IT'S NOT EASY BEING BROWNISH-GREEN: EXPLORING THE RELATIONSHIP BETWEEN SIZE AND ENDOSYMBIONT POPULATION IN THE LARGER, ALGAE BEARING FORAMINIFERA *SORITES DOMINICENSIS*

by

Benjamin J. Ross

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Susan Richardson, and has been approved by the members of her/his supervisory committee. It was submitted to the faculty of The Honors College and was accepted in partial fulfillment of the requirements for the degree of Bachelor of Arts in Liberal Arts and Sciences.

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Abstract

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Sorites dominicensis is a common epiphytic foraminifera living throughout the Carribean and South Florida, and is commonly found living on turtle grass, *Thalassia Testudinum*. *S.dominicensis* plays host to algal symbionts related to those found in coral. Estimates for the numbers in these symbiotic populations are few, of limited scale, and vary widely. In this thesis we performed a large scale survey of the populations of algal symbionts living within the *S. dominicensis* population of Jupiter Sound. We then used this data to propose a linear model for the relationship between foraminiferal size and endosymbiont population, and to suggest explanations for the variability seen in the Jupiter Sound population.

To Dad
Thanks.

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Introduction

What are foraminifera?

Foraminifera are a monophyletic clade of unicellular, largely marine eukaryotes united by physiological and genetic synapomorphies, namely the presence of granuloreticulopodia and a set of certain unique insertions in their ribosomal DNA. Phylogenetically, foraminifera are currently considered to be closely related to the clade Cercozoa, a group that includes an assortment of eukaryotes characterized by pseudopodia and the formation of single chambered tests (Nikolaev et al, 2004). Foraminifera, on the other hand, are unique within the larger, more inclusive clade Rhizaria in that the derived foraminiferal clades secrete very complex, multichambered tests, a trait unseen in other rhizarians (Richardson 2006).

What is Sorites?

Sorites dominicensis (Figure 1) is a largely epiphytic species of marine foraminifera, which can commonly be found living on seagrass blades and macroalgae throughout Bermuda, the Caribbean, and South Florida. In South Florida especially, it is often found attached to the broad, flat blades of turtle grass, *Thalassia testudinum*.

What are its symbionts?

S. dominicensis, like many of the largest, most complex foraminifera, is host to intracellular algal symbionts. Foraminifera have been observed to host a number of different symbiont types, including diatoms, red and green algal symbionts, and dinoflagellates. *Sorites dominicensis* itself possesses dinoflagellate endosymbionts of the genus *Symbiodinium* (Figure 2), which includes zooxanthellae, the photosymbionts seen in stony and soft corals (Pochon and Pawlowki 2006; Richardson 2006).

These symbiotic relationships are not necessarily exclusive; foraminiferal species that play host to diatoms, for instance, have been known to host any of a large number of different diatom species, and some soritines have been observed to contain red cyanobacteria, instead of the more common dinoflagellates (Lee, 2006), and some soritids are known to have secondary symbionts. Even the basic mechanisms for symbiont recognition in *Symbiodinium*-soritine symbiosis are not well understood, being shown to involve different signaling molecule and receptor system than seen in the more studied diatom bearing foraminifera (Lee and Reyes 2006). The identity of the symbionts is further clouded by the difficulty of identifying *Symbiodinium* species, which lack discernible morphological features, especially in the non-motile phases found occupying symbiotic hosts (LaJeunesse 2001).

Importance of endosymbionts

Regardless of their identity, the presence of these symbionts is important to the health of *Sorites*. Endosymbiosis with photosynthetic symbionts has been suggested to be one of the major features allowing for the original evolution of the "larger" foraminifera in which it is common (Lee et al. 1979). This seems to be especially true in soritids, which are host to six *Symbiodinium* groups not generally found in other *Symbiodinium*-bearing hosts. This is thought to be the result of not only a selective recognition system, but vertical transmission from parent to offspring and biogeographical isolation. Vertical transmission ensures that parents and offspring are host to the same symbionts, while isolation increases speciation (Pochon and Pawlowski 2006; Garcia-Cuetos et al 2005).

The symbionts provide an additional source of energy for the foraminiferan. The

endogenous, photosynthetically derived energy from the *Symbiodinium*, combined with the feeding activities of the foraminiferan itself give the individual a surplus of energy beyond simple respiratory and upkeep needs, allowing it to divert more of its energy on growth, contributing to the characteristically larger size. The symbionts, and their additional energy, also work to enhance calcification in the test (Hallock 2000).

These symbionts are integral to the health of the foraminiferan. Although research shows that many species of algal-bearing foraminifera, including *S. dominicensis,* cannot grow while starved even while given access to light, suggesting that, for some species at least, photosynthesis is not enough to keep individuals healthy and growing (Lee 2006). Individuals of large, algal-bearing foraminifera genera, such as *Amphistegina* (which possess endosymbiotic diatoms) that either lack symbionts completely or possess them in limited numbers, have exhibited severe detrimental effects, including test-breakage, test deformation, lesions on the test surface, and reproductive dysfunction (Talge and Hallock 1995).

The incidences of these effects are becoming more common due the increasing occurrence of foraminiferal bleaching. Bleached foraminifera appear completely white or with patchy coloration; this coloration is a result of the loss of their algal symbionts, the photosynthetic pigments of which were responsible for the foraminiferan's coloration. Foraminiferal bleaching has been well documented in *Amphistegina gibbosa*, the Florida Keys population of which was first observed to have undergone bleaching in 1991 (Talge and Hallock 1995; Toler and Hallock 1998; Toler et al. 2001). Although bleaching in *Amphistegina* has been the most studied, it has also more recently been observed in *Sorites dominicensis* (Figure 3). Bleaching has been observed in Belize, in the Florida

Keys (Richardson, Ross, Gaston 2007, unpublished observations) and, originally, in a population in Jupiter Sound, Florida (Richardson 2006). When originally observed, this population had up to a 16% incidence of total or partial bleaching. Prolonged observation of this population after bleaching had been first observed was made impossible after the original population was decimated by two hurricanes in September of 2004. *Sorites dominicensis* has only recently returned in strength to this site, and this population is the focus of the present study.

