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MARFIN Project FINAL Report

Title: The recovering goliath grouper population of the southeastern US: non-consumptive investigations for stock assessment.

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by

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Executive Summary

Goliath grouper, a species indigenous to the southeastern U.S., the Caribbean, Brazil, and West Africa, has been overfished to the extent that the IUCN (World Conservation Union) has classified it as 'critically endangered'. The West African population is thought to have gone extinct (Craig et al. 2009). The species was protected in the southeastern U.S. in 1990 through action by Gulf of Mexico Fishery Management Council and the South Atlantic Fishery Management Council and the National Marine Fisheries Service (NMFS). Since that time there has been a steady recovery of the population (Koenig et al. 2011).

The primary purpose of our studies is to provide an understanding of the reproductive biology of goliath grouper including: the mating system, movement patterns, location of spawning, and timing of spawning. We are also evaluating diet and attempting to rear goliath grouper eggs for purposes of larval description. We are the first to find hermaphrodites in the goliath grouper population, but the function of these hermaphrodites is unknown, as sex ratio (1:1) and size structure are atypical for protogynous species. In our studies we used the FACT (Florida Atlantic Coast Telemetry) system, managed by FWC, off eastern Florida which manages many VR2W receivers on reefs from the St Mary River to Boynton Beach. We found that goliath grouper are capable of migrating great distances to spawning sites where they spawn in spatially and temporally consistent aggregations, primarily on new moon nights of August and September. Spawning takes place on natural and artificial reefs in SE Florida between Stuart and Boynton Beach, and SW Florida off Lee and Collier counties. Night-time sounds produced by goliath grouper during the spawning period (and no other) appear directly related to spawning, as the frequency of spawning and spawning sounds increases on new moon nights and decreases on full moon nights. These sounds may be useful in verifying spawning sites and estimating the size of the spawning population.

Introduction:

Goliath grouper (*Epinephelus itajara*), the largest reef fish in the western Atlantic, reaches total lengths of up to 2.5 m (8.2 ft) and weights up to 400 kg (880 lbs.) (Robins et al. 1986). The species ranges from North Carolina on the Atlantic seaboard (Francesconi and Schwartz 2000) to Texas in the Gulf of Mexico, throughout the Caribbean, to southern Brazil. While they are reported in the eastern hemisphere along the west coast of Africa, Craig et al. (2009) suggested that the African population may have gone extinct because none have been observed or captured in the fishery for over 20 years. Recently, the population in the Pacific, which ranges from the Gulf of California to Peru, has been determined to be a distinct species, *E. quinquefasciatus* (Craig et al. 2009).

In the United States, goliath grouper briefly supported both commercial and recreational fisheries, primarily in the Gulf of Mexico off Florida. Populations were rapidly overexploited and the fishery was closed in 1990 (Sadovy and Eklund 1999). Today, they are considered critically endangered throughout their range globally (IUCN 2008), and continue to be listed as overfished in the United States (NMFS 2009), although the population has made a continuous recovery over the last 24 years (Koenig et al. 2011). Whether or not the adult population has recovered to population equilibrium in still unknown primarily because of poor historical catch records and no catch statistics since fishing was outlawed in 1990. In addition, critical habitat supporting the goliath grouper population has changed significantly over the years—high quality juvenile mangrove habitat has diminished significantly and preferred adult habitat (high-relief artificial reefs) constantly increases as the Florida Fish and Wildlife Conservation Commission (FWC) continues to deploy artificial structures around the state (Koenig et al. 2007, 2011). History has shown that the species is highly vulnerable to exploitation, yet valuable to both the recreational fishing community and the dive eco-tourist community, so management plans should be highly conservative.

Our objectives in this project were tightly associated with spawning—we tagged (acoustic and conventional) adult goliath grouper on spawning sites to determine movement patterns relative to spawning sites; we analyzed gonad biopsies and monitored sound production. Tagging adults with acoustic transmitters also gave us the opportunity to evaluate overall adult mortality via the Kaplan-Meier telemetric method. When we sampled the adults we took as many samples as possible including stomach contents for the evaluation of diet, gonad biopsies for evaluation of reproductive condition, fin rays to determine age structure, genetic samples to examine stock structure, and muscle tissue for isotopic analysis. We also tagged goliath grouper in several ways (in addition to the selected transmitter tagging we used PIT tags, pig-ear tags attached to the posterior base of the dorsal or anal fins, and scars left by removal of dorsal fin rays). The survival rate of captured fish was very high, better than 99%, as estimated from diver observations on catch sites immediately after sampling.

Project objectives:

1. Estimate goliath grouper abundance and size structure on spawning aggregations,

2. Estimate fecundity, reproductive timing (diel, lunar, seasonal) and other aspects of the mating system, such as sexual pattern (hermaphroditic or gonochoristic),

3. Determine the geographic distribution of spawning aggregation sites, as well as the extent of site fidelity and movement patterns of individuals associated with those sites,

4. Determine the relationship between goliath grouper sound production and reproductive patterns.

Part I. Goliath grouper abundance and size structure on spawning aggregations.

Introduction

Goliath grouper spawn in aggregations in southwest Florida off Lee and Collier Counties and in southeast Florida off Palm Beach and Martin Counties. The two areas are very different in many ways. The southeast Florida shelf is very narrow and is swept with the relatively strong currents of the Florida Current. The visibility is generally very good close to shore, but the area is periodically flushed with cold upwelling water during the summertime (Atkinson et al. 1984). The shelf in southwestern Florida is very wide and currents are generally slow. Visibility is poor close to shore, so for diving purposes one must travel far offshore to access goliath grouper spawning aggregations. Another advantage that southeast Florida has in an array of monitored reef sites—this array, called FACT (Florida Atlantic Coast Telemetry) is managed by FWC and provides tremendous advantages in the monitoring of fish movements. It has provided us with a tremendous advantage in understanding the migration and movement patterns of goliath grouper relative to spawning. Thus, working in the southeastern Florida goliath grouper spawning group has given us many insights that we can apply to the study of the much less tractable southwestern Florida spawning area.

The Reef Environmental Education Foundation (REEF) provides accurate and reliable measures of goliath grouper population status within the southeast US—surveys can be used to augment direct density measures on spawning sites. It is possible that regional carrying capacities could be estimated from equilibrium densities in REEF data, similar to what we observed off southwest Florida from 1998 through 2008. That is, trends can be detected in the REEF data that give insight into the changing state of the regional population (Koenig et al. 2011).

Materials and Methods

Catching and sampling

Sampling goliath grouper while the species is protected under federal and state law requires that we catch and release them unharmed. We developed methods to do this efficiently. We use 20-0 circle hooks attached to 900 lb test monofilament leader that is about 6 – 7 m long. Monofilament is far superior to stainless steel cable because with monofilament the fish may escape if it becomes entangled in the reef structure—monofilament will abrade and break whereas cable will not causing extreme stress to the fish and ultimately death. We use heavy swiveled gangion clips at the upper end of the leader and connect the leader to a loop in the lower end of the hand line, which is about 30 m long and made of 3/8 inch diam braided nylon. Hooks are usually baited with dead bait. Cut barracuda, amberjack, or sting ray work very well. Sometimes we use live blue runners or hardhead catfish (spines removed).

We attach a 5 lb weight to the upper end of the leader, and sometimes a polyball float (#50) is attached to the hand line to suspend the bait above the reef. The float also provides a buffer to the impact of the initial strike exerted by a hooked fish and can keep the fish out of the reef structure. We limit our depth of fishing to about 30 m because barotrauma may cause hemorrhage and death if we fish deeper.

After the fish is hauled to the surface, we pull the fish on deck with a short swivel-handle gaff which we hook in the thin membrane behind the lower jaw. The fish is slid onto a dive platform just at the water surface, then onto the aft deck on a stainless steel stretcher frame laced with a nylon mesh bed. Tie-down straps on the stretcher are used to keep the fish from thrashing about on deck--this eliminates the possibility of injury to both the fish and the crew. These fish are large (typically 100 to 250 kg) and can cause serious damage if allowed to flail about on the deck.

Our procedure when the fish is on the stretcher is to tie it down, then put a running seawater hose in the fish's mouth to irrigate the gills and cover the exposed eye to protect it from direct sunlight. We then vent the fish using a stainless steel trocar (9.5 mm diam) & cannula (Figure 1) which is designed for venting cows with bloat http://www.scbt.com/datasheet-362154.html, but works very well on goliath grouper. We puncture the fish with the trocar (with the cannula slid over it) at a point just behind the tip of the pectoral fin (while laid flat against the body) and just below the midline. After puncturing the fish, the trocar is removed leaving the cannula inserted for about 30 sec or until all the gas is expelled.

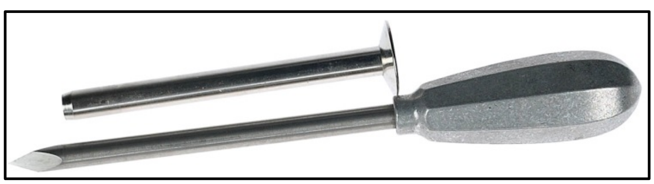

Figure 1. Stainless steel trocar (9.5 mm diam) and cannula used to vent goliath grouper.

We then sample fin rays for aging the fish by cutting dorsal soft rays 6 and 7 (counting from anterior to posterior) at their base, or we may remove the rays at their point of articulation. (If rays are cut, they will grow back in about a year; if they are removed at their articulation, only the membrane connecting the rays will grow back). Fin rays are kept in labeled bags and put on ice until they can be frozen. Stomach contents are sampled by hand (see Part IV). Muscle tissue is taken from the base of the rays for stabile isotope analysis and a small tip of a ray is saved in 95% EtOH for genetic analysis.

