Hypoxia Tolerance of Benthic Foraminifera in Tampa Bay, FL

By

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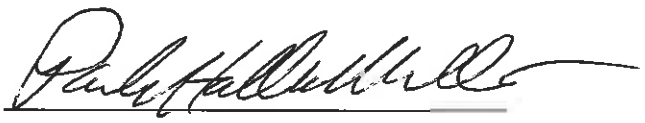

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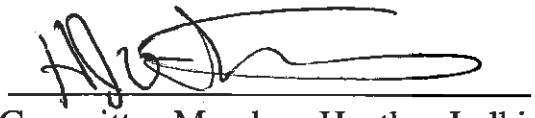

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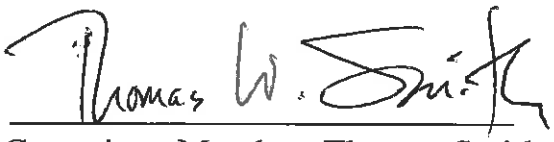

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HYPOXIA TOLERANCE OF BENTHIC FORAMINIFERA IN TAMPA BAY, FL

Protists, some of the most diverse and intriguing organisms on the planet, have managed to occupy niches in virtually every possible environment. It has been said that their diversity is "near-imponderable," with numbers of extant species likely pushing the boundary of 300,000 or more (Foissner 1999, 2009). Within the group, the Foraminifera are some of the most interesting and readily obtained organisms for study. All that is required to observe them is to take sample of sediment from any beach and sort through it under low magnification on a dissecting microscope. A brief scan reveals their intricate shapes standing out against the sea of miniature quartz crystals and the collection process begins.

Foraminifers are fascinating organisms that, at first glance, do not make this apparent. Given a brief 24hr rest period after collection, their remarkable ability to move about is clear. They are easily identified on the sides of a container as small specks that are undoubtedly alive. Sometimes they are even clinging to the surface tension of the water. This movement is made possible by their "reticulopodial network (RPN)," wherein cytoplasmic dexterity presents itself through trunk filopodia (Travis & Allen 1981). The filopodia are in a continual state of flux, where they anastomose, fuse, and branch at a rapid rate to facilitate the movement of the creature. This is all made possible through their remarkable ability to build and extend microtubules within their cytoplasm to serve as a framework upon which the RPN operates. The RPN is so capable that it is even used for the capture of prey items by most foraminifers. *Ammonia tepida*, a classically herbivorous foraminifer, is able to use its RPN to ensnare and

consume metazoans such as copepods, nematodes and even larval gastropods (Dupuy et al. 2010).

Foraminifers are predominantly marine, found in the sediments of the oceans and coastal habitats in great abundance. They are typically benthic and test-building, either from extracted elements dissolved in the water or in agglutinated forms which collect material from their surroundings. However, not all are benthic, test-building or even marine. Many species are planktic, a few do not build tests of any kind, some left the marine realm to occupy freshwater and, recently, it appears some may be terrestrial (Holzmann et al. 2003; Lejzerowicz et al. 2010). They have existed as a group since as early as the Neoproterozoic, with small subunit rRNA phylogenetic analyses suggesting they are among the first eukaryotes to bear mitochondria (McIlroy et al. 2001; Pawlowski et al. 1996). Their existence for such time and their tendency to bear tests capable of surviving through the geologic record has resulted in their extensive use in stratigraphy (Gregory 1973).

The phylum Foraminifera encompasses immense diversity within itself, with everything from the smallest of calcareous-shell-building organisms up to the large deepwater xenophyophores (Pawlowski et al. 2003). This diversity in form and function is in part what makes foraminifers useful to microfossil studies. Foraminifers of particular niches are very distinct, such as those living in the sediments barely offshore and those living in estuarine or marsh environments. Scott and others (2003) capitalized upon this particular dichotomy in an effort to pinpoint when in the recent past hurricanes made landfall in the US state of South Carolina. They took sediment cores and analyzed the layers for their foraminiferal assemblages, elucidating the times in which offshore-dominant species were briefly abundant in a given layer, suggesting a substantial overwash of sediments into the marsh by hurricane. In a similar vein, a

foraminifer-dependent microfossil study by Mathewes and Clague (1994) examined the history of large earthquakes in the US Pacific Northwest. Again, cores were taken and the team used the presence or absence of particular foraminifers to gain insight into the possible occurrence of sudden uplift as a result of major seismic activity.

Adding to their geologic role, the Foraminifera produce large amounts of sediment through their discarded tests after death. Langer (2008) estimates all benthic and planktic foraminifers together produce nearly 25% of the $CaCO_3$ in the world's oceans annually. Even when the percentage of $CaCO_3$ produced by foraminifers is not the highest in a given community, they can still make substantial contributions to the sediment. A coral sand cay in Australia was found to be maintained in large part by benthic foraminifers, wherein approximately 30% of sediment produced in and around the cay was foraminifer tests (Yamano et al. 2000). These are astonishingly high percentages, especially when considering the generally microscopic scale of foraminifers.

Their carbonate sediment production is a testament to their influence on the biogeochemical cycles of the ocean. As calcifying organisms, they are not immune to the effects of ocean acidification. To disrupt the foraminiferal populations of the ocean would wreak havoc on the system. Already, anthropogenic ocean acidification has been demonstrated to cause thinning of foraminiferal shells (De Moel et al. 2009; Guinotte & Fabry 2008). This is disconcerting, as foraminifers are useful indicators, reflecting the status and habitability of the environment around them.

The geologic importance of foraminifers is nearly boundless, with their role as environment-tagging organisms of distinct value. In addition to their ability to indicate the past, we rely on them to help predict the future. Earth's human population is exponentially growing.

We are moving and damming rivers, connecting bodies of water that would otherwise be isolated, spewing vast amounts of chemicals, gasses and other entities into the environment, among numerous other activities. It is of no surprise that this has had a cascade of deleterious effects. However, great strides have been made to curb the incredible influence our species has on the planet through campaigns, technological advancements and a host of other actions. As a part of this, we are continually monitoring the environments we have damaged in an attempt to better understand what capacity to hold life they still retain. This is where foraminifers are highly applicable. The tests discarded after death are typically well preserved and thus, with a core sample, an examination can reveal the assemblages at any given point in time. Using lead and radiocarbon dating, it is possible to develop an understanding of the conditions of an environment at any point in the past such that a baseline for pristine (pre-human impact) conditions can be established (Schönfeld et al. 2012).