The implications of foraminiferal bleaching events have been highlighted by a number of authors. Mostly they arise from the susceptibility of foraminifera to environmental changes, including anthropogenic effects. According to Hallock (2000), foraminifera with algal symbionts can be very susceptible to increasing nutrient flux; symbionts with access to large amounts of nitrogen, for instance, are free to use photosynthetic products for their own growth and reproduction, instead of relying on their host for nutrients, and releasing these products for their host's use. This suggests that increased nutrient levels would have severe effects on the growth and survival of algal-bearing foraminifera, and models and field studies have shown that they do tend to fare best in nutrient deficient waters. Bleaching in foraminifera has also been observed to be related to increased UV radiation, which can be a symptom of ozone depletion. Increased temperatures have also been suggested as a cause of bleaching. The fact that algal-bearing foraminifera are so susceptible to these types of environmental changes, so often linked to human activity leads Hallock to suggest that algal-bearing foraminifera are "harbingers of global change" (Hallock 2000).

Sorites dominicensis could play an even more specific role as a proposed "model

system" for the widespread coral bleaching observed on coral reefs worldwide. Richardson (2006) observes that all sites at which *S. dominicensis* bleaching was observed experienced elevated water temperatures, sub aerial exposure during extreme spring tides during the summer months, and increased irradiance, as well as hurricane disturbance and exposure to heightened levels of UV radiation. These conditions are very similar to those linked to bleaching in corals. These similar conditions, combined with their role as hosts of related *Symbiodinium*, suggest that *S.dominicensis* and its symbionts may react similarly to corals with respect to environmental stress, meaning that it could be considered not only an indicator of deteriorating environmental conditions that could cause damage to corals, but also act as a model for understanding coral health and reactions overall (Richardson 2006).

Despite all of this, baseline data on the relationship between *S. dominicensis* and its *Symbiodinium* symbionts is limited. Although studies have explored the internal mechanisms and dynamics of the endosmybiosis, including internal distribution patterns and possible population regulation (Richardson 2006), little is known of one of the most basic subjects, the internal population of dinoflagellate endosymbionts in the cell, and how this varies within the population.

Algal symbionts in *S. dominicensis* were first noted by Cushman in 1922 from observations of samples taken in the Dry Tortugas. In regards to symbiont density, Cushman noted only that they "packed" the test chambers (Richardson 2006). Later work by Doyle and Doyle (1940) estimated the dinoflagellate population in a 2mm diameter individual to be approximately 16,000. However, confocal microscopy of a 2 mm diameter individual collected from the Jupiter Sound site yielded an estimate of

approximately 4,000 dinoflagellates (Richardson 2006).

These estimates obviously vary widely, and are very limited, being estimates from limited samples for individuals of a single size. The size of *S.dominicensis* can vary greatly, from young individuals with diameters of a fraction of a millimeter to older individuals up to 3 mm in diameter. It seems reasonable to assume that internal symbiont populations will vary with the size of the individual foraminifera, but the specifics of this relationship have not been studied, and symbiont populations over the natural size range remain unexplored.

Quantifying the population shifts as the foraminifera age and grow larger would give researchers another glimpse into the mechanisms controlling symbiont population recruitment in *S. dominicensis*, but is also interesting in light of the suggested role of algal-bearing foraminifera, and *S. dominicensis* more specifically, as model systems and sentinel species for environmental stress. In order to understand and quantify the aberrations in symbiont densities which lead, in extreme cases, to bleaching, knowledge of internal populations across the growth range, and variability within a population, is helpful. The goal of this paper is to explore the natural pattern of internal symbiont populations across the size range of *S. dominicensis*, and to create a model to describe the size-population relationship, as well as to identify inter-populational variability between individuals of similar sizes.

Materials and Methods

Samples were collected from the seagrass beds at the Jupiter Sound site referenced above (Figure 4). The seagrass meadow grows along the eastern edge of the

lagoon, off of south Jupiter Island, adjacent to Coral Cove Park (N26°57', W80°05') (Richardson 2006). Samples of *S.dominicensis* were harvested from blades of *Thalassia testudinum*, collected from a stand dominated by *T. testudinum*, interspersed with patches of mixed *T. testudinum* and *Syringodium filiforme*.

The study site is vulnerable to anthropogenic influences; although buffered from wake action coming off of the Intracoastal Waterway to the West by an oyster bar, it is a popular beach going spot. The meadow commonly plays host to human and canine waders, as well as boaters in vessels ranging in size from kayaks up to large motorboats, which often anchor in the sea grass bed to take advantage of the sandy beach. During the course of this study, direct damage and rhizome destruction was observed. There is also heavy development to the south. Still, the sample site continues to thrive, with shoot densities previously measured as 172 +/- 25 shoots/m^2, with an estimated leaf area index of 1-1.4m^2 of sea grass blade/m^2 of sea floor.

Samples were collected from throughout the meadow, from both the middle of the patch and from the edges. Although *Thalassia testudinum* is found in the low intertidal, and the sample stand is generally immersed, parts of the meadow were observed to be partially exposed during low tides, extreme low tides left portions of the stand, especially on the eastern, shoreward edge, completely exposed. Samples were harvested from throughout the stand at different points throughout the tidal cycle.