Fish are tagged using pig-ear tags which are clipped into the base of the posterior part of the dorsal or anal fin. In our main sampling area off Jupiter we tag males and females with different colors to determine patterns of association on spawning sites. We also tag with PIT (passive integrated transponder) tags which are injected with a little triple antibiotic (prevents infection and holds the PIT tag in the syringe) into the dorsal musculature just below the juncture of the spinous and soft dorsal fins. We also use acoustic tags (Vemco V16s, 8-year battery life)—we tagged 50 goliath grouper on spawning sites off Jupiter, FL (see details under Part III of this report) with these tags by making a small incision just anterior to the vent region, then inserting the tag into body cavity and using surgical staples to close the wound. (Also, this year we tested a speargun-application method of attaching transmitters using 300 lb test monofilament attached to a stainless steel Tee-bar that anchors the tag about 10 cm into the muscle. The success of the speargun-tagging method will be evaluated over time.) Amputated fin rays also serve as a non-specific tag—we can easily see if we previously caught and tagged a fish because the fin-ray sampling leaves a distorted fin-ray pattern during and after grow-back. If a fish loses an external tag, a distorted fin pattern alerts us to look for a PIT tag.

Just before releasing the sampled and tagged fish, we photograph the vent region with a metric ruler and a label that identifies the date and the fish by its tag number. The photographs are used to distinguish between genital openings of males and females and of spawning females (the opening to the oviduct of a spawning female is usually large and red).

REEF abundance patterns

We used REEF (Reef Environmental Education Foundation , http://www.reef.org/) volunteer reef survey data to determine general increases in abundance of goliath grouper on spawning reefs during spawning times. These data were analyzed and graphed for the eight regions of Florida. Because REEF volunteers score abundance as zero, 1, 2-10, 10 to 100 and over 100, we used the median of each interval above one to describe the increase in abundance around spawning times.

The coastal zone of Florida is covered by eight REEF zones (Figure 2). Zone 1 covers the western panhandle from the Florida-Alabama state line to Cape San Blas; zone 2 from Cape San Blas to the Pasco-Pinellas county line; zone 3 extends to the Sarasota-Charlotte county line; zone 4 covers the rest of peninsular southwest Florida; zone 5 covers the Florida Keys and Florida Bay; zone 6 from Key Biscayne National Park to Jupiter Inlet; zone 7 from Jupiter Inlet to Cape Canaveral; and zone 8 from Cape Canaveral to the St. Mary's River (the Florida-Georgia state line). Using REEF zone designations allowed us to make regional comparisons of distribution and abundance of goliath grouper. However, the spawning areas of goliath grouper may span more than one zone and are therefore not defined by REEF's zones, so we modified zone designations. Off SE Florida, the upper part of zone 6 (northern Palm Beach County) and the lower part of zone 7 (southern Martin County) is the key east coast spawning area. This spawning area extends from Stuart to Boynton Beach, FL. Off SW Florida, the most important spawning area is off Lee and Collier Counties (zone 4), but that spawning area may extend north into the southern part of zone 3.

We compared abundances in spawning areas from non-spawning times (mid-October through late July) with spawning times (late July through mid-October). The data off SW and SE Florida show increases due to aggregation formation.

Figure 2. Map of REEF (Reef Environmental Education Foundation) survey zones of Florida.

We use the Roving Diver Technique (RDT) to estimate relative abundance of goliath grouper. RTD involves the diver swimming freely throughout the site and recording all the goliath grouper seen.

It is considerably more difficult to obtain absolute abundance estimates. We know from past studies that some of the goliath grouper will be away from the reef site foraging—absolute abundance estimates will include these fish in the abundance estimate, but the RDT method will not. We estimate absolute abundance (absolute site density) by using the Petersen tag-resight approach (= Petersen mark-recapture). The Petersen method requires a highly skilled tagger (e.g,, Don DeMaria) and also requires that a large proportion (up to 50% tagged to get high precision) of the population on a site be tagged (details of the method in Koenig et al (2011)). We assume that the population is closed (i.e., the fish remain on site) overnight after tagging and that fish are randomly assorted on the day after tagging. The first assumption is supported by our observations of transmitter-tagged fish which show generally low rates of movement on spawning sites and the second assumption is supported by our method of sending 3 divers to swim RDT transects over the reef to determine marked to unmarked fish—if the proportion marked is similar among divers, the assumption holds, if not, it is likely that the marked and unmarked fish are segregated, i.e., not randomly assorted. The diver counts are summed to estimate the proportion of tagged fish on the reef. Fish can be resighted multiple times by the divers, which is equivalent to sampling with replacement in a Petersen mark-recapture study.

We estimated population size (N) using the following equation:

$N = T(C + 1)/(R + 1)$,

where T = total number tagged, C = total number observed, marked and unmarked, on the day after initial tagging, and R = total number of tagged fish observed on the day after initial tagging.

Size structure

We determined total length (TL, cm) of individual goliath grouper either directly from captured fish using a tape measure (straight-line distance from tip of upper jaw to end of caudal fin) or indirectly from digital videos taken underwater using a video camera equipped with paired lasers. The two lasers are arranged so that the beams are parallel and 20 cm apart. During scuba dives, lasers and the attached video camera are aimed at the fish when it is perpendicular to the beams. The lasers appear as two green dots on the side of a fish separated by 20 cm. We use dividers on the video/computer screen in the lab to estimate the total length of each laser-measured fish. This technique is especially useful for measuring fish in spawning aggregations because many can be measured in a very short period of time. The method is also useful for evaluating whether or not there is size selection (=age selection) in the catch. That is, the determination of the age structure of the stock depends on the assumption that there is no bias in sampling. Data are presented in the results section of Part I of this report that shows no size bias in sampling.

Age structure

We have captured, sampled, and tagged over 500 goliath grouper from off Jupiter, Florida, mostly during the spawning seasons of 2010, 2011, 2012, and 2013. We excise dorsal fin rays 6 and 7 at their base for determining the age of the fish (Dr. Debra Murie, U of FL, an expert in aging fish, has re-evaluated age estimates from spawning seasons 2010 and 2011, and now estimates all goliath grouper ages). We currently are using the methodology set out in Murie et al. (2009). Processing involves: cleaning the soft tissue off the cartilaginous rays, drying the rays, embedding the rays in epoxy, and then sectioning the rays at widths of about 1.0 to 1.4 mm. After several sections are mounted to a slide, they are read under a compound microscope using a green filter which enhances contrast between translucent and opaque zones (annuli).

Although not explicitly part of the objectives of this study, we have sampled dorsal rays from the start of the project in 2010. It is so difficult to catch and sample goliath grouper that we use the opportunity to maximize sampling. Thus, non-destructive sampling for age has been part of our work from the beginning.

In addition, fin rays received from FWRI (Angela Collins), sampled from goliath grouper collected from fish kills (red tide or winter cold kills, etc.) are also being processed for comparison of rays with otoliths from the same fish to validate fin-ray aging. We use additional fin rays from recaptured fish at liberty for over a year to validate fin ray aging method.

Results

Abundance on spawning sites

REEF data provide an estimate of mean site densities over time (1990s to present) of goliath grouper in non-spawning months and all shelf reefs throughout Florida—these data are depicted in Figure 3. Goliath grouper prefer high-relief structure (Koenig et al 2011) so site densities increase and the pattern changes if only high-relief sites are used in the site-density estimates (Figure 4). If we compare mean site densities during the spawning season (primarily August and September) with those of the non-spawning season in spawning areas (Figure 5; northern part of zone 6 and southern part of zone 7), we see that there is a significant increase in mean site density during spawning times and an overall increasing trend in the abundance of spawning fish (mean number of surveys in zone 6-7 for years 1997 to 2012 = 46.7 for spawning months and 92.9 for non-spawning months.) A similar graph for zone 4 (off Lee and Collier Counties on the SW coast of Florida) shows a similar, but much more variable picture (Figure 6). The variability can be attributed to low sample size for the spawning season (mean number of annual surveys from 1997 to 2012 = 10.1 for spawning season and 39.9 for non-spawning times.) That is, relatively few divers visit sites off SW Florida partly because of the inaccessibility of those sites. The shelf off SW Florida encompasses a much greater area and the underwater visibility is generally poorer than the shelf off SE Florida. So, the density of REEF surveys (number per unit area) is much greater off SE Florida relative to SW Florida.

In January 2010 there was an intense cold event that reduced the water temperatures of south Florida to well below 15 ^{o}C, the limit of tolerance for goliath grouper (Sadovy and Eklund 1999). The air temperature sustained for several days at about 8 ^{o}C. This temperature was clearly lethal to those juveniles in the shallow mangrove habitat of SW Florida and other mangrove areas. Creel survey data from the Everglades National Park Service provide evidence of a near 90% loss of juveniles throughout the Park (Figure 7), and undoubtedly in other mangrove areas as well. Goliath grouper juveniles spend the first 5 to 6 years of benthic life in the mangroves and leave that habitat to reside on offshore reefs at a size of about 1.0 m TL (Koenig et al. 2007). It is likely that larger individuals left the mangroves in response to the declining temperature, whereas smaller ones died. Thus, the spike in abundance seen in 2010 in Figures 3, 4, and 6 are likely the result of larger juveniles moving offshore in response to the cold event.

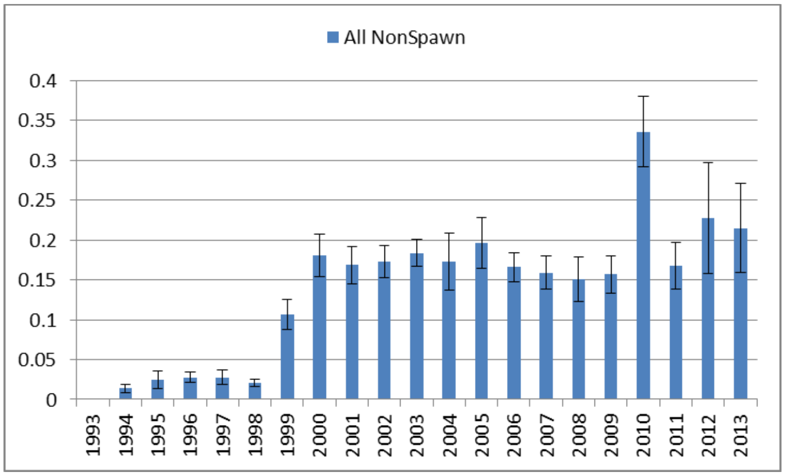

Figure 3. REEF mean site density for all years and all habitats in all 8 zones of Florida, excluding the spawning months of the spawning zones. The spike in 2010 is assumed to be due to the intense freeze that occurred in January 2010 which may have driven larger juveniles from their juvenile mangrove habitat to offshore reefs.