The Foraminifera are excellent bioindicators. Studies have demonstrated the use of foraminifers in determining the impacts of deforestation on waterways, general water and sediment quality, heavy metal and PAH concentrations, trace elements, and a variety of other pollutants (Du Châtelet et al. 2004; Frontalini et al. 2009; Luan & Debenay 2005; Ross 2012). The FORAM (Foraminifera in Reef Assessment and Monitoring) index is calculated based upon the numbers of particular types of foraminifers found in a given area and scales how conducive an environment is to supporting algal-symbiotic-dominant communities (Hallock et al. 2003). This index is extremely useful in that it can be calculated quickly and affordably by nearly anyone with access to a stereomicroscope to rapidly determine the quality of the environment at hand. Foraminiferal assemblages can be used to characterize the nature of oxygen-stressed environments through the use of the AEI (*Ammonia-Elphidium* Index), which is based solely

upon its two namesake genera (Sen Gupta et al. 1996). The two genera are both tolerant of hypertrophic conditions, however *Ammonia* can withstand the hypoxia better than *Elphidium*.

Expanding on this, foraminifers were recently found to be able to fall into a state of dormancy upon exposure to toxins (Ross 2012). This is of particular interest, as it suggests a potential for foraminifers to survive temporary drastic shifts in habitat quality and be able to rebound with abundance once conditions improve. Ross's original study was limited in its scope of detailing the nature of foraminiferal dormancy in that it was not the study's primary objective and it only focused on *Amphistegina gibbosa*, a relatively large, symbiont-bearing foraminifer.

Another environmental stressor, hypoxia, is a notoriously detrimental affair for marine ecosystems. It is often the cause of large-scale fish kills and various environmental breakdowns. Foraminifers are highly influenced by the condition, given that most require oxygen for survival. Bouchet and others (2012) suggest there is a "strong link between the benthic foraminiferal diversity and the bottom-water oxygen gradient" in the North Sea foraminiferal assemblages, lending credence to the notion. Platon and others (2005) demonstrated that worsening of the seasonal hypoxia in Louisiana forced the near disappearance of the genus *Quinqueloculina* from certain areas, while other taxa, interestingly, appeared unaffected overall. Coinciding with this study, Blackwelder and others (1996) found the foraminifer *Buliminella morgani*, a particularly hypoxia-tolerant organism, to increase toward the upper end of recent cores in the Mississippi River plume. In an additional study, *Elphidium magellicum* and *Stainforthia fusiformis* appeared to handle anoxic, or at least extremely hypoxic, conditions for five months, enough time to outlast all other assemblages and prevent their establishment (Gustafsson & Nordberg 1999).

This study will delve further into the ability of the foraminifers to survive significantly disruptive events by going dormant. Specifically, I will attempt to determine if any foraminifers

are capable of transitioning to and from dormancy when subjected to hypoxic conditions for one month's time. I hypothesize that more taxa than those outlined above are able to survive sudden hypoxia for at least four weeks' time through a temporary dormancy mechanism.

METHODS

SAMPLE COLLECTION

Locations for sampling were selected through an overview of satellite imagery, colleague recommendation, prior exploratory testing, and a quest for variety (Figures 1 and 2). Established criteria were:

- 3m depth or less
- Accessible by kayak or foot after traveling
- Within fifty kilometers of Egmont Key at the mouth of Tampa Bay, FL
- Either highly likely to experience periods of depleted oxygen levels (e.g., shallow, secluded harborage) or unlikely to experience periods of depleted oxygen levels (i.e., coastal open-water)

As a result, locations selected included:

Table 1
Locations of samples taken with GPS coordinates

Location Code	General Area	Latitude	Longitude
BP1	Mullet Key – Canoe Launch	N 27° 37.375'	W 82° 44.080'
LP2	Lassing Park	N 27° 45.295'	W 82° 37.807'
GDY	Gandy Bridge Causeway	N 27° 46.922'	W 82° 35.668'
MKB	Mullet Key Bayou	N 27° 37.641'	W 82° 43.897'
MKG	Mullet Key – Gulf Side	N 27° 36.783'	W 82° 44.243'
VPK	Vinoy Park	N 27° 46.922'	W 82° 37.498'