According to long-term water quality measurements from the Loxahatchee River District management program, water temperatures from this area (near the RiverKeeper Water Quality station #25) range from ~20°-23°C on average for the period of this study (January through March), with a higher yearly average of 25.35°C (Loxahatcheeriver.org,

RiverKeeper Water Quality Data to 2001). Temperature data taken during sampling followed these recorded trends. During low tides, and especially the extreme low tides that leave tracts of sea grass meadow subaerially exposed, temperatures are likely locally elevated, but due to restraints on the timing of sampling opportunities, no reliable data was collected for these extremes during this study. Other factors, such as salinity and turbidity, have been shown by data (reinforced by observations in the case of turbidity and light transference) to vary on relatively small time scales, understandable in an estuary (Loxahatcheeriver.org, RiverKeeper Water Quality Data to 2001).

Blade Collection

Whole blades of *T. testudinum* were harvested by hand. Blades were plucked at their base, with only one blade removed per blade bundle. If multiple blades were accidentally harvested, all would be kept for observation. Blades of multiple lengths were collected via haphazard sampling. The blades were kept in a bucket with sea water taken from the site at the time of sampling, and exposed to natural light and aerated with a small aquarium bubbler. Although this was not conducive to the long term health of the foraminifera, samples were generally processed within 2 days of sampling; if the foraminifera began to die off in large numbers or show signs of extreme stress (i.e. inability to remain on the blades), the sample was discarded; this was to avoid skewing data by sampling a large number of heavily stressed, and possibly bleached or dying, individuals.

Sample Preparation and Counting

Individual foraminifera were located on the *T. testudinum* blades using a dissecting microscope. When located, their diameters were measured across the two

most obvious major axes (treating the foraminifera as an ellipse, with a surface area of π*a*b, where a and b are the radii of each major axis). Although *S. dominicensis* is not a flat disc, but instead is a very thin cylinder, growth occurs mainly laterally as chambers are added along the edges. The thickness of the test changes very little, and relevant differences are difficult to record with an optical micrometer. In addition, even a measurement of internal volume would be only an estimate, since an unknown internal volume is occupied by the septa that subdivide the interior of test. However, since the volume of a cylinder is a function of the surface area of the face multiplied by the thickness, if thickness is considered to be constant, as suggested by the growth pattern of *S. dominicensis*, then the volume changes in proportion to the surface area. Since cellular material generally fills the test completely, making cellular volume roughly proportional to test volume, we consider differences in surface area to be a valid approximation of differences in cellular volume.

A micrograph was taken for each specimen using a digital camera, for future reference. The individual foraminiferan was then removed with a metal pick, and placed in a microfuge tube. The length of the blade was measured and the overall density of epiphytic growth estimated as light, medium, or heavy. If there were more foraminifera on the blade, it was set aside or placed back in the water; if not, it was discarded. Individuals that were obviously dead (these individuals are nearly transparent, and devoid of almost any pigment) were not sampled; individuals with some pigment-free splotches (i.e. lightly bleached) or overall lighter pigmentation were sampled, and accepted as natural variation within the population.

If the collected foraminiferan was very small (one or both axes ~.6-.5mm) or

obviously lightly pigmented, suggesting a low *Symbiodinium* population, a very small amount of seawater was micropippeted into the microfuge tube with the foraminifera (< 10 µl). For larger foraminiferans, 80 µl of seawater was added to the tube. The foraminiferan was then crushed with a micro pestle, in order to destroy the test, and release the cellular matter into solution in the water.

If the foraminiferan had been small and crushed with a small amount of water, all of the water was pipetted onto a slide, and a full observation of all of the water with suspended cellular material was performed, counting all of the dinoflagellates in solution.

If the foraminiferan had been large enough to crush with the larger volume of water, counts were performed using a hemacytometer. Due to generally low symbiont densities, dinoflagellates were counted within all nine of the large cells (a total volume of .9 µl). Six counts were performed for each foraminiferan, except in cases of human error. These counts were then averaged, and this number was used to estimate the number of symbionts in the entire foraminiferan; the average was used to compute the number of symbionts per µl, and then multiplied by the total volume in the microfuge tube (80 µl).

Following the end of sample collection, the photo records were used to divide the foraminiferans into categories based on Condition and Color. Condition was defined as being either uniform (1 in the attached figures), lightly mottled (individuals having a patch of lighter pigmentation or lack of pigmentation-2), medium mottling (more than a few lightened spots, but pigmented overall- 3), or heavily mottled (dominated by lightened spots, but not completely bleached-or 4) (Figure 5). Completely bleached individuals were excluded from the samples. Color was defined as being either light (1 in the figures), medium (2), or dark (3). Unfortunately, technical difficulties resulted in the

loss of approximately 20 photographs, and these individuals were excluded from analysis of these characteristics.

Results

Analyses were performed using Vernier Software's Graphical Analysis application and SPSS Inc.'s SPSS 16.0. For all analyses, a significance value of .05 was considered significant. Initial analysis was concerned with identifying the relationship between surface area and symbiont populations (Figure 6).

Based on initial impressions, an exponential relationship seemed a likely possibility (Figure 7). However, the strength of this relationship appears to rest mainly in a relatively small number of very large individuals.

With the lack of data points at the upper limits of the observed size range, and with such large amounts of variation observed over the entire range, we did not feel that the exponential relationship was necessarily strong enough to accept out of hand. Instead, if symbiont populations increase in synchronization with cell growth, a linear relationship would be expected. As discussed above, surface area is roughly proportional to cellular volume. If symbiont populations increase proportionally with increased cell volume, then they should also increase linearly with increasing surface area. With this in mind, a linear curve fit was made (Figure 8). The Pearson's correlation coefficient for this relationship (r) was 0.683. This suggests a relatively highly correlated positive relationship between increased surface area and symbiont popullation. With a Pearson's correlation value of .683 and 84 (n-2) degrees of freedom, this relationship is significant at $n < .001$; in other words, statistically speaking, there is less than a .1% chance that this relationship occurred by chance in the natural population.