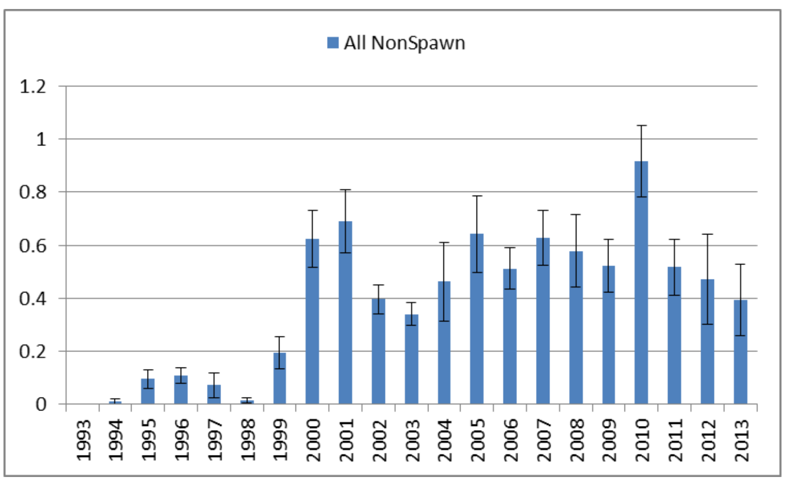

Figure 4. Graph depicts REEF data, mean (± SE) number of goliath grouper per high-relief sites, in all 8 zones of Florida, excluding the spawning months of the spawning zones. The spike in 2010 is assumed to be due to the intense freeze that occurred in January 2010 which may have driven larger juveniles from their juvenile mangrove habitat to offshore reefs.

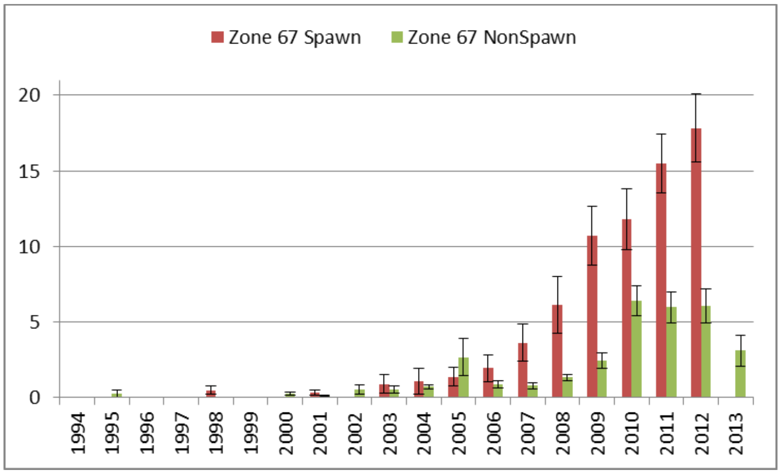

Figure 5. Graph depicts REEF data from upper Palm Beach County (upper zone 6) and Martin County, Florida (lower zone 7) showing where goliath grouper spawning aggregations have been documented off the southeast coast of Florida. Graph shows mean (± SE) goliath grouper site densities during spawning months ("Zone 67 Spawn"; August and September) and non-spawning months ("Zone 67 NonSpawn"). (Mean number of surveys for years 1997 to 2012 = 46.7 for spawning months and 92.9 for non-spawning months). Spawning season data for 2013 is not available.

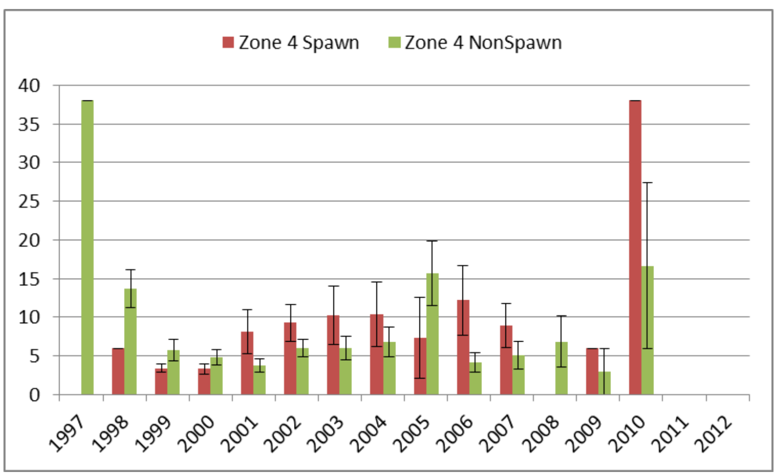

Figure 6. Graph depicts REEF data from off Lee and Collier Counties, Florida (zone 4) where goliath grouper spawning aggregations have been documented. Graph shows mean (± SE) goliath grouper site densities during spawning months ("Zone 4 Spawn"; August and September) and non-spawning months ("Zone 4 NonSpawn"). Data show considerable variability primarily because of low number of REEF surveys per year off SW Florida (mean number of surveys from 1997 to 2012 = 10.1 for spawning season and 39.9 for non-spawning). Data are not available for 2011, 2012, and 2013.

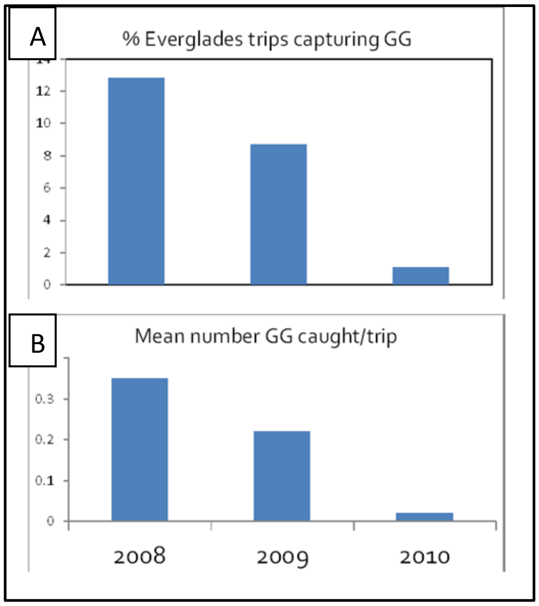

Figure 7. Everglades National Park creel survey data showing decline in juvenile goliath grouper due to cold winters. The creel surveys for 2008 (N = 3,537), 2009, (N = 3,245) and 2010 (N = 2,695) show a clear decline in the percent of trips catching goliath grouper (A) and in the mean number caught per trip (B). These data provide evidence for the spike in mean site density in REEF data for goliath grouper in 2010.

Analysis of movement data from transmitter-tagged fish indicated that new moon phases were most important for aggregating behavior on spawning sites (see Part III for details) and for spawning. To determine absolute abundance on these sites, we used the Petersen tag-resight method with speargun tagging (Table 1). Two sites are very large, encompassing nearly 100 m in a north-south line each. So, on those sites we searched for aggregated fish and tagged each aggregation separately, then summed the abundance estimate for the entire site (Table 1).

Table 1. Petersen tag-resight estimates of absolute abundance of goliath grouper on spawning sites off Jupiter, FL during September 6, 7, and 8 (new moon phase) 2013. Fish were tagged with a spear-gun on each site—the following day three divers scored tagged to untagged. Because of the large size of Zion Train and MG111 sites, two aggregations on each site were tagged and data from each were summed (bold).

Site	**Number Tagged**	**Observed tagged**	**Observed total Tagged & untagged**	**Population size**	**95% c.l.**
3-holes	13	11	49	54	36 - 110
208 wreck	17	37	47	23	20 - 30

Sun Tug	8	19	57	23	17 -37
Zion Train (N)	12	17	109	73	50 - 128
Zion Train (S)	12	11	67	70	45 - 146
Zion Train-total	**24**	**28**	**176**	**147**	**108 - 221**
MG111 (S)	21	19	45	48	36 - 76
MG111 (piles)	21	27	147	111	82 - 168
MG111-total	**42**	**46**	**192**	**173**	**137 - 254**

During the October new moon, goliath grouper appear to move off many of the traditional spawning sites (e.g., Zion Train and MG111) and aggregate on just a few spawning sites like Gary's Greys or Castor wreck. In the new moon of October (4, 5, and 6) the abundance of goliath grouper on MG111 and Zion Train was reduced to about 25 fish each, and the abundance on Gary's Greys increased to about 50 fish. A similar pattern occurred to the south of Jupiter—goliath grouper abundance on the Mizpah and Bud Bar, holding about 50 fish each on the September new moon, held only about 10 on the October New Moon, but the abundance on the Castor wreck remained high on the October new moon. Apparently the spawning season was over for many individuals, but a few still aggregated and spawned on select sites.

Size structure

We captured and measured 580 goliath grouper, mostly during the spawning seasons of 2010, 2011, 2012, and 2013 (Figure 8). Size distribution for all captures are depicted in Figure 8. The overall mean length was 162 cm TL (SD = 24). The mean sizes of goliath grouper catches for 2010 to 2013 (Figures 9, 10, 11, and 12) were compared using the Kruskal-Wallis non-parametric method and pairwise comparisons were made using Dunn's procedure. Mean size (TL) for 2010 (Figure 9; mean = 154 cm, SD = 25; N = 76) was significantly lower ($p<0.01$) than the mean size for 2012 (Figure 10; mean = 165 cm, SD = 21, N = 242) and 2013 (Figure 12; mean = 165 cm, SD = 24, N = 92) but not 2011 (Figure 9; mean = 161, SD = 24, N = 161).

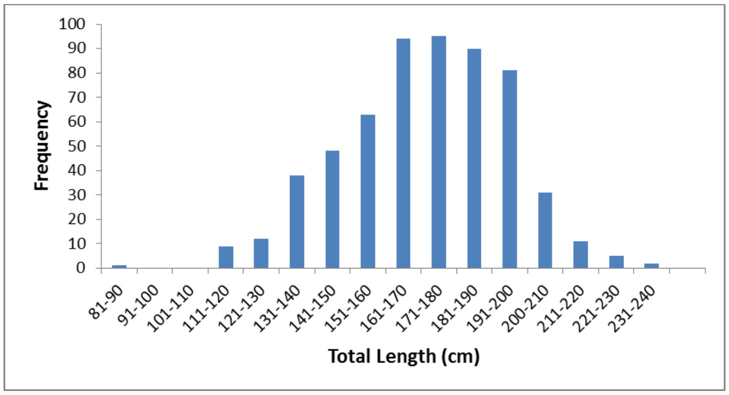

Figure 8. Size frequency distribution of goliath grouper captured during 2010, 2011, 2012, and 2013 (mean = 162 cm, SD = 24, N=580).

