A Report of Research Activities in the Gray's Reef National Marine Sanctuary for Calendar Year 2003

Submitted by:

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Permitted activities conducted in the Gray's Reef National Marine Sanctuary (GRNMS) during calendar year 2003 have been focused primarily in three areas:

1) development of a web-based guide of the benthic invertebrates and cryptic fishes of the GRNMS, 2) determination of the recruitment dynamics of benthic marine invertebrates inhabiting the sanctuary, and 3) assessment of the role of antipredator defenses in the persistence of sponges that dominate the hard bottom community of the GRNMS. Individuals directly involved in these activities, all from the Department of Biology at Georgia Southern University, are as follows:

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Dr. Alan W. Harvey, Associate Professor

Dr. Stephen P. Vives, Professor

Mr. R. Robert Ruzicka, Masters degree candidate

Ms. Lauren M. Wagner, Masters degree candidate

Activities conducted in each of the three research areas listed above were completed through the assistance of two research cruises (May 30–June 6, 2003 and June 10-17, 2003) on the NOAA research ship Nancy Foster and day-trips on research boats provided by the GRNMS. Activities conducted in each of the research areas during calendar year 2003 are detailed below. Note that the information provided below details activities completed within the boundaries of the GRNMS. Additional activities, in some cases highlighted briefly below for comparisons with the GRNMS, carried out in support of each of the research areas were conducted outside the GRNMS boundaries at J Reef (31° 36.056 N, 80° 47.431 W) and the R2 Tower live bottom (31° 24.305 N, 80° 35.490 W).

1) Development of a web-based guide of the benthic invertebrates and cryptic fishes

Considerable effort has been expended in the study of the benthic invertebrate fauna of Gray's Reef National Marine Sanctuary (GRNMS) and other southeastern hardbottom communities. However, the resulting information exists primarily in scattered gray literature and is difficult to access. Likewise, although Milton Gray and his associates made extensive collections of invertebrates from the area, much of the supporting data, or in some cases the specimens themselves, are apparently missing or at least not readily available. For all practical purposes, then, the invertebrate fauna of GRNMS is poorly known, and identification of specimens collected from GRNMS and other southeastern hardbottom communities can be a difficult task. Less effort has been directed toward understanding the benthic cryptic fish community of GRNMS. Census methods in current use at GRNMS (i.e. video transects, diver transects and point counts) are known to under estimate this component of the fish fauna. Species composition and temporal dynamics of these fishes is, therefore, poorly known.

We are developing new resources that will facilitate the identification of benthic invertebrates and cryptic fishes in GRNMS and consolidating literature associated with those species. Our ultimate goal is to construct comprehensive and illustrated identification keys, usable by both specialists and nonspecialists, for the GRNMS benthic invertebrate and cryptic fish fauna. This information is being made available in a web site for Gray's Reef invertebrates. Ultimately, this web site will include an annotated, searchable taxonomic list; interactive, illustrated identification keys; and individual species pages, with photographs and basic biological information. In addition, the site will provide researchers working at GRNMS with a data entry form that will allow information, such as taxonomic revisions and new records, to be updated.

Development of this web site involves photographic documentation of organisms *in situ* and establishment of a museum collection of the species identified from GRNMS. Establishing this museum collection requires that we gather a minimum of one individual representing each species. To avoid damage to GRNMS, during 2003 we concentrated our collections of organisms on hard bottom areas outside of the sanctuary boundaries that house a similar species composition (mainly J-reef and the R-2 tower sites). However, when a unique species of benthic invertebrate (i.e., one not yet found in our collection) was spotted within GRNMS, one individual was collected. During 2003, this resulted in the collection of one sea urchin, two species of sea cucumbers, and several species of sponges, tunicates, and crustaceans.