Despite the relatively strong correlation, there is obviously a large amount of variation within the sample. To try and identify the source of this variability, a series of ANOVA tests were performed, using a number of independent factors for which data had been collected during sampling.

Date was identified as a possible source of variability for a number of reasons. Although the sampling period was relatively short, there was still a marked change in the length of days from the beginning to the end; it is possible that symbiont populations could have increased in reaction to the increase in available light. In addition, the end of the sampling period coincided with the start of an observed period of increased growth and reproduction for *S. dominicensis*. As a result, it was suggested that some of the observed variability could be the result of overall symbiont populations changing over time. However, an ANOVA analysis of symbiont populations as a factor of date returned a F-value of 1.044, corresponding to a significance of .407, which is not statistically significant for this study, suggesting that collection date did not have an effect on the observed populations.

Although graphs of the symbiont population as functions of the coloration of the individuals (Figure 9) and density of epiphytes on the grass blade(Figure 10) show a large amount of variation (expressed in the the range of the 95% confidence intervals), as well as differences in the means of each category,there is no statistically significant difference (significance levels of .469 and .664, respectively). However, an ANOVA test of symbiont population as a factor of foraminifera condition returned a significance value of .023, suggesting that incidences of mottling do affect the overall symbiont populations. ANOVA tests also show a significant difference between populations living on seagrass

blades of different length (significance of <.000); however, there is also a strongly positive relationship between the length of the seagrass blades and surface area of the resident *S. dominicensis* (significance = .012). This is likely a result of the growth form of *Thalassia testudinum*; blades grow from the base, so that older blades are longer (unless they are broken); foraminiferans on older blades are more likely to be older, and larger; it is likely that this relationship is responsible for the relationship between blade length and symbiont population.

Discussion

From the data, it seems certain that there are factors affecting symbiont populations that were not measured in this study. Although the general relationship seems to be an increased endosymbiont population in response to increased cellular volume, there is obviously more to the story.

First, as mentioned in the results, although the relationship is statistically solid, there is a very large amount of variation within the population. This is not unexpected in a natural population, but it suggests that there could be factors other than size contributing to the relationship, and identifying and enumerating these will require further study. As presented above, we looked for differences related to a number of possible confounding factors, but only found a significant effect as a result of the condition of the foraminifera. This was expected, because areas largely or completely devoid of symbionts will likely lower the values for overall population in relation to surface area of the foraminifera as a whole. It would be interesting to see how the overall relationship might change if, instead of looking at the endosymbiont population as a factor of the total surface area, it was plotted as a function of pigmented surface area

only; that may account for some of the observed variation.

It was interesting to find that the observed color of the *Sorites* did not show a significant effect; one of the most obvious shortcomings with the post mortem method of symbiont survey presented in this paper is that it can only measure the current symbiont population at the time of sample preparation. It is possible that, as individuals grow larger, and symbiont populations increase, the growth of those populations themselves may increase exponentially, allowing for greater ability to recover after symbiont loss, to fill newly formed chambers, or to increase photosynthetic ability with increased light availability. Possible differences in recruitment rate are missed in this technique, which rests on the assumption that the observed population is the final endpoint for that individual, which is not necessarily true. This could be another source of variation in the population. We had hoped that by comparing relative coloration, these possible effects could be mediated; if individuals with a full suite of symbionts were more darkly colored, then individuals with lighter pigmentation may be in the midst of recruitment. In terms of health, we had also considered that lightness, caused by a lower symbiont population, could be a symptom of stress or damage at levels that may not cause total bleaching. The statistics suggest that coloration may not be a powerful enough indicator for symbiont population to use in this regard. Of course, for both coloration and condition, the aforementioned technical problem resulted in a smaller sample size for comparisons using these factors; in addition, assessments were crude, broadly relative, and made by eye. It is possible that with larger samples and more accurate assessment techniques, more significant effects could be found.

As presented above, we finally decided on a linear model to describe the

relationship seen in the data, despite the somewhat exponential shape of the overall graph (Fig. r2 and r3). The logic for the linear relationship is discussed above, and holds given the assumptions of our study; in addition, a logarithmic transformation of the data did not produce a distinctly straight line, and the linear correlation for the resulting transformation was lower than that for the original data. Our assumptions, though, are based on limited data, and made out of a necessity born of limited measurement ability. Although we hold thickness constant and consider surface area directly proportional to volume, this may not be true when put to the test with more accurate instruments. The thickness may change; in addition, volume may not be as directly related to surface area as we assume. The internal cellular volume is likely to be different than what we would calculate, which would be the volume of the test as a whole; even if we assume that the cellular material is distributed uniformly, the septa of the chambers occupies internal volume. How this may affect the available residency volume of individuals of different sizes has not been studied; with more information along these lines, we may find a more complex relationship will more fully describe the observed relationship.

The observed variability was in fact one of the main rationales for rejecting a more complex, power-based model. Variation seems to increase with the size classes of the individuals. Whether this is a measured trend or a statistical artifact was not examined here. Certainly, increased population variability with age makes sense. Offspring are seeded with symbiodinium via vertical transmission from the parent, suggesting that young *Sorites dominicensis* are initially recruiting with similar endosymbiont populations. As they grow older and larger, there are more chances for variability to be introduced. Recruitment is not necessarily equitable, and determinants

of recruitment ability, whether physiologically within the foraminiferan itself or via environmental effects, have yet to be studied. Older individuals are also more likely than the very young to have recently reproduced and to be experiencing a temporary symbiont shortage post-transmission. It is also possible that older individuals have undergone more exposure to environmental stressors, and are more likely to be unhealthy. Survivorship curves (Susan Richardson, unpublished data) have shown that after initial mortality of new recruits, young populations undergo low mortality before undergoing a sudden drop; if mortality is higher among older individuals, then the variability in our data may be reflecting this.