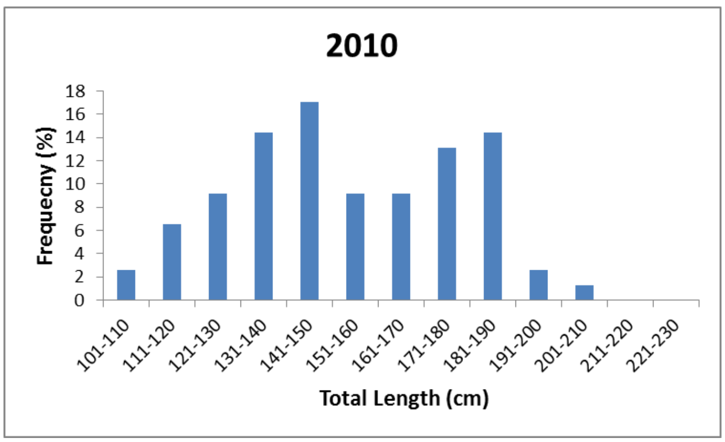

Figure 9. Percent size frequency of goliath grouper captured in 2010 (mean = 154 cm, SD = 25, N = 76).

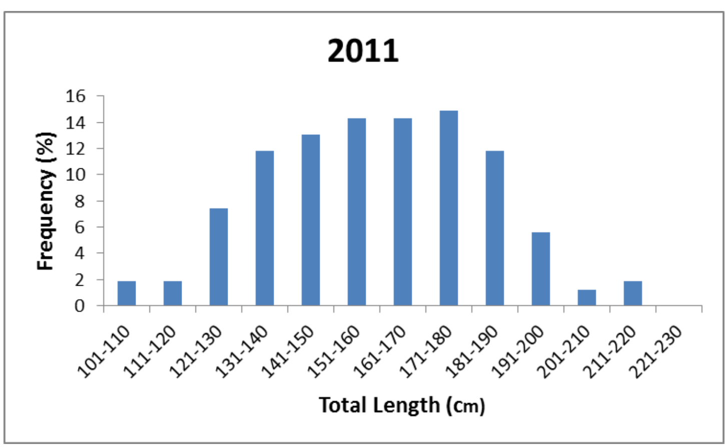

Figure 10. Percent size frequency of goliath grouper captured in 2011 (mean = 161 cm, SD = 24, N = 161).

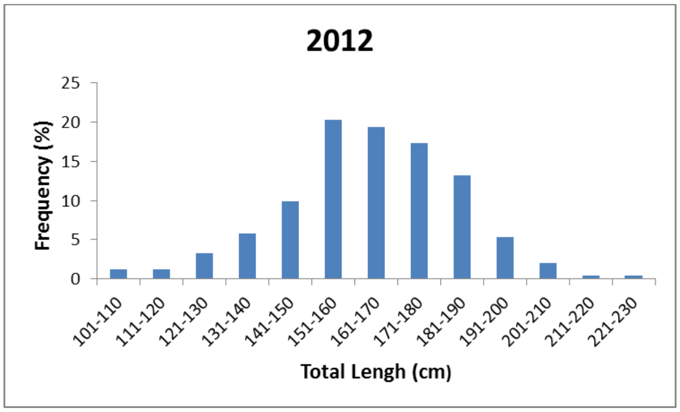

Figure 11. Percent size frequency of goliath grouper captured in 2012 (mean = 165 cm, SD = 21, N = 242).

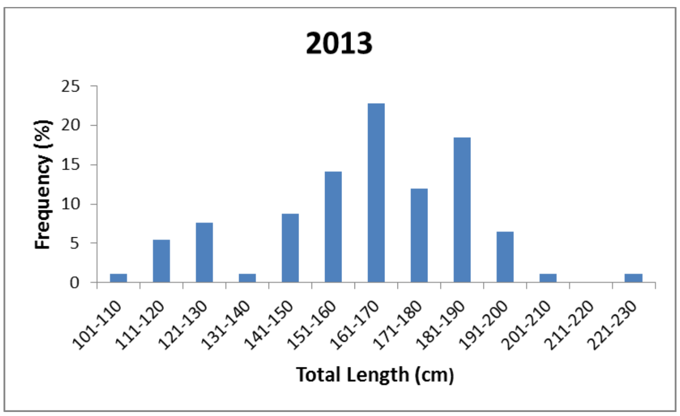

Figure 12. Percent size frequency of goliath grouper captured in 2013 (mean = 165 cm, SD = 24, N = 92).

In 2013 we measured 174 goliath grouper *in situ* with lasers on 5 spawning sites during the spawning season and compared the sizes of those fish with the size of catches (N = 82) taken in the same season and on the same 5 spawning sites in order to see if our fishing was size selective. The mean size of the fish captured in the 2013 spawning season (mean = 164.3, SD = 24.2, N = 82) was not significantly different (p>0.05) than the mean size of the fish measured with lasers on the same sites (mean = 165.8, SD = 23.7, N = 174) (Figure 13).

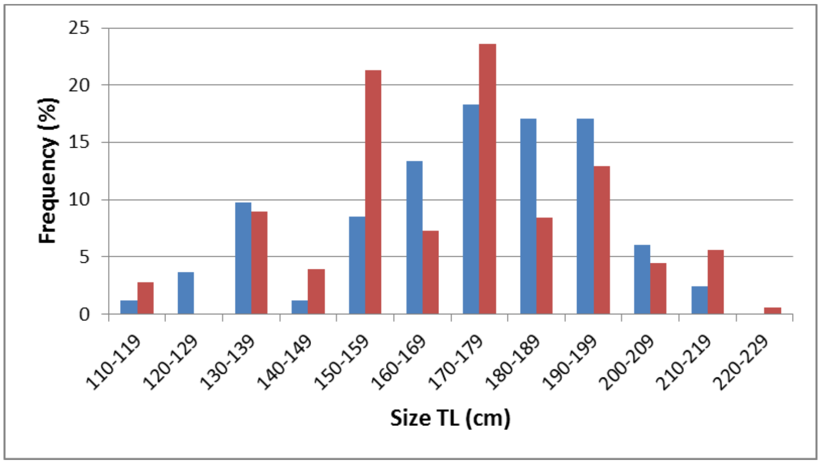

Figure 13. Percent size frequency comparison of *in situ* laser-measured goliath grouper (red; N = 178) and catch (blue; N = 82), both sets sampled on 5 spawning sites during the spawning season of 2013. Mean size of the catch (= 164.3 cm TL, N = 82) is <u>not</u> significantly different (t-test, p=0.89) than mean size of laser-measured fish (= 165.8 cm TL, N = 178).

Age structure

Dorsal fin rays of goliath grouper had clearly distinguishable annuli (Figure 14). Ages of goliath grouper from spawning aggregations sampled in 2012 ranged from 6 to 15 years, with the majority of fish between 9 and 12 years of age. The maximum size of fish caught and sampled for fin rays from the Jupiter spawning sites was 225 cm TL. This was larger than any goliath grouper sampled by Bullock et al. (1992), who obtained samples from fish ranging from 33.8 to 216 cm TL and 0 to 37 years of age. The ages (Figure 15) of goliath grouper caught in spawning aggregations off Jupiter should therefore give an age and size range that would provide a complete and contemporary von Bertalanffy growth curve (Figure 15).

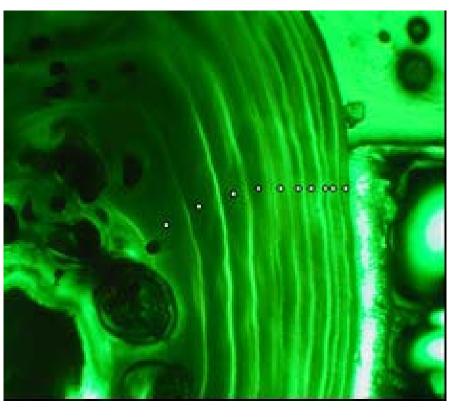

Figure 14. Sectioned fin ray from a 10-year old goliath grouper caught during the 2012 spawning season off Jupiter, FL. This fish was 166 cm total length and sexed as a female. Dots denote the opaque zone of the aging structure, with an opaque zone on the edge of the structure. The fin ray section is viewed using a compound microscope with a green filter to enhance the contrast among translucent and opaque zones.

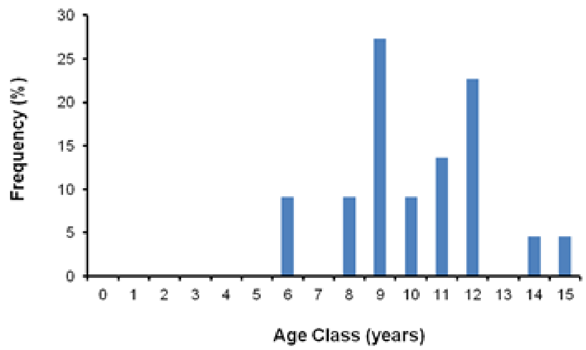

Figure 15. Age frequency distribution for some of the goliath grouper sampled on spawning sites during the 2012 spawning season off Jupiter, FL. Fish were aged using dorsal fin rays.

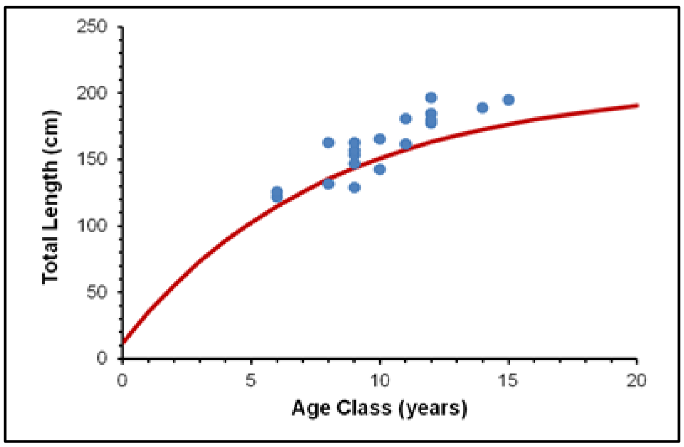

Figure 16. Total length as a function of age for goliath grouper caught during the 2012 spawning season off Jupiter, FL, and aged using fin rays (blue dots). Reference von Bertalanffy growth curve from Bullock et al. (1992) is given by the red curve. Note: only a small fraction of the fin rays in our collection are represented here.