In addition to collecting specimens directly from the reef, continual expansion of the species database requires us to deploy artificial structures that will encourage settlement by cryptic species of invertebrates and fishes and that can be collected at a later date. During 2003, we deployed traps made of either 5 cm or 1.9 cm diameter x 25 cm length polyvinyl chloride (PVC) pipe within the sanctuary at the GRNMS monitoring site. These traps were secured with cable ties to 0.5 cm diameter x 15 cm length galvanized bolts that had been anchored in holes drilled in the substrate with a pneumatic drill and a 9.5 cm diameter concrete drill bit. A total of five tubes of each size were deployed June 11-14, 2003 and were collected on July 30, 2003. Retrieval of the PVC tubes resulted in the capture of one belted sandfish, 2 blennies, and 2 cardinal fish. Five more PVC tube traps of each size were redeployed on August 22, 2003 and have yet to be retrieved.

The web site now has approximately 75 species of benthic invertebrates and cryptic fishes linked to their corresponding photographs. Many of these species have multiple images so the total number of photographs available on the web site is approximately 170. The web site is a "work in progress," but the current status can be viewed at the following link: http://www.bio.gasou.edu/gr-inverts/grlist.html

In association with development of this field guide, during 2003 we also began monitoring physical factors that may exert control on the distributions and abundances of benthic invertebrates on hard substrata in GRNMS. Specifically, we started monitoring sedimentation rates and bottom temperatures. Both of these monitoring programs were established in June 2003. Sediment traps consisting of 5 cm diameter x 25 cm length PVC tubes, capped at one end, were deployed by attaching them to stainless steel rods that had been glued into the substrate with underwater epoxy. These traps were deployed on June 11, 2003 at the GRNMS monitoring site and were retrieved on July 30, 2003. Samples obtained from these traps have been processed, but the data has yet to be analyzed. A second set of sediment traps were deployed at this site on August 22, 2003 and have yet to be retrieved.

Bottom temperatures are being monitored with a StowAway Tidbit Temperature Logger (Onset Computer Corporation, Pocasset, MA). The logger is programmed to take readings once per hour. This temperature probe was deployed on June 11, 2003 and retrieved on July 30, 2003. Data downloaded from this temperature logger (as well as others deployed at J-reef and the R-2 tower) suggest that there was a body of unseasonably cold water that moved into GRNMS during this time period. Unfortunately, lack of a historical data base at the reef surface has prevented us from reaching a definitive conclusion. However, this event provides strong justification for monitoring water temperatures at the reef surface. This temperature logger was redeployed at the GRNMS monitoring site on August 22, 2003 and will be retrieved in early 2004.

2) Recruitment dynamics of benthic invertebrates

The specific goals of this project are two-fold: 1) to follow recruitment of benthic marine invertebrates to newly established patches of bare reef substrata and document changes in patch community structure over time and 2) to determine if recruitment of benthic marine invertebrates to GRNMS is primarily the end product of larval dispersal from adjacent, but physically separated hard bottom communities outside the sanctuary, or the result of recruitment of propagules produced within the sanctuary. The first objective is being addressed by following reestablishment of cleared plots on both flat top ledges and scarps. The second is using allozyme markers to assess population genetic structure and infer dispersal patterns in adult populations of the temperate coral *Oculina arbuscula*. This project has practical implications for management of the GRNMS because it will provide baseline information that can be used to assess what short and long-term impacts might result from user-induced damage to the benthos.

To date, only the second objective (i.e., assessment of population genetic structure in *O. arbuscula*) has been initiated. During both cruises on the Nancy Foster (May 30–June 6, 2003 and June 10-17, 2003) we collected data on the density and dispersion of colonies of *O. arbuscula* at the GRNMS monitoring site (31º 23.815 N, 80º 53.461 W). We conducted five belt transects (10 m x 1 m) to determine if the corals are dispersed in a clumped or random fashion. A clumped distribution may indicate one of the following: 1) a population consisting mainly of asexually produced fragments that settle close to the parent colony, 2) a population of sexually produced larvae that rely on very specific settlement cues resulting in establishment of new individuals within the vicinity of conspecifics, or 3) a population structured by non-random postsettlement mortality whereby larvae settling on unsuitable substrate die shortly after establishment. The data were divided into 160 separate 1m x 1m plots and the corals within each plot were totaled. Similar data were collected outside the GRNMS boundaries at J-reef and the R-2 tower sites.