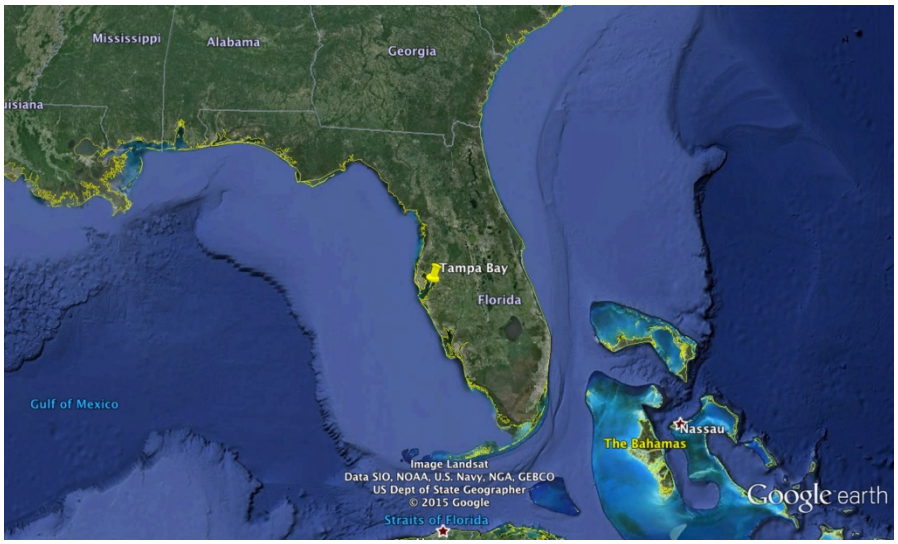

Figure 1
Small scale view of Tampa Bay, FL locale

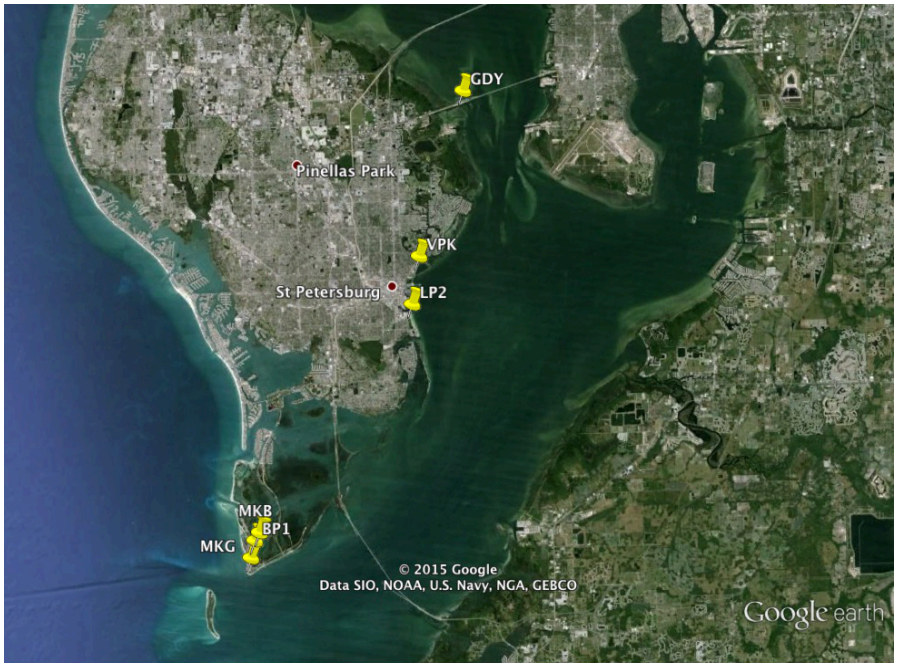

Figure 2
Large scale view of Tampa Bay and stations

Figure 3
Close-up view of stations near the mouth of Tampa Bay

Sediment sampling was conducted using a custom-designed stainless steel four-section grid (Figure 4). This grid holds approximately 9cm^3 of material in each quarter and samples the top 2.5cm of sediment. The grid was pressed down into the seafloor by hand until the top became level with the surrounding bottom. Then, a stainless steel spatula was slid underneath the grid such that all sections are effectively capped at the bottom. The grid was then covered by hand on its top and carefully brought to the surface for processing at the field base.

Figure 4
Stainless steel grid

Sample Processing

One 9cm^3 subsample from each stainless grid taken was sieved on-site through 2mm and 63µm sieves. That which was trapped in the 2mm sieve was washed with water from the sampling station into a labeled petri dish. The flow-through was captured and passed through the

63µm sieve, where the trapped material was also washed into a labeled petri dish. The finest portion that passed through the 63µm sieve was discarded as foraminifers smaller than 63µm in size are exceedingly difficult to identify to the species level with a high degree of confidence. Additionally, the 63µm lower limit maximizes the useful assemblage obtained and reduces the discard to a minimum (Schröder et al. 1987). This sample served as the control, as it was never subjected to hypoxia beyond that which was present in the environment at the time of sampling. All petri dishes were then placed in a cooler to protect from the elements until the end of the collection day.

Two of the $9cm^3$ samples were flushed into airtight blue pop-top containers on-site, with the final fourth $9cm^3$ sample flushed into a small petri dish and kept in long-term storage for future sampling or reference. The containers were filled to the top with water from each station, sealed, and covered with aluminum foil to prevent light entry and to induce hypoxia. Upon completion of the field excursion, all samples from all sites were placed into an environmental chamber at 25°C with a 12hr photoperiod. One blue pop-top container from each station was left in the chamber for one week, and the other for four weeks. After the prescribed time, the containers were then opened and received the same sieving treatment as the control.

For all subsamples, including those processed in the field, a 72hr recovery period in the environmental chamber was administered following sieving. Upon completion of the recovery period, subsamples were stained for 48-72hrs using rose Bengal at a concentration of $1g\ L^{-1}$ 70% EtOH.

Following staining, all samples were washed until the rinse water ran clear with deionized water onto double-layered coffee filters and allowed to air dry in a vent hood for 72-96hrs. Once drying was complete, all samples and grades of material were massed and

subsequently stored in plastic vials. Prior to picking, sediments were split on a shaker table using a microsplitter such that a fraction between 0.5-1g was obtained. This was then massed for use in later calculations and set aside for picking.

Picking of the Foraminifers

The <1g fraction of material was spread out onto a gridded glass petri dish under a dissecting microscope at 12-50X magnification. Using a damp 000 artist's paintbrush, foraminifers were hand picked from the sediment and placed onto a micropaleontological slide coated with an approximately 5% Elmer's glue-water mixture for adhesion. Two such slides were prepared: one for foraminifers classifiable as living at the time of staining and one for all others. In categorizing specimens, the following rules were observed:

- Miliolid specimens must exhibit staining within the chambers of the test and have little or no staining on the exterior to be labeled as living
- *Ammonia* and *Elphidium* specimens must appear yellowish throughout the majority of the older chambers, with only the last two or three staining pink to be counted as living
- Broken foraminifers are classified as nonliving
- In all other foraminifers, if staining is abundant around the aperture or inside and essentially absent elsewhere, it is categorized as living

For each subsample, all foraminifers were picked from the fraction of sediment and placed at a density of 1-2 individuals per cell on the micropaleontological slide. Once all foraminifers had been removed from the fraction, it was stored in a separate, identified plastic vial for future reference.