Discussion

Spawner abundance

REEF data from the southeastern Florida spawning area show a clear increase in the abundance during the spawning season, but this increase has a dynamic nature to it (details in Part III). That is, adults move among spawning sites, but tend to accumulate on their "home" spawning sites on the new moons of August and September (but not October). On the east coast there are also effects from periodic upwellings which bring cold water onto the shelf. Goliath grouper are sensitive to cold water (temperatures below 15 ^{o}C are stressful or lethal; Sadovy and Ecklund 1999). On east coast spawning sites, where upwellings are relatively common during the summer months, the fish move into shallower water away from the deeper cold water flushing onto the shelf. There also appears to be an effect of severe storms on movement—goliath grouper in shallow water move into deeper water when inshore waters become too rough. Late in the spawning season (October), the mass of fish may move off many of the traditional spawning sites and aggregate on a relatively few sites. Thus, the abundance on spawning sites may be quite variable throughout the season.

Size and age structure of goliath grouper on spawning sites.

During the 2013 spawning season we concentrated on *in situ* laser measuring of goliath grouper on confirmed spawning sites to determine possible sampling bias in size of goliath grouper captures on the same sites. A fishing bias in size would translate to a bias in age structure, so comparison of measures, catch and *in situ* laser, is important. We showed no significant difference between mean size of the catch relative to the mean size of the laser-measured fish, indicating that our sampling is unbiased relative to size.

Mean size of goliath grouper catches increased from 2010 to 2013 (Figures 9, 10, 11, and 12). The reduced mean size during 2010 and 2011 may be due to the cold winter of 2010 which appears to have driven larger juveniles (70 – 100 cm TL) out of the mangroves and onto the shelf, as assumed from the REEF data. If this happened, then the contribution of the smaller individuals would have lowered the mean size on the shelf. The mean sizes for catches of 2012 and 2013 appear to have leveled off at about 165 cm TL.

Although the data presented here on goliath grouper ages is preliminary, the von Bertalanffy growth curve developed by Bullock et al. (1992) is somewhat lower than the preliminary curve we developed from a sample of goliath grouper caught off Jupiter (Figure 16). That is, contemporary samples show a larger size at age for the fish caught off Jupiter. However, the fin-ray aging objective is intended for another MARFIN project and will be reported in another final report (MARFIN, Award No. NA11NMF4330123), which will be due next year. These preliminary data are presented to show that fin ray aging is a successful non-destructive technique to arrive at the age structure of the population.

Part II. Reproductive timing (diel, lunar, seasonal) and sexual pattern (hermaphroditic or gonochoristic), sex ratio, reproductive behavior including sound production.

Introduction:

Assessing the reproductive capacity of fish populations is important to their long-term management (Goodyear 1993, Myers and Barrowman 1996). Current management practices in U. S. domestic marine fisheries require that managers determine the level of fishing pressure that results in recruitment-overfishing (i.e., the point at which the depletion of adult stock exceeds the point at which it can replace itself under natural spawning conditions (Powers 1996). This requires an in-depth knowledge of a stock's reproductive biology at a time when the stock is perceived as being at great risk. Although the southeastern U.S. goliath grouper population has been recovering since the 1990 harvest ban (Porch et al. 2006), the current lack of life history data makes it difficult to determine either the extent of recovery, the population size, or the level of harvest that is sustainable.

Sexual pattern can affect a species' vulnerability to fishing pressure. Sequential hermaphrodites, for instance, may be more vulnerable to overfishing than gonochorists if there are sex-specific fishing mortality rates (Coleman et al. 1996). The evidence defining sexual pattern in goliath grouper is inconclusive. Smith (1971) described goliath grouper testes as having a lumen and peripheral sperm-collecting sinuses like the males of most protogynous hermaphrodites, and Bullock and Smith (1991) found at least one testis with a few regressed oocytes. However, Bullock et al. (1992) found no sexually distinct size or age pattern distribution among the many males and females they collected and even reported that males matured at slightly smaller and younger ages than females. None of these patterns are what would be expected if goliath grouper were sequential hermaphrodites.

Knowing the size and age of sexual maturity is important because it is closely linked to stock productivity (Hunter and Macewicz 2003). Shifts in maturation to smaller and younger individuals are well known among heavily exploited fish stocks. The only estimates of size and age of maturity for goliath grouper (Bullock et al. 1992) occurred when the species was severely overfished. To understand both the level of the stock's recovery as well as its vulnerability to fishing, it will be necessary to have current estimates. It is particularly important to know the minimum sizes of fish in the spawning aggregations, as it is likely that spawning takes place only on the aggregations and the smaller individuals are likely spawning for the first time.

Materials and Methods:

Gonad histology

We developed a method to biopsy the gonads of goliath grouper (and other reef fish) using a flexible plastic catheter, a plastic collection cup, and a hand-operated vacuum pump (Figure 17; designed for testing automotive vacuum hoses). The procedure involves inserting a polyethylene catheter (6.3 mm OD, 4 mm ID) into the oviduct (for males we use a 2 mm OD catheter) while increasing the vacuum with the pump. The tube is drawn back and forth in the lumen of the gonad so that it continuously removes tissue from the inner wall. The tube is withdrawn while still under vacuum and the gonad tissue is sucked into the collection cup. We preserved gonad tissue immediately in 10% formalin. After several days of fixing, the tissue is collected in plastic tissue cassettes, washed with 70% EtOH, put into plastic bags, sealed, and shipped off to Crowder Histology Consulting, 4952 Alvin Dark Ave., Baton Rouge, LA 70820. The resulting slides are examined by Murie (UF) for a description of reproductive features.

Figure 17. Vacuum pump used to take gonad biopsies from goliath grouper.

A total of 228 gonad biopsies were sampled from goliath grouper during the spawning seasons of 2010 (44 samples), 2011 (69 samples), and 2012 (115 samples) —histological sections were made from biopsies and examined under a compound microscope. Among the various characteristics of the sections, we noted early Post Ovulatory Follicles (ePOFs), hydrated oocytes, late POFs, germinal vesicle breakdown (GVB), germinal vessicle migration (GVM), final oocyte maturation (FOM), cortical alveoli, (CA), primary growth oocytes (PG), advanced vitellogenic oocyte (Vtg 3), moderately advanced vitellogenic oocyte (Vtg 2), and initital stage vitellogenic oocyte (Vtg1). Biopsies were also evaluated for the occurrence of male and female tissue, i.e., indications of hermaphroditism. Transitional fish were scored as 25% male, 50% male, or 75% male as an indication of progression of the transitional state. Also, testicular samples were analyzed for remnant ovarian structures, such as regressing oocytes.

The ePOF and hydrated egg data were most useful to explain lunar patterns of spawning because they provided indications that spawning had occurred very recently. Hydrated oocytes in the lumen of the ovary may mean that spawning is imminent or that spawning has just occurred. That is, the biopsy method may sample hydrated oocytes that had not been fully shed--remnants of unshed ovulated oocytes may remain in the lumen of the ovary after spawning. However, ePOFs are certain indicators that spawning had occurred very recently.

The ePOF and hydrated egg data were standardized to percent frequency and displayed in relation to the moon phase at the time the biopsy was taken during the spawning seasons of 2010, 2011, and 2012. The frequency of ePOFs and hydrated eggs among moon phases was compared using a X^2 test and significant differences among the 4 lunar phases was determined by using the Marascuilo comparison procedure.

Sound production

All recordings were made with a DSG-Ocean (Figure 18, http://loggerheadinstruments.com/) or a H2n recorder and an H2a hydrophone (http://www.aquarianaudio.com/index.php). The DSG-Ocean is a low-power underwater acoustic recorder that records to SD memory cards card using a FAT32 file system and makes high-quality acoustic recordings over long time periods. The DSG-Ocean can sample continuously at rates up to 80 kHz or intermittently, for example, 10 sec per 10 min. The H2n recorder can only record continuously for up to 20 hrs on two AA batteries—it cannot be programed to record intermittently. It is therefore useful for overnight recordings on specific sites, but not for long-term recordings.

Long term acoustic time series data were recorded on the east and west coasts of Florida at known or suspected goliath grouper spawning aggregation sites during the 2010 – 2012 spawning seasons. On the Atlantic coast, off Jupiter, FL, recordings were made mainly at three shipwreck sites (MG111, Zion Train (DSG recorder failed), and Gulfland), located within a 10 km range. Water depths ranged from approximately 20 m at the Gulfland site to 40 and 50 m at the MG111 and Zion Train sites, respectively. In the Gulf of Mexico acoustic data were recorded off Lee County on the Fantastico wreck (35 m deep) and the Stoney wreck (40 m deep) in 2011. The Fantastico wreck is a documented goliath grouper spawning site (Koenig and Coleman, 2009) and the Stony is a suspect spawning site as assumed from the build-up of large adults during the spawning season.

Figure 18. DSG-Ocean acoustic datalogger (hydrophone on top).

Acoustic recorders were either attached directly to the wrecks/reefs or to a buoyed mooring cable and suspended 2-3m above the bottom. Because DSG recorders remained on the selected sites for several months, they were programmed to be conservative of battery power—they recorded 10 seconds every 10 minutes within a frequency range of 0 to10 kHz—goliath grouper calls are within 0-100 Hz. Data were treated with a Fast Fourier Transform to examine the concentration of acoustic energy in the 0-100Hz range. All analyses were done with MATLAB R2009b and Adobe Audition 2.0.

Sex ratio

We calculated the sex ratio for goliath grouper captured in 2010, 2011, and 2012. These data are displayed in histograms and data were analyzed using χ^2 analysis.