There were more corals per plot at the GRNMS monitoring site and J-Reef than at the R-2 tower where the rocky substrate is interrupted by sand patches every few meters (Figure 1). Furthermore, the highest densities of *O. arbuscula* were found at the GRNMS monitoring site.

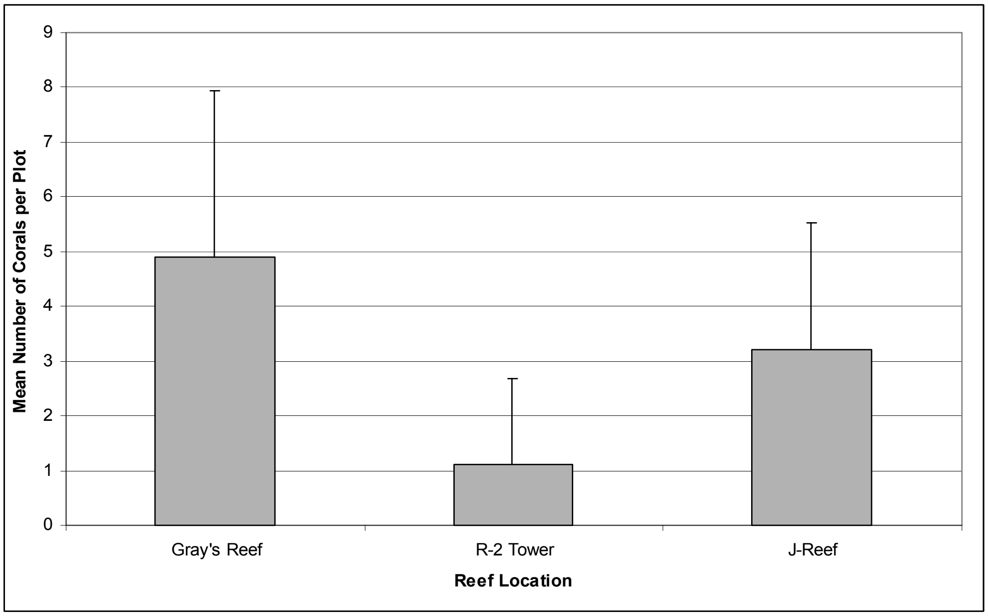

Figure 1. Mean (±s.d.) number of *O. arbuscula* colonies per 1x1 m plot at three Georgia reefs

To determine if *O. arbuscula* colonies are dispersed in a clumped or random fashion, data from these 1x1 m plots were incorporated into a frequency distribution (i.e. the number of plots with 0, 1, 2, 3, 4, 5, 6, etc. corals) and compared to expected frequencies based on the Poisson distribution (Figure 2).