IDENTIFICATION

Foraminifers on each slide were sorted such that each taxon present was confined to a specific cell on each microslide. This served to allow direct comparison between individuals and thus facilitate distinguishing between highly similar taxa. Where possible, SEM images from Poag (2015) were used for identifying to the species level. Some taxa collected were not in Poag (2015) and so, secondarily, Bock (1971) was used. In certain instances, a dilute, approximately 5% green food coloring, dye mixture was used to emphasize ill-defined morphological features to aid in identification. Broken foraminifers were not included in the counts for analysis – despite being collected and stored – due to inability to identify fragments to even the level of genus. In some cases, the differences among taxa of certain genera were so minute (e.g., *Ammobaculites*) that identification only to this level was possible.

RESULTS

Data from this experiment were insufficient as to test the hypothesis with any statistical significance. Live foraminiferal numbers collected were all far below the standard of 300 specimens. From three of the six sampling sites, total foraminifers numbered less than 12 individuals per gram of sediment. These three sites (GDY, VPK and MKG) were eliminated from the remainder of the project due to the lack of data. The remaining three stations each yielded higher numbers of foraminifers, though in total yielded no more than an average of 160 individuals per gram per subsample (Table 2). Overall, foraminiferal abundance was rather low, with treatments yielding anywhere between 25 and 177 foraminifers per gram of sediment (Table 4). Thus, further analysis and characterizations by indices were based upon station foraminiferal totals, as opposed to treatment totals.

Table 2
Average foraminiferal densities (individuals/gram sediment) by station

Average Absolute Densities by Station			
	LP2	**BP1**	**MKB**
Density (ind./g)	62	41	160

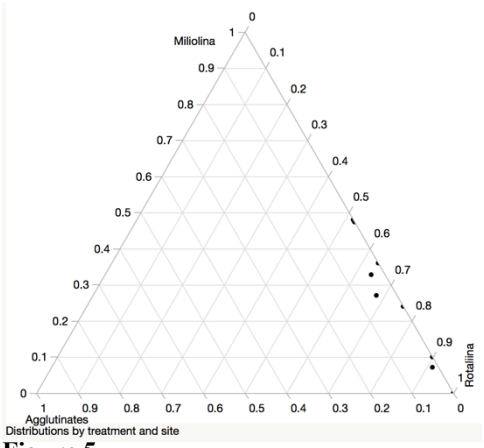

Figure 5
Ternary plot of assemblages by treatments and site

As depicted in Figures 5 and 6, the Miliolina and Rotaliina were well-represented throughout all treatments and stations, accounting for, on average, >99% of the taxa present. Agglutinated foraminifers were notably absent or rare across all sites, averaging a mere 0.64% overall representation.

The *Ammonia-Elphidium* index (Table 3) was chosen because of the large abundance of both genera in all stations sampled and used as a metric for quantifying the hypoxic nature of the sampling sites (Sen Gupta et al. 1996). Simpson's diversity index, *D*, as defined by:

$$D = \frac{\Sigma n(n-1)}{N(N-1)}$$

where n = number of individuals of a given taxon and N = total number of individuals of all taxa, was used in determining the diversity at each station. Simspon's diversity index values ranged from 0.33 at site LP2 to 0.14 at site MKB (Table 3). In addition, richness values increased with decreasing Simspon's diversity index values, ranging from 5.3 at site LP2 up to 12.3 at site MKB (Table 3). The number of individual taxa present increased with increasing

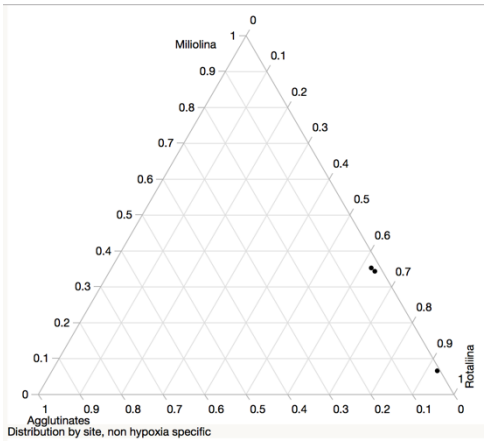

Figure 6
Ternary plot of assemblages by station

Table 3
Indices characterizing the sampled foraminiferal assemblages by station
*Unknown miliolid (Table 4) removed from analyses

Average Indices by Station			
Index	**LP2**	**BP1**	**MKB***
Ammonia-Elphidium	66	92	69
Simpson's Diversity	0.33	0.21	0.14
Species Richness	5.3	8.3	12.3

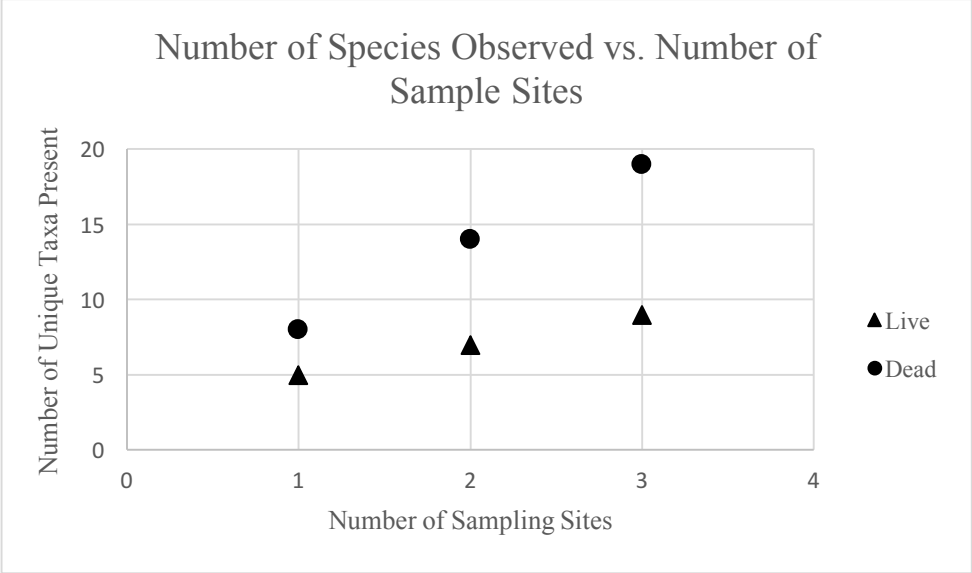

Figure 7
Plot of species richness as a function of number of sampling sites

numbers of sites sampled (Figure 7). This trend was observed both in the live and dead assemblages of each station.