Of course, the data could be reflecting this survivorship pattern in other ways. Older, larger individuals are rarer; *Sorites dominicensis* the size of the three extraordinarily large individuals sampled for our data, which contribute heavily to the overall non-linear shape of the data, are even rarer. As a result, we have far less data for larger size ranges. We may simply be observing greater variability as a result of a smaller data set. Like the other points discussed here, statistical exploration of these possibilities was beyond the scope of this initial study, but is a ripe area for further study.

A final source of variability may be a result of sampling techniques. One of the main goals of this study was to establish and to perform data collection using a low-tech symbiont counting method. Although adapted from a sampling technique commonly used by researchers of coral symbiodinium, it was nowhere near perfect, and a lot of testing and tweaking went on before data collection began and, unavoidably, afterwards, as the researcher became more familiar with the techniques. Although with practice it became effective and efficient, error is always a worry. Care needs to be taken especially

in the grinding of the samples; large test fragments can block pipette tips, or obscure the view in a hemacytometer, and researchers should become familiar with the preparation techniques before attempting counts, to minimize such error. Attempts should also be made to refine sampling of extraordinarily small individuals. Counts via a hemacytometer will probably not be accurate for individuals of the sizes which we counted completely, but there is a possibility of error introduced when multiple sampling techniques are used for a single data set. ANOVA tests did show a significant difference (.043) between the means of samples counted via hemacytometer and those counted by eye, although this could be because the individuals counted by full observation were uniformly small, and the mean of this subset was simply lower than larger individuals. Still, further refinement of these sampling methods could go a long way towards eliminating random and systematic error that may be inherent in the current incarnation.

Illustrations

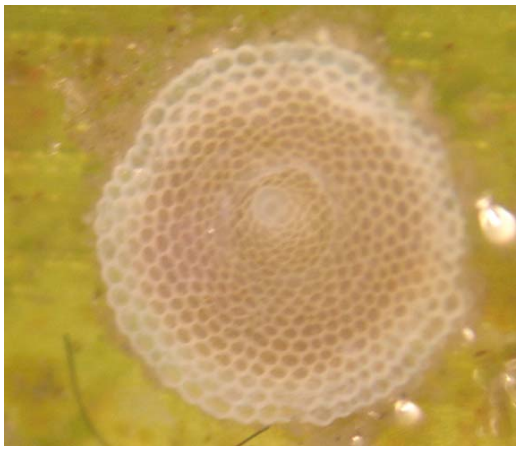

Fig 1: *Sorites dominicensis*

Fig 2: Dinoflagellate symbionts of *S. dominicensis* under a microscope

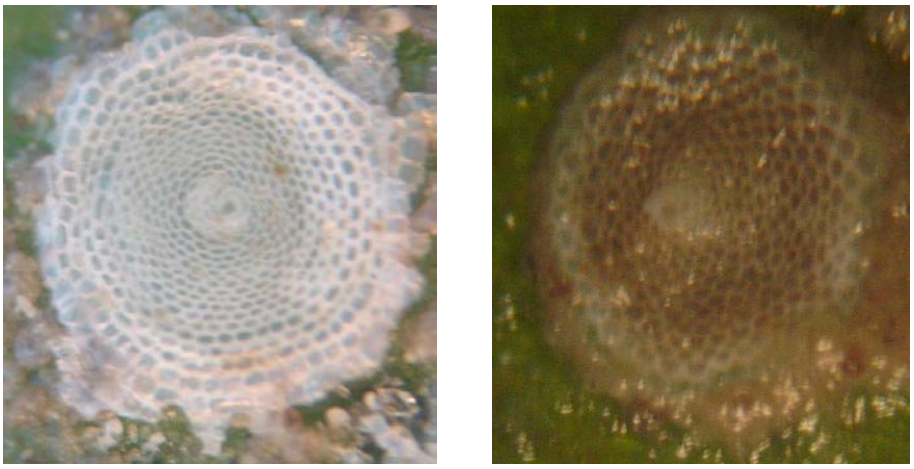

Fig 3: Bleached foraminifera (left) vs. healthy foraminifera (right)