Results

Gonad histology

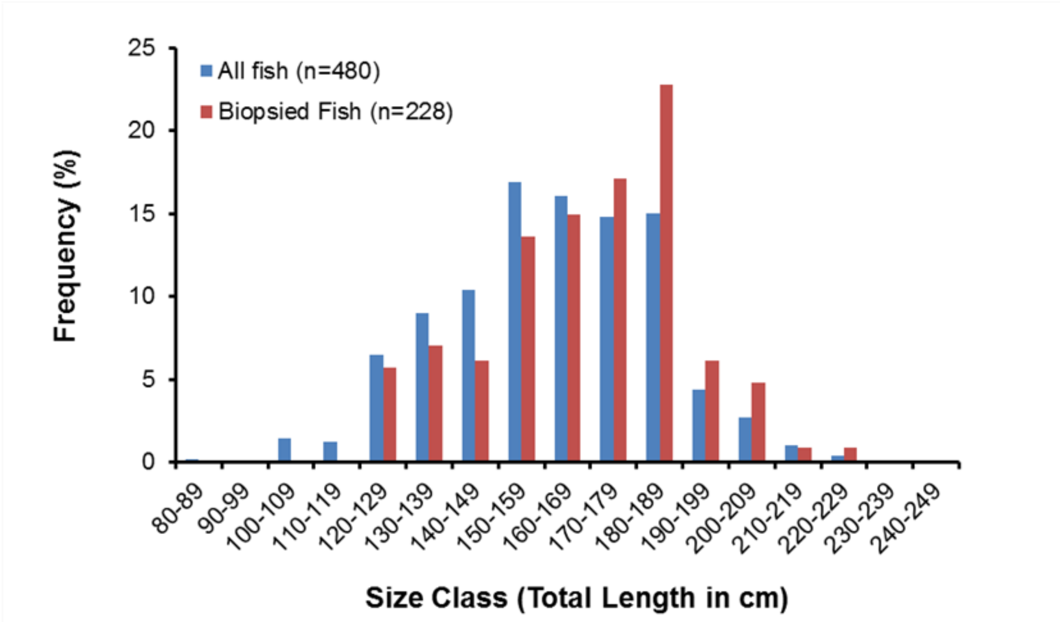

Figure 19. Percent frequency of size classes of goliath grouper for all fish caught from 2010 to 2012 in comparison to fish that were biopsied for reproductive tissue. The means of the two distributions are not significantly different (χ^2, p>0.05)

Size frequency distributions of gonad-biopsied goliath grouper and the size frequency distribution of the total catch of the 2010-2012 spawning seasons were not significantly different (Figure 19; χ^2, p> 0.05). This comparison indicates that gonad samples were not subject to size selection biases.

Hermaphrodites were relatively common among the biopsied adults sampled during the spawning season. (Note: only biopsied fish are included here—biopsies of many males were unsuccessful, but they were identified as functional males in the field by the expression of copious amounts of sperm. Sex ratio (see below) was calculated using all captured fish, biopsied and not) There was no discernible size-related pattern of female, transitional, or male; their size distributions overlapped completely (Figures 20 and 21). Females (with oocytes only, no male tissue) ranged from 121-225 cm TL (Figure 20). Fish with a majority of female tissue and some male tissue present (< 25% male tissue) ranged in size from 140-192 cm TL and represented only 3% of the gonad biopsy samples. Fish that had a relatively equal amount (50 %) of both male and female tissue (Figures 20 and 21) ranged from 127-186 cm TL and comprised 4% of the fish biopsied. These fish had oocytes that were in all stages of development, including later vitellogenic stages (Vtg3) and spermatogenic tissue interspersed through the gonad. Thus, they appeared capable of spawning as a female, but they contained male gonad tissue (Figures 22 and 23). These fish were referred to as "bisexuals", using the definition of Fennessy and Sadovy (2002) without their additional condition of the fish being "inactive". Fish that had a majority of male tissue but some oocytes (<25% female tissue) were 127 to 205 cm TL and represented 9% of the fish biopsied. Some of these fish had only one or two oocytes embedded in otherwise all male tissue. Fish that were biopsied and determined to be male only had no remnants of oocytes and were 126-220 cm TL and comprised 17% of the biopsied fish.

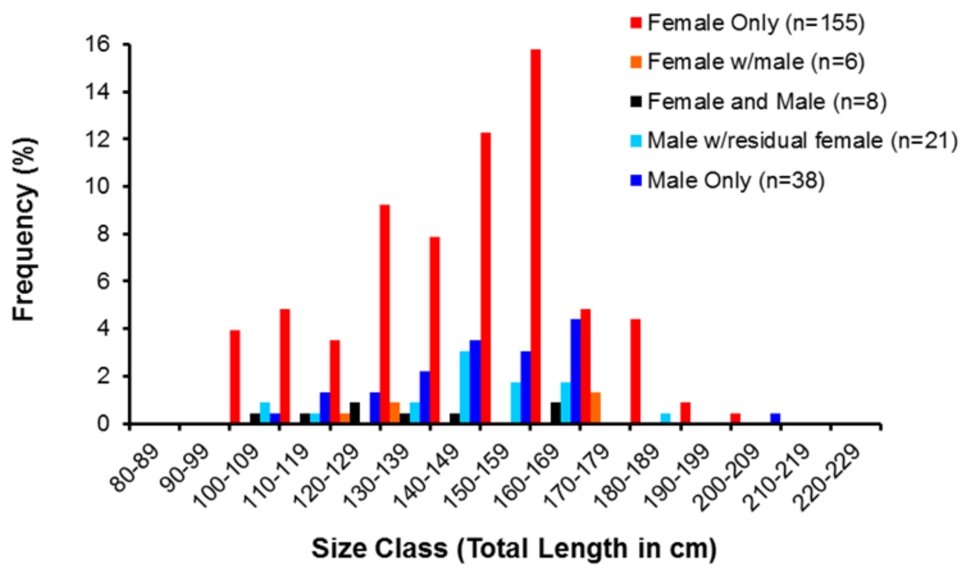

Figure 20. Percent frequency of reproductive stages of goliath grouper as a function of total length for biopsied fish (un-biopsied fish not included) in 2010-2012 off Jupiter, Florida. Key: Female Only=no male tissue observed in biopsy; Female w/male=male tissue represents <25% of tissue in biopsy; Female and Male=male and female tissue equally represented in biopsy sample; Male w/residual female=female tissue <25% of tissue in biopsy (may include only a few oocytes); and Male=no oocytes observed in biopsy tissue.

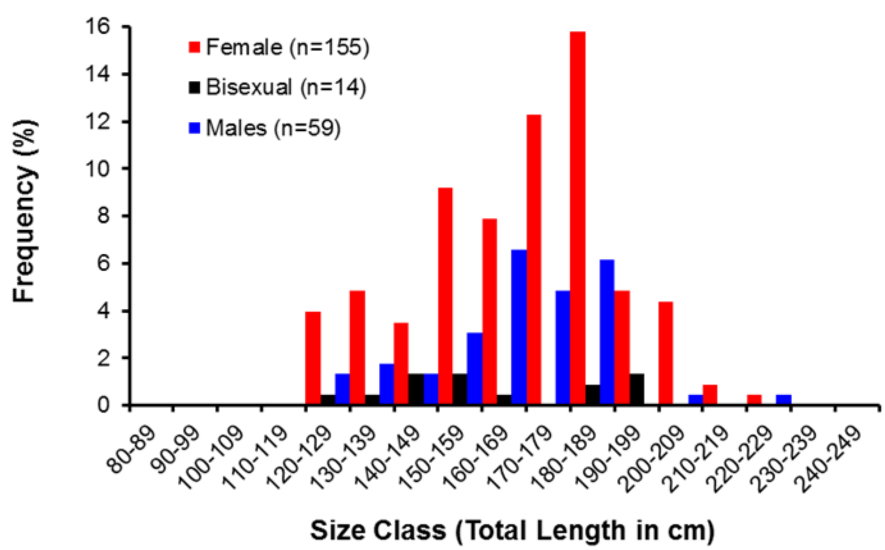

Figure 21. Percent frequency of functional female, male, and female-male (bisexual) goliath grouper as a function of total length for biopsied fish only sampled in 2010-2012 off Jupiter, Florida.

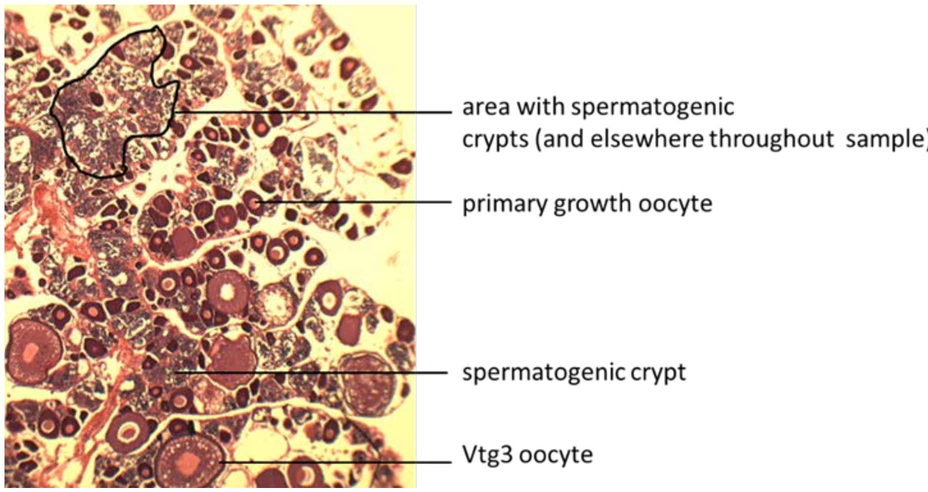

Figure 22. Reproductive biopsy from a goliath grouper that had both female and male reproductive tissue (bisexual). Oocytes were in various stages of development, including Vtg3 oocytes, which indicate a spawning capable phase for the fish. Male tissue was interspersed throughout the sample but was more completely developed at the periphery of the lobules where primary growth oocytes were dominant. Spermatogenic crypts were common throughout the sample.