Overall, a minimum of 19 taxa were observed in total across all stations. Of particular note is the abundance of *Palmerinella palmerae* at stations MKB and BP1 (Table 4). This particular taxon was also found to survive at least some exposure to hypoxia, with a density of eight living individuals per gram sediment even after four weeks of hypoxia at station MKB (Table 4).

Table 4
Absolute abundance of individual taxa per gram sediment for all treatments – Dead counts enclosed in parenthesis

Live and (Dead) Absolute Abundance per Gram Sediment by Species									
Station	**LP2**			**BP1**			**MKB**		
Species	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**
Affinetrina bermudezi	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ammobaculites sp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (3)
Ammonia parkinsoniana	4 (18)	3 (30)	1 (34)	0 (13)	2 (8)	9 (19)	2 (21)	0 (37)	4 (33)
Bolivina lowmani	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (5)	0 (0)	0 (0)
Broken Miliolids	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (4)	0 (0)	0 (0)	0 (0)
Elphidium galvestonense	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (2)	5 (0)	0 (6)	0 (8)
E. poeyanum	5 (8)	0 (12)	0 (20)	0 (1)	0 (0)	0 (0)	5 (19)	0 (2)	3 (0)
Haynesina germanica	0 (6)	1 (12)	1 (16)	0 (0)	0 (3)	0 (6)	0 (12)	4 (8)	0 (9)
Liebusella soldanii	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)	0 (3)
Miliolinella labiosa	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)	0 (2)	0 (0)
M. subrotunda	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)
Palmerinella palmerae	0 (0)	0 (0)	0 (0)	0 (3)	0 (5)	0 (4)	2 (16)	0 (31)	8 (36)
Quinqueloculina bosciana	0 (0)	1 (3)	0 (1)	0 (1)	1 (1)	1 (1)	16 (16)	10 (20)	4 (18)
Q. impressa	0 (0)	0 (0)	0 (0)	0 (8)	0 (2)	2 (4)	0 (12)	0 (0)	0 (0)
Q. poeyanum	0 (0)	0 (0)	0 (0)	1 (0)	0 (1)	0 (0)	0 (0)	2 (6)	1 (0)
Q. seminula	0 (0)	3 (0)	0 (2)	0 (1)	0 (1)	3 (4)	5 (12)	2 (12)	1 (8)
Rosalina bahamaensis	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (7)	0 (6)	0 (6)
Triloculina linneiana	0 (0)	0 (0)	0 (0)	0 (4)	0 (0)	0 (1)	0 (14)	0 (0)	0 (8)
T. oblonga	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (7)	0 (0)	0 (0)
Trochammina japonica	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (3)
Unknown Miliolid	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)
Standing Crop	**8 (32)**	**8 (58)**	**2 (77)**	**1 (31)**	**3 (23)**	**16 (48)**	**35 (142)**	**18 (129)**	**21 (136)**

DISCUSSION

MKB, a small mangrove island in the "No Internal Combustion Engine" zone of Mullet Key Bayou, yielded the greatest average density of foraminifers at 160 per gram of sediment (Table 2). It was the most secluded of stations and likely only rarely experienced any major erosive events. Interestingly, this site was not the least hypoxic by AEI. LP2, a shallow beach near the port of St. Petersburg dotted with "No Swimming" signs, was the least hypoxic and least diverse by both Simpson's Diversity index and species richness (Table 3). *Ammonia* and *Elphidium* were heavily dominant at this station, an observation consistent with those of Poag (2015) and Dix (2001). Throughout all treatments, it was consistently demonstrated that at least some foraminifers were able to tolerate hypoxia for four weeks (Table 4). Of note, site MKB had very little change in the densities of live and dead foraminifers between one and four weeks of hypoxia, whereas site BP1 actually had an increase in the number of surviving foraminifers by the end of the four weeks of hypoxia (Table 4). It is possible that from either of these sites, foraminifers flushed in during major storm events may have been in a dormant state due to the relatively high hypoxia. Following the 72hr recovery period, these foraminifers may have resumed normal activity levels.

Rose Bengal staining is not a perfect system, in that it is known to stain nonliving foraminiferal cytoplasm (Bernhard et al. 2006). On this note, Walker and others (1974) suggest the tendency to stain nonliving foraminifers is, in some cases, a result of the detritus that clings to the tests after death. In light of this, the criteria were put in place in an attempt to reduce the possibility of categorizing a dead foraminifer as living. If anything, the strict nature of the criteria likely resulted in some foraminifers that were alive at the time of staining being labeled as dead. This may have contributed to the dearth of picked living foraminifers, thereby

artificially reducing the number of foraminifers that were able to survive the various hypoxic treatments.

The methodology of this investigation proved problematic especially in that the sampling sites chosen yielded insufficient foraminiferal abundance. Some of the sites had been quickly scanned for foraminifers prior to collection of sediments, though the density of foraminifers had not been determined. Part of the reasoning behind having collected and treated the foraminifers in their natural benthic microenvironment was to establish a baseline for hypoxia tolerance similar to what may be found in the field. In light of the limited abundance, a different study examining the tolerance of specific taxa to hypoxia in a controlled, sterile substrate may be more appropriate for characterizing such a baseline. This could be accomplished by picking live foraminifers from wet sediment samples and setting them into new, prescribed microenvironments for treatments.

However, this is not to entirely discount the original investigation's techniques. As a pilot study, it demonstrates the ability to subject foraminifers and their associated microenvironments to periods of hypoxia with simplicity. With more resources than a single investigator, sieving and picking foraminifers from low-density substrates would no longer be a major constraint, as it would be possible to work through volumes much greater than the 0.5-1g examined in this study in short periods of time. Given the densities of foraminifers from the sites sampled in this study, volumes of sediment around 10g may be necessary to reach 300 foraminifers for analysis on sites at the edges of Tampa Bay. One method for processing such large volumes of sediment makes use of heavy liquids to separate out low-density populations of foraminifers and is described by Murray (2014).