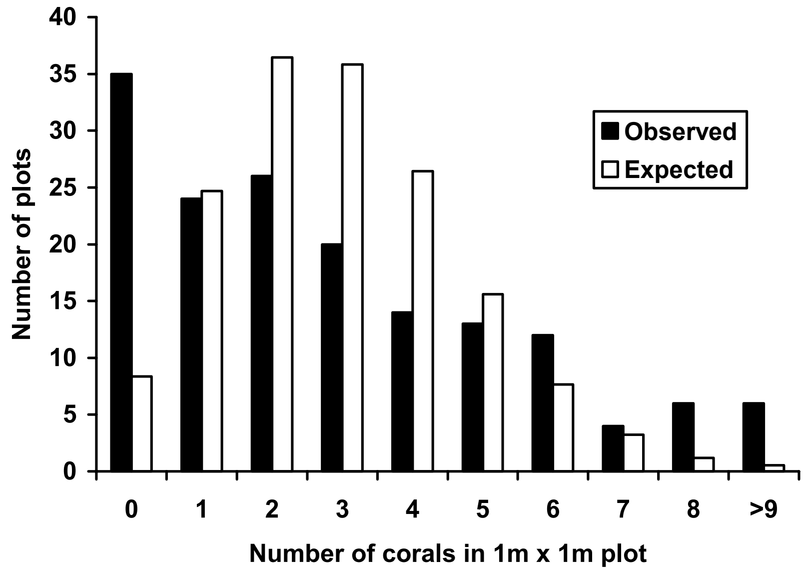

Figure 2. Observed frequencies of *O. arbuscula* colonies in 1x1 m plots versus the expected frequencies under the Poisson distribution. Data represent the combined results from observations at the GRNMS monitoring site, J-reef, and R-2 tower study sites.

Observed and expected frequencies were compared using a Chi-square test. A clumped distribution is indicated if the observed χ^2 value is greater than the expected χ^2 value. Based on these criteria, when data from all three sites are combined, *O. arbuscula* shows a clumped distribution pattern (Table 1). However, when each site is analyzed separately, colonies at J-reef and the R-2 tower are clumped whereas colonies at the GRNMS monitoring site are randomly distributed.

Table 1. Results of the Poisson test for dispersion of *O. arbuscula* colonies at three sites off the Georgia coast and the same test when the data from all three sites are combined.

	All Sites Combined	**GRNMS**	**R-2 Tower**	**J-Reef**
χ^2 observed	177.5537	13.4593	190.4697	14.16761
χ^2 expected (Poisson)	15.507	15.51	12.59	14.07
Degrees of Freedom	8	8	6	7
Probability	$p < 0.001$	$p = 0.097$	$p < 0.001$	$p = 0.048$
Conclusion	Clumped	Random	Clumped	Clumped

The role that local and long-distance dispersal processes play in the clumped and random patterns detected above is currently under investigation through the incorporation of allozyme electrophoresis. A total of 80 coral fragments were collected at GRNMS [50 fragments from the monitoring site and 30 from patch #1 (31º 24.340 N, 80º 51.983 W)] during summer and fall 2003. In all cases only a small portion of the colony was sampled by breaking off a branch approximately 5 cm in length. Subsequent observations indicate that survivorship of colonies subjected to this sampling protocol is high. Additional *O. arbuscula* samples were collected at J-reef and the R-2 tower for comparative purposes. To date, we have screened a total of 8 enzyme systems on *O. arbuscula* and have identified 4 that show promise as polymorphic markers.

3) Sponge antipredator defenses

The main objective of this project is to determine whether or not predation by spongivorous fishes exerts direct control on the distributions and abundances of sponges on temperate hard bottom reefs such as GRNMS. During summer 2003, we initiated studies of the sponge fauna of GRNMS and surrounding hard bottoms. Preliminary data were collected to assess sponge abundances and diversity, sponge predator densities, and sponge palatability.

Sponge abundance, diversity, and distribution

Water temperatures at GRNMS extend seasonally below the threshold for survival of scleractinian corals. An anticipated outcome from a lack of spatial competition with corals and a potential reduction in the abundance of predators would be an increase in the density of sponges. We conducted preliminary surveys of sponge abundance and diversity at the GRNMS monitoring site during summer 2003 and the results support the anticipated outcome. Although we recorded a total of only 20 sponge species on the immediate reef and adjacent live-bottom habitats, as opposed to >80 species in the Caribbean, the number of species per m^2 is almost identical to that found in the Florida Keys and St. Croix (Table 2). In contrast, sponge density at sites in and near GRNMS with moderate rocky relief (0.5-1.0 m height) is almost five times greater than those reported from Florida or Caribbean reefs (Table 2).