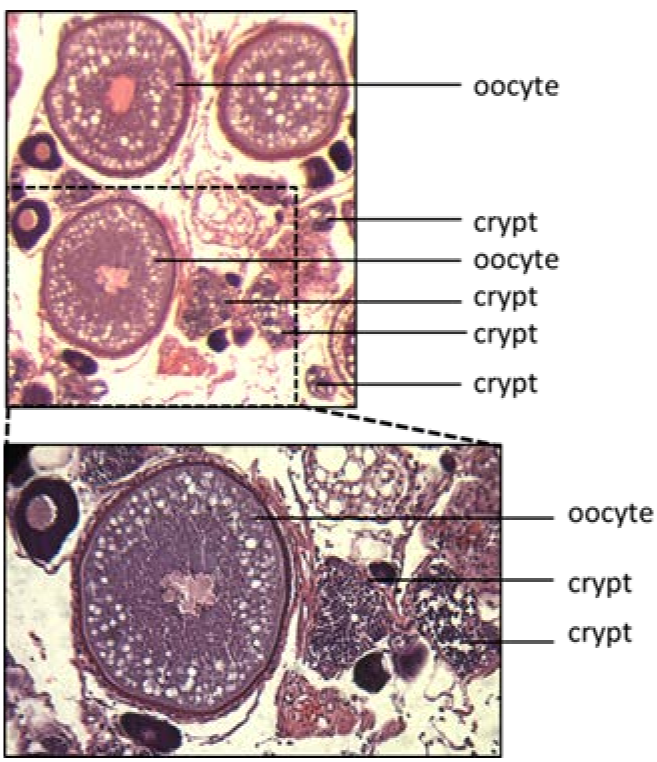

Figure 23. Reproductive biopsy from a goliath grouper showing multiple vitellogenic oocytes (Vtg3), with spermatogenic tissue interspersed throughout sample.

It is important to note that many males (as determined by the production of copious amounts of sperm upon capture) could not be successfully biopsied because of the difficulty of penetrating the sperm duct with a catheter. Thus, the calculation of the sex ratio of the catch includes these non-biopsied males.

Spawning sounds

Patterns of sound production by spawning-site goliath grouper in the Gulf of Mexico and Atlantic generally conformed to those described previously by Mann et al. (2009). Goliath grouper generally start chorusing (increased sound pressure levels sounding like "booms") after sundown and may persist through the night or may increase in intensity late at night and persist for several hours before declining. Variation in signal amplitude among sites was probably related to the distance of the receiver from the sound source (i.e., higher signal attenuation over greater distances) or sound production varying with the number of fish on the site.

Goliath grouper display distinct patterns of night-time chorusing ("booms", as described in Mann et al. 2009; Figure 24) in which sound production ceases, or is dramatically diminished during full moon nights. These sounds were recorded on sites off SW and SE Florida during the spawning season (Figures 25, 26, and 27). Sites off SW Florida, such as Stony and Fantastico wrecks (Figures 25 and 26), and off Jupiter, MG111 (Figure 27), show intense night-time chorusing. Some sites, like the Gulfland wreck off Jupiter, Florida, showed no sign of night-time chorusing (Figure 28). These night sounds occur only during the spawning season on spawning sites, as was observed by Mann et al. (2009). Thus, it appears that night-time chorusing is related to spawning, possibly part of courtship behavior.

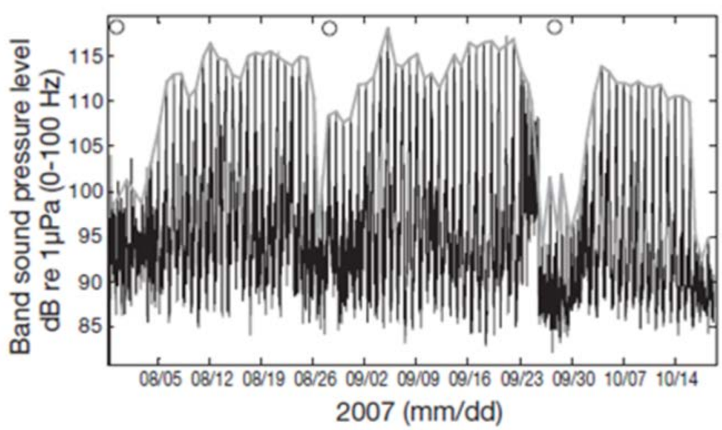

Figure 24. Figure taken from Mann et al. (2009) showing night-time chorusing of goliath grouper on a spawning aggregation (Fantastico wreck) throughout the spawning season (August to mid October). There was no night-time chorusing prior to or after the spawning season. Decline in chorusing can be seen on full moon nights (depicted as open circles). Peaks are defined as sound energy (dB re 1uPa for sound frequencies from 0 to 100 Hz, the range of goliath grouper sound energy.

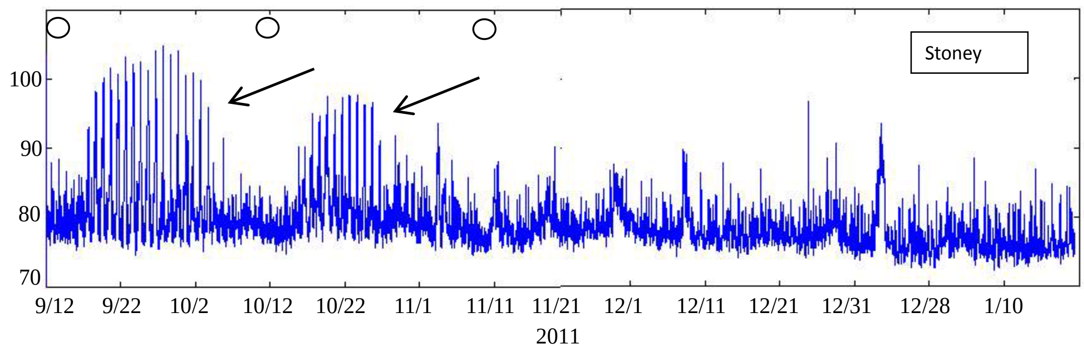

Figure 25. Acoustic time series recorded at the Gulf of Mexico wreck site, the Stony wreck, located off southwest Florida. Data show diel (night-time), seasonal, and lunar periodicity documented previously for goliath grouper by Mann et al. (2009). Circles above acoustic series indicate times of full moon. Arrows indicate night-time sounds being produced by goliath grouper in the 50-100 Hz range. Sound patterns indicate near cessation of sounds on full-moon phases, and lack of sounds after October. Note: Y-axis= band level sound pressure levels dB re: 1µ Pa.

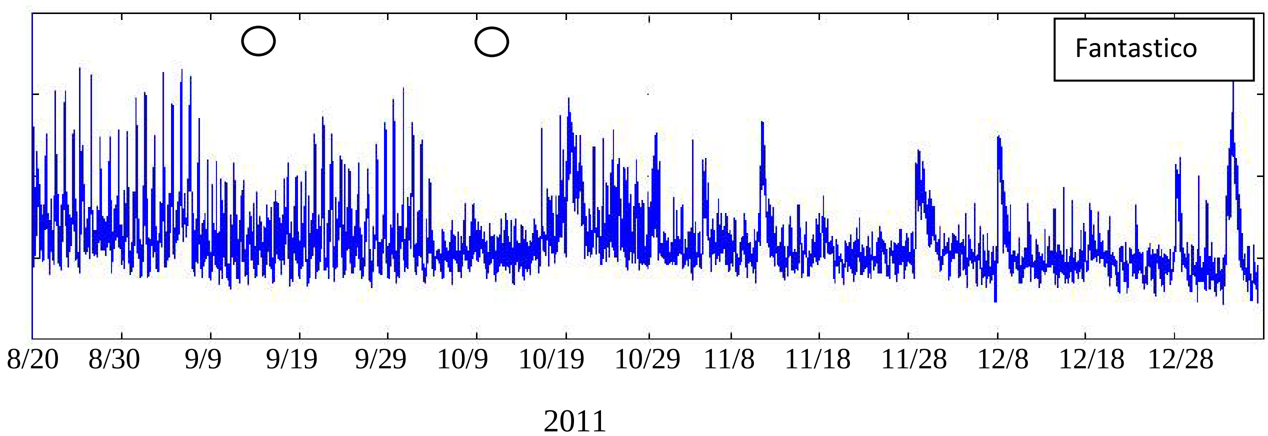

Figure 26: Acoustic time series data recorded on the Gulf of Mexico wreck site, the Fantastico, located off southwest Florida about 18 km to the east of the Stony wreck. Data show diel (night-time), seasonal, and lunar periodicity documented previously for goliath grouper by Mann et al. (2009). Circles above acoustic series indicate times of full moon. Night-time sounds are produced by goliath grouper in the 50-100 Hz range. Sound patterns indicate near cessation of sounds on full-moon phases, and lack of sounds after October. Note: Y-axis= band level sound pressure levels dB re: 1µ Pa.

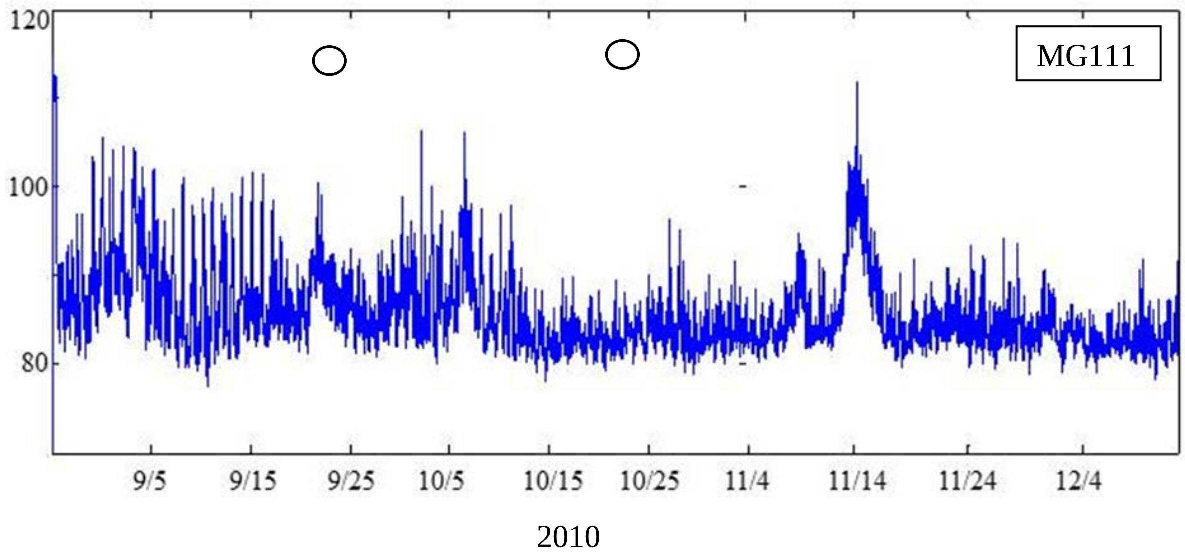

Figure 27: Acoustic time series data recorded on MG111 wreck site off Jupiter, Florida during the latter part of the 2010 goliath grouper spawning season. Data show diel (night-time), seasonal, and lunar periodicity documented previously for goliath grouper by Mann et al. (2009). Circles above acoustic series indicate times of full moon. Night-time sounds are produced by goliath grouper in the 50-100 Hz range. Sound patterns indicate near cessation of sounds on full-moon phases, and lack of sounds after October. Note: Y-axis= band level sound pressure levels dB re: 1µ Pa.