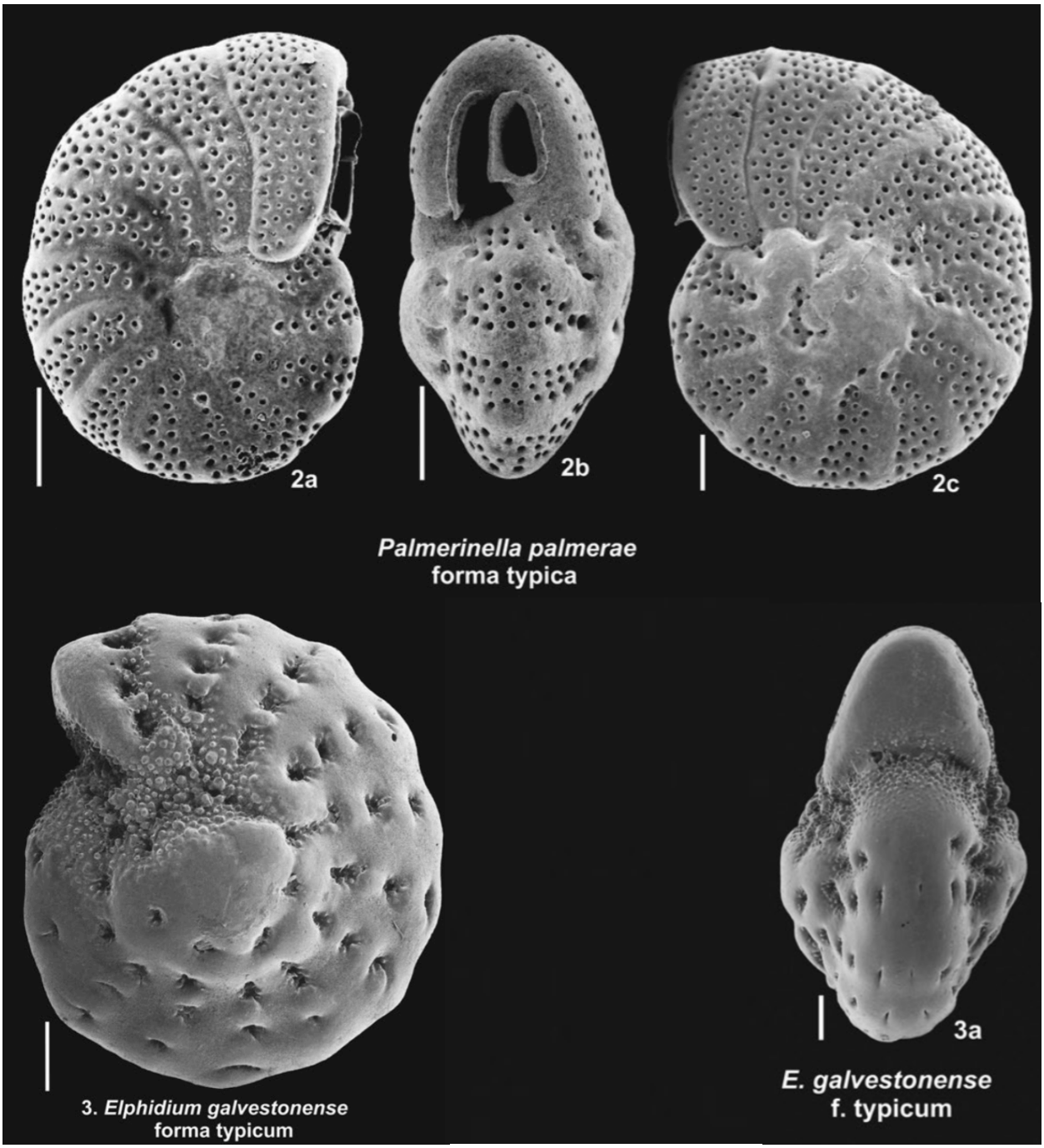

Figure 8
SEM images (100µm scale bar) of *Palmerinella palmerae* f. typica and *Elphidium galvestonenense* f. typicum for comparison; adapted from Poag (2015).

Of particular interest in this study was the abundance of *Palmerinella palmerae* at stations BP1 and MKB. Poag (2015) indicates *P. palmerae* to be a "generally minor, but distinctive component" of assemblages of estuarine margins of the Gulf of Mexico; my

observations are consistent with this. Both sampling sites where *P. palmerae* were present were at the margins of Tampa Bay in estuarine, mangrove-dominated habitats and *P. palmerae* was generally clearly present, but not typically the dominant taxon. In line with this, the foraminiferal assemblage of site BP1 was dominated primarily by *Ammonia parkinsoniana* and *P. palmerae*, with notably fewer *Elphidium* spp. compared to other sites. This is worth looking into further, as the potential for an index employing abundance of *Palmerinella* may be possible to develop as another hypoxia metric.

In addition, *P. palmerae* has had very little, if any, biological review in scientific literature. Poag (1978) describes *P. palmerae* as exhibiting two distinct ecophenotypes in San Antonio Bay, Texas defined along salinity and temperature curves of the bay. Here, *P. palmerae* is demonstrated to survive periods of hypoxia lasting for up to four weeks, an indication of its tolerance to poor water quality and potential ability to remain dormant under stressful conditions. None of the studies Poag (2015) reviews indicate a population of *P. palmerae* in Tampa Bay and Dix (2001) does not report finding even the genus *Palmerinella* in the 75 Tampa Bay cores used in his study. It is possible *P. palmerae* was present for some of these studies, though was misidentified as *Elphidium galvestonense*. The two taxa appear highly similar when viewed only under a dissecting microscope and are extremely challenging to separate. Figure 8 uses SEM images of both taxa from Poag (2015) and displays them alongside each other for comparison. The profiles of both taxa are remarkably similar and each has a distinctive boss at its center, further complicating the identification process.