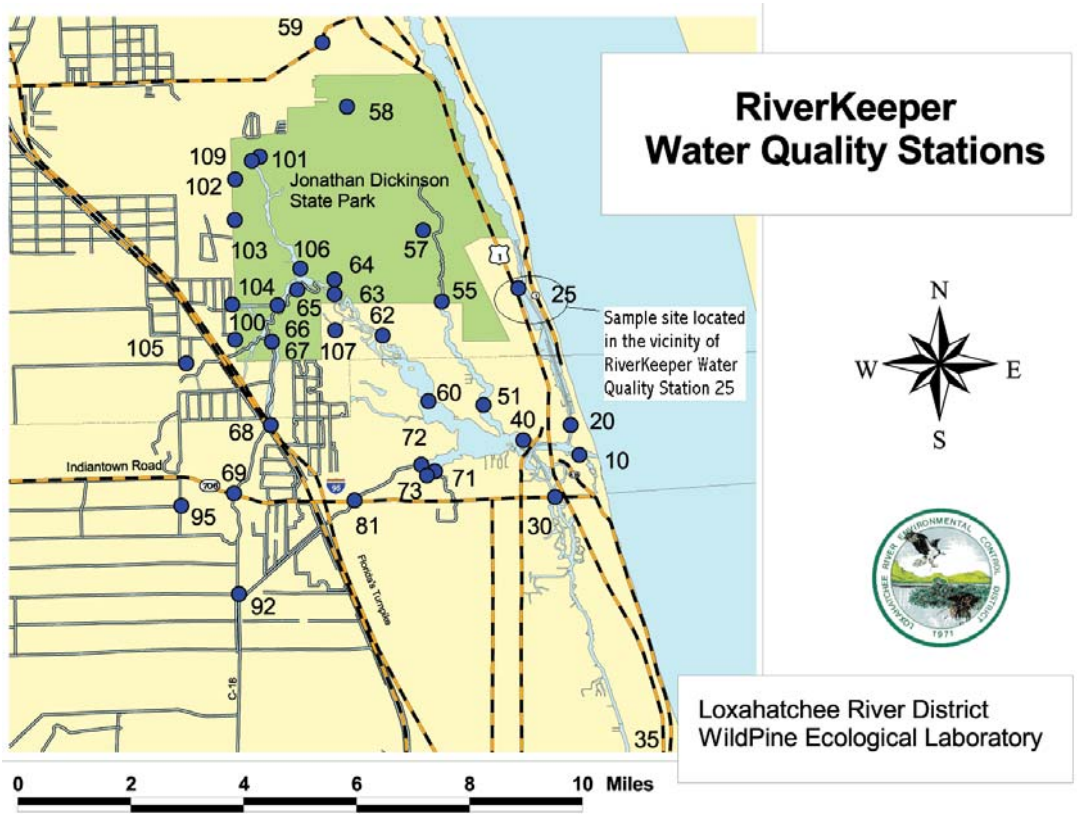

Fig .4: Map from Loxahatchee River District, showing study site in relation to major water and roadways

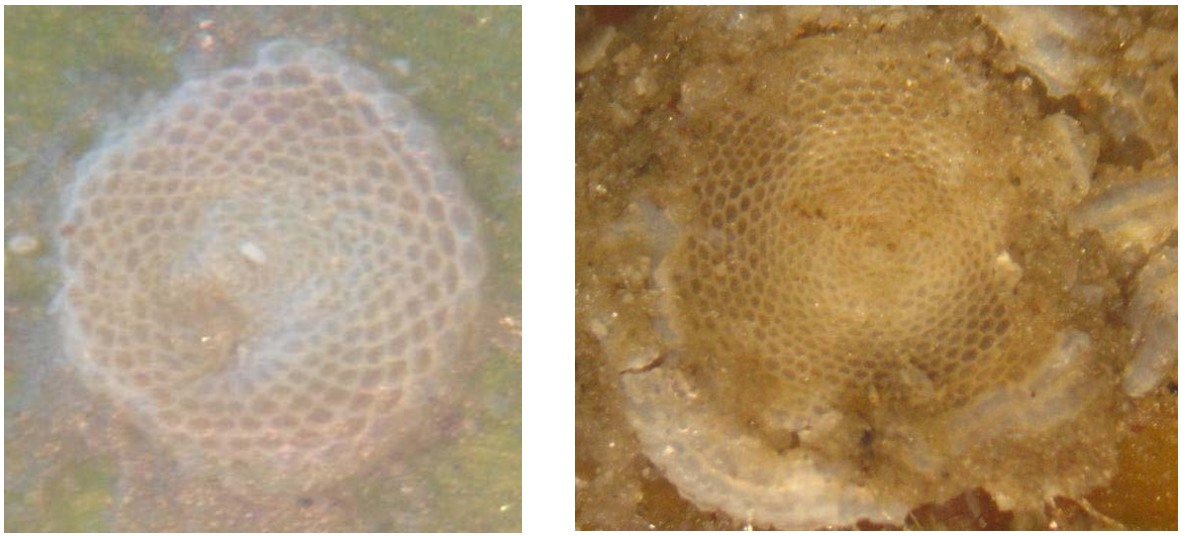

Fig.5: Two Mottled individuals; individual on the left is lightly mottled; individual on the right is medium mottled, with many unpigmented spots