Table 2. Comparison of diversity and density of sponge populations from three different reef locations. *N* = 32 quadrats of 0.25 m^2 at GRNMS. Data from the Florida Keys are adapted from Schmahl (1991) and for St. Croix are from Targett and Schmahl (1994)

Location	**Species/m^2**	**Individuals/m^2**	**Total Species**
Florida Keys	10.5	17.5	>80
St. Croix	9.8	19.4	>80
GRNMS	9.8	125.5	>20

Of the 20 species counted, *Chondrilla nucula* was the most abundant followed by an as yet unidentified species of *Ircinia* (Table 3). A total of 14 species are presented in Table 3. Six other species were observed on live-bottom habitats, but were not recorded in our preliminary surveys. These six species; that include *Speciospongia vesparium*, *Iricinia sp. A, Haliclona sp., Psuedaxinella sp., Psuedoceratina crassa,* and *Homaxinella sp.*; were not recorded because our preliminary assessments only sampled along areas with moderate relief (0.5-1.0 m in height) adjacent to scarps, but not live-bottom habitats on the tops of ledges that are 5-15 m distant of these areas. The important point is that there appear to be very different sponge communities as you progress from the area near the scarp to the ledge. Sponges located adjacent to the scarp are primarily encrusting species like *Chondrilla nucula, Chondrosia sp.*, and *Aplysilla sp.*, or massive individuals from the noxious genus *Ircinia* (Table 3). On the tops of ledges, density decreases and many sponges are branching or palmate in growth form rather than encrusting. Whether this change in growth form and species composition is the result of predation or an environmentally-induced response remains untested.

Table 3. Mean (±s.d.) abundance of sponges counted at GRNMS within areas of moderate relief adjacent to rock scarps. Sponges were surveyed with $0.25m^2$ quadrats (n=32) along 25 m transects running parallel to the reef.

Sponge	**Number/m²**	**Sponge**	**Number/m²**
Verongid sp.	2.13±0.90	*Aplysilla sulfuracea*	0.13±0.18
Iricinia sp. B	0.27±0.25	*Aplysilla sp.*	6.00±1.37
Iricinia sp. C.	10.67±1.24	*Ptilocaulis sp.*	0.27±0.26
Ircinia sp. D	1.73±0.68	*Spongia sp.*	0.40±0.40
Microciona junipera	2.67±0.92	*Chondrosia sp.*	3.73±1.95
Chondrilla nucula	86.13±11.21	*Aplysina sp.*	2.40±1.13
Dysidea etheria	1.07±0.65	Orange encrusting	7.60±2.67

Spongivorous fish surveys

Although Green (1977) hypothesized that a decrease in the toxicity of sponges at temperate latitudes coincides with a reduction in temperate predators, Becerro et. al. (2003) found that chemical defenses from temperate sponges are equally effective in deterring predators as tropical sponges. These contrasting results beg the question, does spongivory pressure vary with latitude? Comparison of our preliminary fish surveys with those conducted on Caribbean reefs suggest not. Densities of spongivorous fishes on eight Florida Keys reefs (0.21 ± 0.04 per m^2, Hill 1998) are similar to those observed at GRNMS and another coastal Georgia temperate reef, J reef, (0.22 ± 0.04 predators per m^2). Hill, however, only counted angelfishes while our survey included fish species listed in Randall and Hartman's (1968) survey and those found by Dunlap and Pawlik (1996). These fish other than angelfishes were deemed spongivorous because >6% of their gut contents contained sponge material. Species on this list that were found at GRNMS include *Holocanthus bermudensis, Holocanthus sp.* (townsend angelfish), *Cantherhines macroecerus, Lactophrys quadricornis,* and *Chaetodopterus fiber*. As with our surveys of sponge diversity and abundance, these preliminary results suggest spongivory pressure may be high on temperate reefs.