CONCLUSIONS

This study exemplifies how simple it is to subject an array of foraminifers to environmental stressors and successfully segregate them based upon rose Bengal staining. In

addition, it serves to characterize the foraminiferal assemblages some of the different benthic environments around the west side of Tampa Bay. In addition, it explores the possibility of treating foraminifers in their respective microenvironments within a lab setting. It is feasible, though not without abundant time or more than one investigator. There is indication that the developed coastal areas of Tampa Bay are seemingly extremely hostile environments for foraminifers, as foraminiferal densities were extremely low for half of the sampling sites. Finally, this study is the first of its kind to test the tolerances of *Palmerinella palmerae* to hypoxia and elucidates its ability to survive under such conditions for extended periods of time.

APPENDIX

Table 5
Total relative abundance of foraminiferal groups by subsample

Total Relative Abundance (as Percent) by Group									
Station	**LP2**			**BP1**			**MKB**		
Group	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**
Agglutinates	0	0	1	0	0	2	0	0	3
Miliolina	0	27	4	73	28	35	52	54	29
Rotaliina	100	73	96	27	72	63	48	46	68

Table 6
Total absolute abundance of foraminifers per gram sediment by group

Total Absolute Abundance by Group			
	Station		
Group	**LP2**	**BP1**	**MKB**
Agglutinates	1	2	6
Miliolina	10	38	96
Rotaliina	143	71	171

Table 7
Total relative abundance of foraminifers as percentage of each group

Relative Abundance by Group (as Percent)			
	Station		
Group	**LP2**	**BP1**	**MKB**
Agglutinates	1	2	2
Miliolina	6	34	35
Rotaliina	93	64	63

Table 8
Relative abundances of each taxon for all treatments

Live and (Dead) Relative Abundance, as Percent, by Species									
Station	**LP2**			**BP1**			**MKB**		
Species	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**	**No Hypoxia**	**1w Hypoxia**	**4w Hypoxia**
Affinetrina bermudezi	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ammobaculites sp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)
Ammonia parkinsoniana	43 (56)	33 (52)	50 (44)	0 (42)	67 (36)	60 (39)	7 (15)	0 (29)	19 (25)
Bolivina lowmani	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (3)	0 (0)	0 (0)
Broken Miliolids	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (9)	0 (0)	0 (0)	0 (0)
Elphidium galvestonense	0 (0)	0 (0)	0 (0)	0 (0)	0 (5)	0 (4)	13 (0)	0 (5)	0 (6)
E. poeyanum	57 (26)	0 (20)	0 (26)	0 (4)	0 (0)	0 (0)	13 (13)	0 (2)	13 (0)
Haynesina germanica	0 (19)	17 (20)	50 (21)	0 (0)	0 (14)	0 (13)	0 (8)	22 (6)	0 (7)
Liebusella soldanii	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (4)	0 (0)	0 (0)	0 (2)
Miliolinella labiosa	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)	0 (2)	0 (0)
M. subrotunda	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)
Palmerinella palmerae	0 (0)	0 (0)	0 (0)	0 (8)	0 (23)	0 (9)	7 (11)	0 (24)	38 (26)
Quinqueloculina bosciana	0 (0)	17 (5)	0 (1)	0 (4)	33 (5)	7 (2)	47 (11)	56 (15)	19 (13)
Q. impressa	0 (0)	0 (0)	0 (0)	0 (25)	0 (9)	13 (9)	0 (8)	0 (0)	0 (0)
Q. poeyanum	0 (0)	0 (0)	0 (0)	100 (0)	0 (5)	0 (0)	0 (0)	11 (5)	6 (0)
Q. seminula	0 (0)	33 (0)	0 (3)	0 (4)	0 (5)	20 (9)	13 (8)	11 (9)	6 (6)
Rosalina bahamaensis	0 (0)	0 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (5)	0 (5)	0 (5)
Triloculina linneiana	0 (0)	0 (0)	0 (0)	0 (13)	0 (0)	0 (2)	0 (10)	0 (0)	0 (6)
T. oblonga	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (5)	0 (0)	0 (0)
Trochammina japonica	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)
Unknown Miliolid	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)

REFERENCES

Bernhard, J. M., D. R. Ostermann, D. S. Williams, and J. K. Blanks. "Comparison of two methods to identify live benthic Foraminifera: A test between rose Bengal and CellTracker Green with implications for stable isotope paleoreconstructions." *Paleoceanography* 21.4 (2006): DOI: 10.1029/2006PA001290

Blackwelder, P., T. Hood, C. Alvarez-Zarikian, T. A. Nelsen, and B. Mckee. "Benthic Foraminifera from the NECOP study area impacted by the Mississippi River plume and seasonal hypoxia." *Quaternary International* 31 (1996): 19-36.

Bock, W.D., 1971, A handbook of benthonic foraminifera of Florida Bay and adjacent waters. In: J.I. Jones and W.D. Bock (Editors), A Symposium of Recent South Florida Foraminifera. Miami Geol. Soc. Mem., v. 1, p1-72.

Bouchet, V. M. P., E. Alve, B. Rygg, and R. J. Telford. "Benthic Foraminifera provide a promising tool for ecological quality assessment of marine waters." *Ecological Indicators* 23 (2012): 66-75.

De Moel, H., G. M. Ganssen, F. J. C. Peeters, S. J. A. Jung, D. Kroon, G. J. A. Brummer, and R. E. Zeebe. "Planktic foraminiferal shell thinning in the Arabian Sea due to anthropogenic ocean acidification?" *Biogeosciences* 6.9 (2009): 1917-925.

Dix, Thomas L. *The Distribution and Ecology of Benthic Foraminifera of Tampa Bay, Florida*. Thesis. University of South Florida, (2001). Tampa, FL: U of South Florida, 2001, 197 pp.

Du Châtelet, É. Armynot, J-P. Debenay, and R. Soulard. "Foraminiferal proxies for pollution monitoring in moderately polluted harbors." *Environmental Pollution* 127.1 (2004): 27-40.

Dupuy, C., L. Rossignol, E. Geslin, and P.-Y. Pascal. "Predation of mudflat meio-macrofaunal metazoans by a calcareous foraminifer, *Ammonia tepida* (Cushman, 1926)." *The Journal of Foraminiferal Research* 40.4 (2010): 305-12.

Foissner, W. "Protist diversity: estimates of the near-imponderable." *Protist* 150.4 (1999): 363-368.