Figures

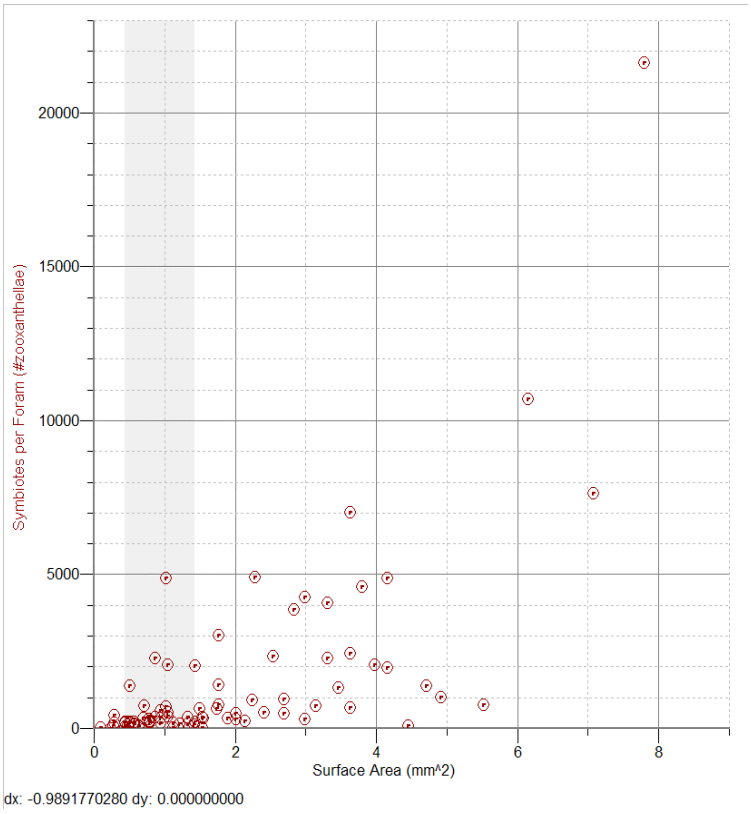

Fig. 6: Symbiont population vs. Surface Area of the Foraminifera

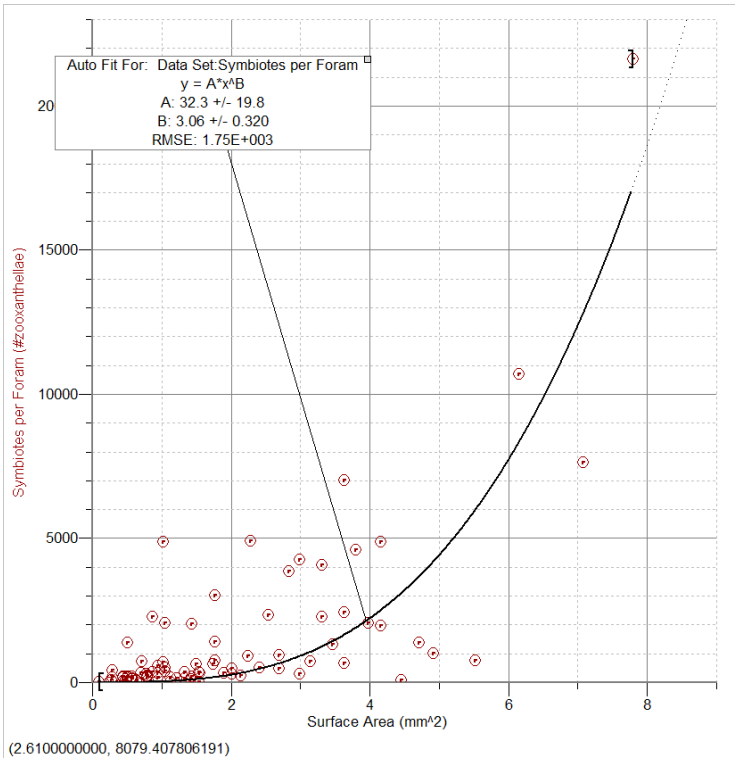

Fig. 7: Exponential curve fit for the relationship between SA and symbiont population