Feeding assays

During spring and summer 2003, we obtained an initial assessment of the palatability of crude organic sponge extract to natural assemblages of reef fish at GRNMS. These preliminary experiments showed mixed results that need further examination. Sponge samples were obtained by cutting a small section (5 ml or less total volume per sample) from larger sponges. In total during 2003, crude organics were extracted from 105 samples obtained from 9 different sponge species found within GRNMS. Preliminary feeding assays were conducted with six of these sponge species and three types of controls. Food pellets (5 cm^3 in volume) were prepared using a 0.78 g Type I carageenan:agar mix (85:15), 1.25 g powdered squid mantle, 2 ml of 70% EtOH containing 25 ml sponge extract, and 25 ml distilled water. Control food cubes were prepared in the same manner but with solvent only. The amount of powdered squid mantle used was based upon the mean nutritional value of sponges from Pawlik's et. al. (1995) survey. To test if the organic solvents were non-deterrent, one set was prepared with double the amount of EtOH, and another with 2 ml of MeOH. All control cubes were eaten. In the field, food cubes were dispensed individually and were considered unpalatable if rejected by fish three or more times. Species responsible for consumption were most commonly black sea bass, *Centropristus striata,* and slippery dicks, *Halichoeres bivittatus*, and less often tomtate grunt, *Haemulon aurolineatum* and gray triggerfish, *Balistes capriscus* (Table 4).

The sponge species used in the preliminary feeding assays were chosen because they were either (1) conspicuous on the reef (2) observed being consumed by predators or (3) noticeably absent in areas of greatest relief and found only on live bottom patches away from the scarp. The most abundant sponge at GRNMS, *Chondrilla nucula*, was readily consumed. This is interesting because this species shows a high level of intraspecific variability, but is deterrent more often than not in the tropics (Pawlik et al. 1995, Swearingen and Pawlik 1998). A red branching sponge, *Ptilocaulis sp.*, which is very rare on the reef, but more common on live bottom adjacent to the reef, was also quickly consumed. *Homaxinella sp.*, however, which is absent along the reef scarp, but grows nearby was completely unpalatable. Colonies of this palmate species become larger further away from the central reef. *Spheciospongia vesparium*, collected from live bottom habitats away from the reef was the only other sponge completely unpalatable. Both *Aplysina sp.* and *Psuedaxinella sp.* demonstrated intraspecific variability where cubes were accepted and rejected. Although previous studies with *Aplysina* have shown sponges within the genus to be highly deterrent in the Caribbean (Pawlik et al. 1995) this does not appear to be the case at GRNMS. Both *Aplysina sp.* and *Psuedoaxinella sp.* are found on the reef scarp.

Table 4. Palatability of crude organic extracts supplied in artificial food cubes from six different sponge species at GRNMS and three controls. Food cubes were fed to natural assemblages of reef fish at GRNMS. A cube was considered deterrent if rejected by fishes three or more times (*n* = 5).

Sponge	**# cubes consumed**	**# cubes rejected**	**% consumed**
Spheciospongia vesparium	0	5	0.00
Aplysilla rosea	3	2	60.00
Homaxinella sp.	0	5	0.00
Aplysina sp.	3	2	60.00
Chondrilla nucula	5	0	100.00
Ptilocaulis sp.	5	0	100.00
Control 2 ml 70% Etoh	5	0	100.00
Control 4 ml 70% Etoh	5	0	100.00
Control 2 ml MeoH	5	0	100.00

Our preliminary data collected in GRNMS during 2003 demonstrates the following three points: (1) sponges are a numerically important part of the benthos at GRNMS and surrounding live bottom areas with the number of individuals per m^2 far exceeding those found on tropical coral reefs. (2) Spongivorous fish densities are similar to what has been observed on some Florida Keys reefs suggesting that predation rates on sponges may be as high as those found on tropical coral reefs. (3) Sponges at GRNMS show inter- and intraspecific variability in their ability to produce secondary metabolites that deter fish predation, but the allocation of chemical defense does not necessarily reflect observed sponge distributional patterns.

GRNMS Diving Summary 2003

The table below represents the number of dives made by each participant within the boundaries of the GRNMS during calendar year 2003.

Participant	**# Dives**
Daniel F. Gleason	10
Alan W. Harvey	N/A (provides surface support only)
R. Robert Ruzicka	26
Stephen P. Vives	1
Lauren M. Wagner	17
Total	**54**