Foissner, W. "Protist diversity and distribution: some basic considerations." *Protist Diversity and Geographical Distribution*. Springer Netherlands, 2009. 1-8. Web.

Frontalini, F., C. Buosi, S. Da Pelo, R. Coccioni, A. Cherchi, and C. Bucci. "Benthic Foraminifera as bio-indicators of trace element pollution in the heavily contaminated Santa Gilla Lagoon (Cagliari, Italy)." *Marine Pollution Bulletin* 58.6 (2009): 858-77.

Gregory, M. R. "Benthonic Foraminifera from a mangrove swamp, Whangaparapara, Great Barrier Island." *Tane* 19 (1973): 193-204.

Guinotte, J. M., and V. J. Fabry. "Ocean acidification and its potential effects on marine ecosystems." *Annals of the New York Academy of Sciences* 1134.1 (2008): 320-342.

Gustafsson, M., and K. Nordberg. "Benthic foraminifera and their response to hydrography, periodic hypoxic conditions and primary production in the Koljö fjord on the Swedish west coast." *Journal of Sea Research* 41.3 (1999): 163-178.

Hallock, P., B. H. Lidz, E. M. Cockey-Burkhard, and K. B. Donnelly. "Foraminifera as bioindicators in coral reef assessment and monitoring: the FORAM index." *Coastal Monitoring through Partnerships*. Springer Netherlands, 2003. 221-238.

Holzmann, M., A. Habura, H. Giles, S. S. Bowser, and J. Pawlowski. "Freshwater foraminiferans revealed by analysis of environmental DNA samples." *The Journal of Eukaryotic Microbiology* 50.2 (2003): 135-39.

Langer, M. R. "Assessing the contribution of foraminiferan protists to global ocean carbonate production." *Journal of Eukaryotic Microbiology* 55.3 (2008): 163-169.

Lejzerowicz, F., J. Pawlowski, L. Fraissinet-Tachet, and R. Marmeisse. "Molecular evidence for widespread occurrence of Foraminifera in soils." *Environmental Microbiology* 12.9 (2010): 2518-526.

Luan, B. T., and J.-P. Debenay. "Foraminifera, environmental bioindicators in the highly impacted environments of the Mekong Delta." *Hydrobiologia* 548.1 (2005): 75-83.

Mathewes, R. W., and J. J. Clague. "Detection of large prehistoric earthquakes in the Pacific Northwest by microfossil analysis." *Science* 264.5159 (1994): 688-691.

McIlroy, D., O. R. Green, and M. D. Brasier. "Palaeobiology and evolution of the earliest agglutinated Foraminifera: Platysolenites, Spirosolenites and related forms." *Lethaia* 34.1 (2001): 13-29.

Murray, John William. *Ecology And Palaeoecology Of Benthic Foraminifera*. London: Routledge, (2014). *Discovery eBooks*.

Pawlowski, J., I. Bolivar, J. F. Fahrni, T. Cavalier-Smith, and M. Gouy. "Early origin of Foraminifera suggested by SSU RRNA gene sequences." *Molecular Biology and Evolution* 13.3 (1996): 445-50.

Pawlowski, J., M. Holzmann, J. Fahrni, and S. L. Richardson. "Small subunit ribosomal DNA suggests that the Xenophyophorean Syringammina corbicula1 is a foraminiferan." *The Journal of Eukaryotic Microbiology* 50.6 (2003): 483-87.

Platon, E., B. K. Sen Gupta, N. N. Rabalais, and R. E. Turner. "Effect of seasonal hypoxia on the benthic foraminiferal community of the Louisiana inner continental shelf: The 20th century record." *Marine Micropaleontology* 54.3-4 (2005): 263-83.

Poag, C. Wylie. "Paired Foraminiferal Ecophenotypes in Gulf Coast Estuaries--Ecologic and Paleoecologic Implications." *Gulf Coast Association of Geological Societies Transactions* 28 (1978): 395-421. *AAPG Datapages*.

Poag, C.W. 2015. Benthic Foraminifera of the Gulf of Mexico: Distribution, Ecology and Paleoecology. Texas A&M University Press, College Station, Texas, 239 pp.

Ross, Benjamin J. *Responses to Chemical Exposure by Foraminifera: Distinguishing Dormancy From Mortality*. Thesis. University of South Florida, 2012. Tampa, FL: U of South Florida, (2012).

Scott, D. B., E. S. Collins, P. T. Gayes, and E. Wright. "Records of prehistoric hurricanes on the South Carolina coast based on micropaleontological and sedimentological evidence, with comparison to other Atlantic coast records." *Geological Society of America Bulletin* 115.9 (2003): 1027.

Schönfeld, J., E. Alve, E. Geslin, F. Jorissen, S. Korsun, and S. Spezzaferri. "The FOBIMO (FOraminiferal BIo-MOnitoring) initiative—towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies." *Marine Micropaleontology* 94-95 (2012): 1-13.

Schröder, C. J., D. B. Scott, and F. S. Medioli. "Can Smaller Benthic Foraminifera Be Ignored in Paleoenvironmental Analyses?" *The Journal of Foraminiferal Research* 17.2 (1987): 101-05.

Sen Gupta, B. K., R. E. Turner, and N. N. Rabalais. "Seasonal oxygen depletion in continental-shelf waters of Louisiana: Historical record of benthic foraminifers." *Geology* 24.3 (1996): 227-230.

Travis, J. L., and R. D. Allen. "Studies on the motility of the Foraminifera. I. Ultrastructure of the reticulopodial network of Allogromia laticollaris (Arnold)." *The Journal of cell biology* 90.1 (1981): 211-221.

Walker, D. A., A. E. Linton, and C. T. Schafer. "Sudan Black B; a superior stain to Rose Bengal for distinguishing living from non-living Foraminifera." *The Journal of Foraminiferal Research* 4.4 (1974): 205-215.

Yamano, H., T. Miyajima, and I. Koike. "Importance of Foraminifera for the formation and maintenance of a coral sand cay: Green Island, Australia." *Coral Reefs* 19.1 (2000): 51-58.