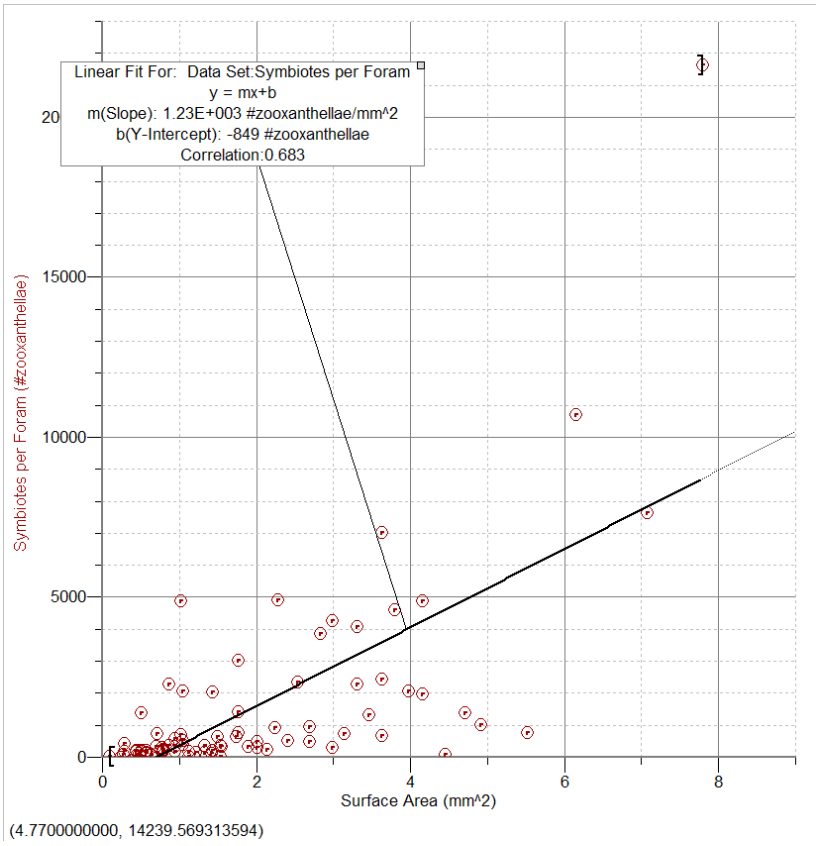

Fig. 8: Linear relationship for the SA:SP relationship

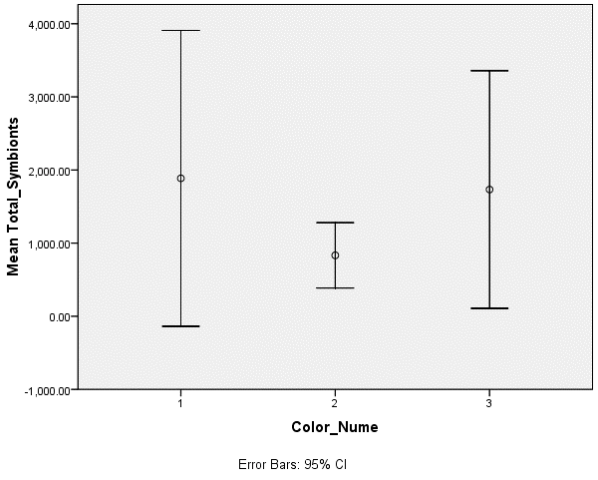

Fig. 9: Symbionts as a function of color

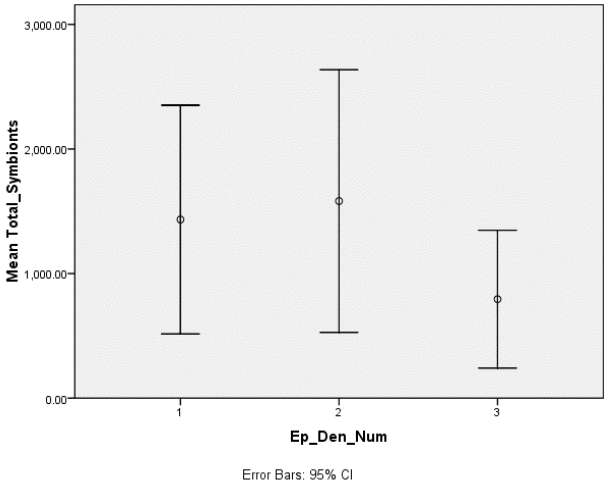

Fig 10: Symbionts as a function of epiphyte density on seagrass blade

References

Appendix 1: Data Set

Sample Number	Date Collected	Count Type	Epiphyte Density	Blade Length	Surface Area	Total Symbionts	Condition	Color
1	10-Feb	all	.	19	1.532	22.00	4.0	1.0
2	10-Feb	all	.	12.4	0.691	335	1.0	3.0
3	10-Feb	all	.	13	3.456	1334	3.0	2.0
4	15-Feb	all	h	8.7	1.123	176	2.0	1.0
5	15-Feb	all	h	18.2	0.628	73	2.0	1.0
6	15-Feb	all	m	9.5	1.532	371	1.0	2.0
7	15-Feb	all	m	16.9	4.155	1974	1.0	1.0
8	2-Mar	hem	l	7.5	1.429	11.432	1.0	3.0
9	2-Mar	hem	m	18	4.155	37.395	1.0	3.0
10	2-Mar	hem	m	18	3.299	32.99	1.0	2.0
11	2-Mar	hem	m	18	4.712	51.832	1.0	2.0
12	2-Mar	all	m	18	0.275	93	1.0	2.0
13	2-Mar	all	m	18	0.471	121	1.0	
14	2-Mar	all	m	18	0.094	42	1.0	
15	2-Mar	hem	l	18.7	7.069	106.035	4.0	3.0
16	2-Mar	hem	l	18.8	0.778	12.448	1.0	2.0
17	2-Mar	hem	.	12.3	6.15	104.55	1.0	1.0
18	2-Mar	all	h	13.2	4.453	84	3.0	3.0
19	2-Mar	all	l	7	0.503	206	1.0	2.0
20	2-Mar	hem	m	17.1	3.134	62.68	1.0	1.0
21	2-Mar	hem	l	8.6	0.864	18.144	1.0	1.0
22	2-Mar	hem	m	18.1	1.759	38.698	1.0	3.0
23	5-Mar	hem	l	12.6	2.403	55.269	2.0	2.0
24	5-Mar	hem	m	4.5	0.275	6.6	2.0	2.0
25	5-Mar	hem	l	15.6	0.707	17.675	1.0	1.0
26	5-Mar	hem	m	13.1	0.636	16.536	1.0	2.0
27	5-Mar	all	m	13.1	0.283	437	1.0	1.0
28	5-Mar	all	m	16	0.503	193	1.0	1.0
29	5-Mar	all	m	16	0.44	193	3.0	2.0
30	5-Mar	hem	h	16.2	2.003	60.09	1.0	2.0
31	5-Mar	hem	.	12.5	0.942	29.202	1.0	2.0
32	5-Mar	all	.	12.5	0.236	8	1.0	1.0
33	5-Mar	hem	l	12.5	3.793	125.169	2.0	2.0
34	5-Mar	hem	l	12.5	1.037	35.258	2.0	1.0
35	5-Mar	hem	l	12.5	0.565	19.775	1.0	1.0
36	5-Mar	hem	m	8.9	0.785	28.26		
37	5-Mar	hem	m	8.4	1.492	55.204		
38	5-Mar	hem	m	20.6	2.003	76.114		
39	5-Mar	hem	m	17	0.503	19.617		
40	5-Mar	all	m	17	0.495	3		
41	5-Mar	hem	m	16.4	1.429	58.589		
42	5-Mar	hem	m	16.4	0.565	23.73		
43	5-Mar	hem	h	22.8	0.864	37.152		
44	5-Mar	hem	m	20.5	3.629	159.676		
45	5-Mar	hem	m	11	1.21	54.45		
46	5-Mar	hem	h	24.4	1.759	80.914		
47	5-Mar	all	l	17.4	0.283	175		
48	9-Mar	hem	l	1.1	3.629	174.192		
49	9-Mar	hem	l	11.1	3.974	194.726		
50	9-Mar	hem	l	15.9	0.636	31.8		
51	9-Mar	hem	m	22.9	1.319	67.269		
52	9-Mar	hem	m	22.9	2.827	147.004		
53	9-Mar	hem	h	18.3	5.513	292.189		
54	9-Mar	hem	l	18.3	3.629	195.966		
55	9-Mar	hem	l	17.8	1.021	56.155		
56	9-Mar	hem	l	17.8	2.985	167.16		
57	19-Mar	hem	h	28	0.565	32.205	1.0	1.0
58	19-Mar	hem	h	28	0.864	50.112	2.0	2.0
59	19-Mar	hem	m	17.5	1.037	61.183	2.0	1.0
60	19-Mar	hem	m	17.5	0.778	46.68	1.0	2.0

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