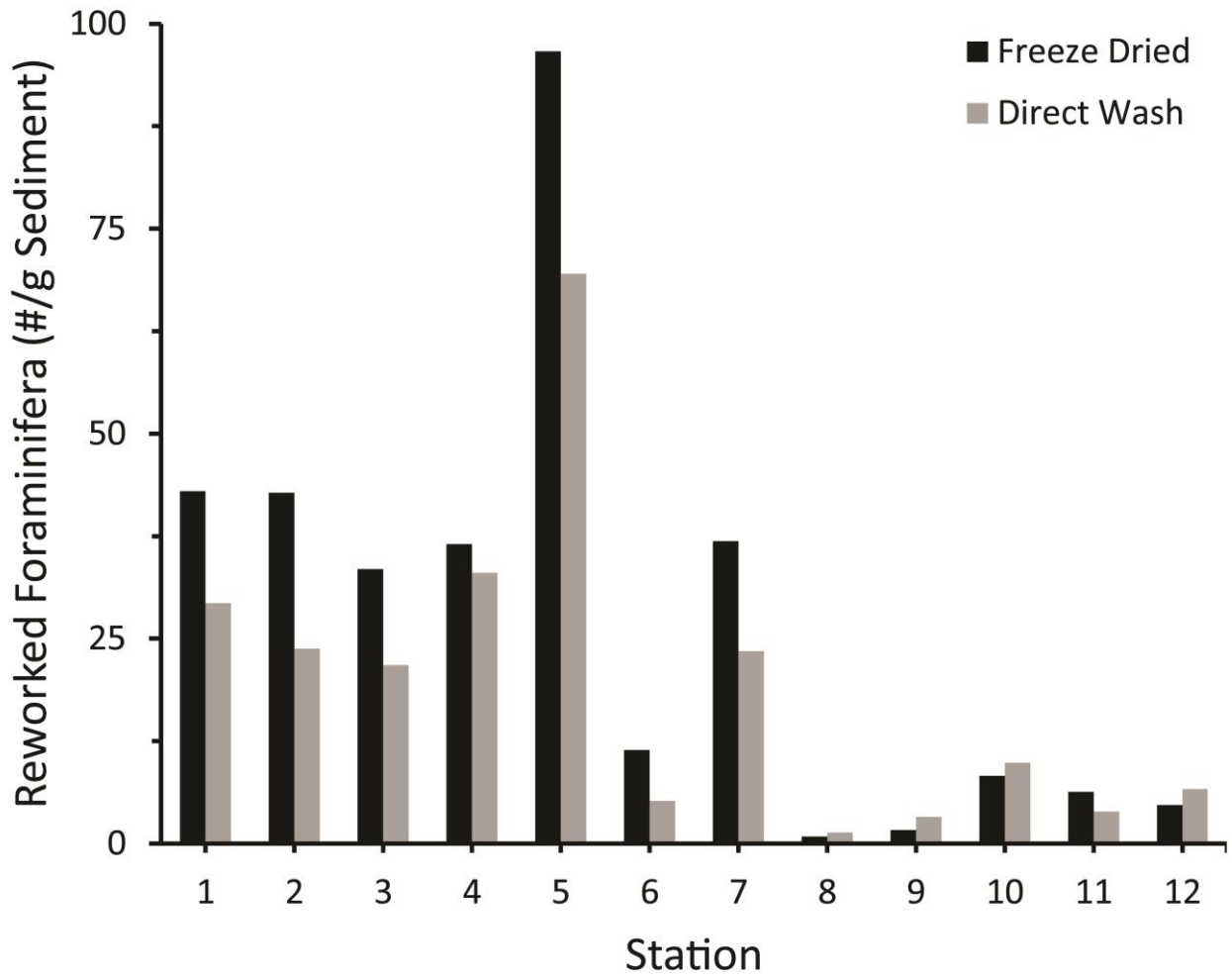


**Figure 4**





**Figure 5**



**Figure 6**