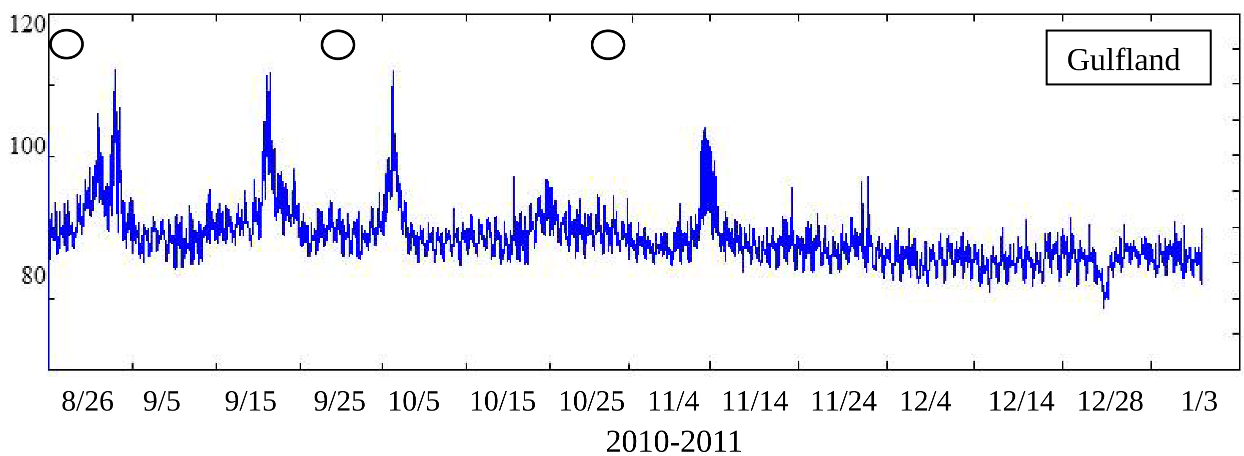

Figure 28: Acoustic time series data recorded at the Gulfland wreck off Jupiter, Florida during 2010-2011. Gulfland wreck is the shallowest and most shoreward east coast site; it is approximately 12 meters deep and within 800 meters of the shore. Goliath grouper occupy this wreck but the acoustic time series data do <u>not</u> indicate a spawning site. The large, broad peaks are likely associated with weather conditions. Circles above acoustic series indicate times of full moon.

Lunar spawning

Early POFs (ePOFs) in ovarian biopsies are indicative of recent spawning, so the occurrence of these histological structures would be a strong evidence for local spawning (on the site where captured) in the past day or two. The percent frequency of ePOFs relative to moon phases during the spawning seasons of 2010, 2011, and 2012 indicate that spawning is most intense on the new moon phase (Figure 29). The frequency of ePOFs in ovarian biopsies from new moon captures was significantly greater (χ^2 test, $p<0.0001$) than the frequency of ePOFs from full moon captures, but not quarter moons (Marascuilo comparison, $p>0.05$, but sample sizes (and statistical power) of quarter moon phases were low).

There was a significantly higher (χ^2, $p<0.05$) occurrence of hydrated oocytes in ovaries sampled on the new moon phase relative to the full moon phase (Figure 30) in females that were biopsied in 2010 through 2012 off Jupiter, FL (Figure 14).

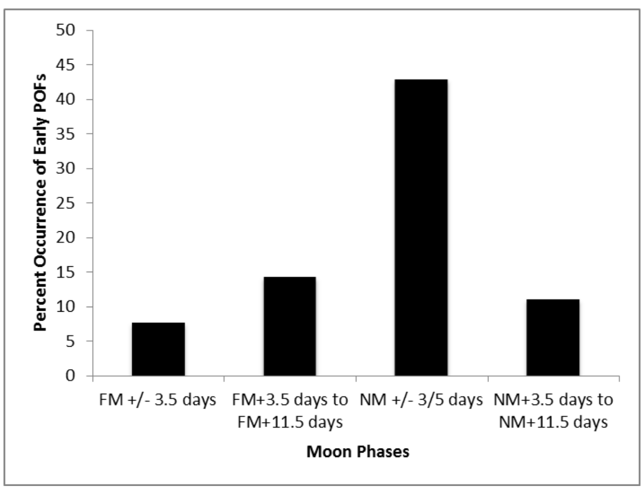

Figure 29. Percent of female goliath grouper with early post-ovulatory follicles (ePOFs) in ovarian biopsies relative to lunar phases (FM = full moon, NM = new moon). Fish were captured on spawning sites during the spawning seasons of 2010, 2011 and 2012 off Jupiter, FL. The frequency of new moon ePOFs was significantly greater than full moon ePOFs (χ^2 test, $p< 0.0001$; Marascuilo comparison, new moon significantly great than full moon, but not quarter moons).

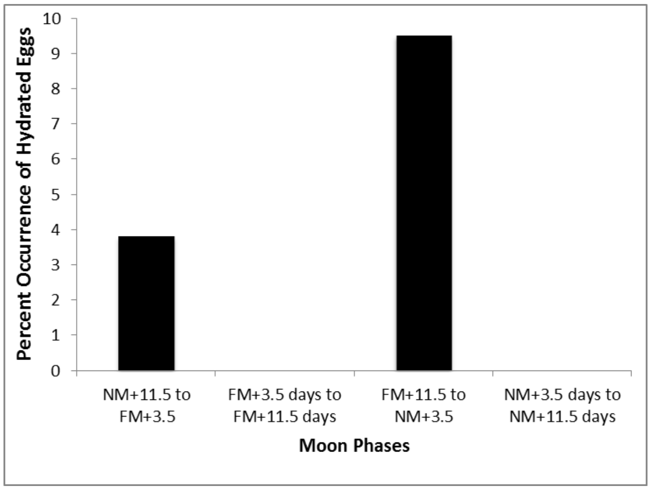

Figure 30. Percent of female goliath grouper captured on spawning sites during the spawning seasons of 2010, 2011, and 2012 off Jupiter, FL with hydrated oocytes in ovarian biopsies is displayed relative to lunar phases (FM= full moon, NM=new moon). The frequency of new moon hydrated oocytes is significantly greater than full moon ePOFs (χ^2 test, $p < 0.05$).

Sex ratio

The sex ratio of captured goliath grouper that were functional females versus functional males (i.e., excluding hermaphrodites) during the spawning season was 1:1 in 2010 (25F:25M), 1.1:1 in 2011 (21F:19M), and 1.1:1 in 2012 (95F:83M) (Figure 30) . When calculated for year-round catches the ratio was similar 1:1 in 2010 (27F:28M), 1:1 in 2011 (32F:33M), and 1:1 in 2012 (102F:107M) (Figure 31). Over all three years, the ratio on spawning sites was 1.1:1 (141F:127M) and all catches 1:1 (161F:168M). None of the ratios are significantly different from 1:1 (z-test, p>0.05).

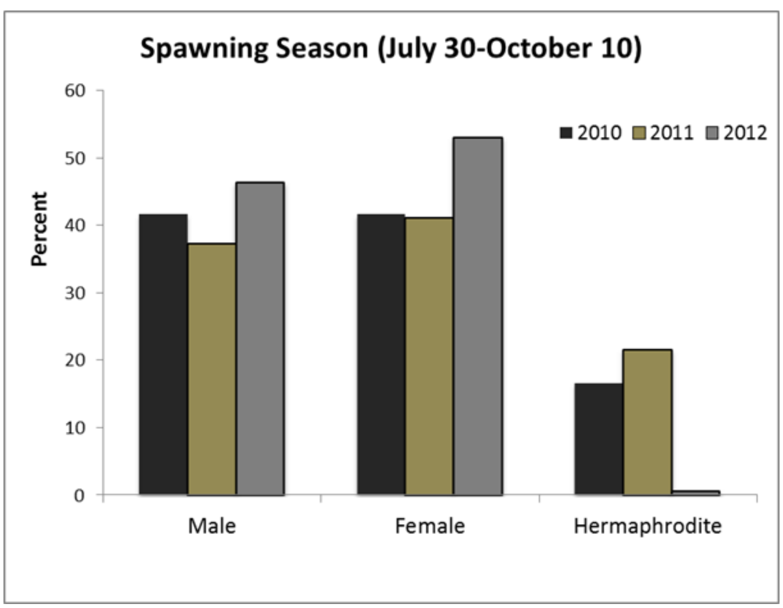

Figure 31. Percent males, females, and hermaphrodites in catches made in the spawning seasons of 2010 (N = 25 (M), 25 (F), 10 (H)), 2011 (N = 19 (M), 21 (F), 11 (H)), and 2012 (N = 83 (M), 95 (F), 1 (H)) off Jupiter, FL.

Hermaphrodites were common during 2010 and 2011, but not during 2012 (Figures 31 and 32). Overall, there were 15% hermaphrodites (% of both males and females). In 2010, 19% of the catch was hermaphrodites; in 2011, 18% were hermaphrodites, but in 2012 only about 1% were hermaphrodites. This dramatic drop in the percent hermaphrodites was significantly (χ^2, $p<0.01$) lower than 2010 and 2011.

The frequency of hermaphrodites was significantly higher (χ^2; $p<0.05$) in the later part of the spawning season (24 Sept – 7 Oct) than in the earlier part (30 July – 23 Sept; Figure 34). This suggests that sex change was initiated during the aggregation period and continued throughout that period.

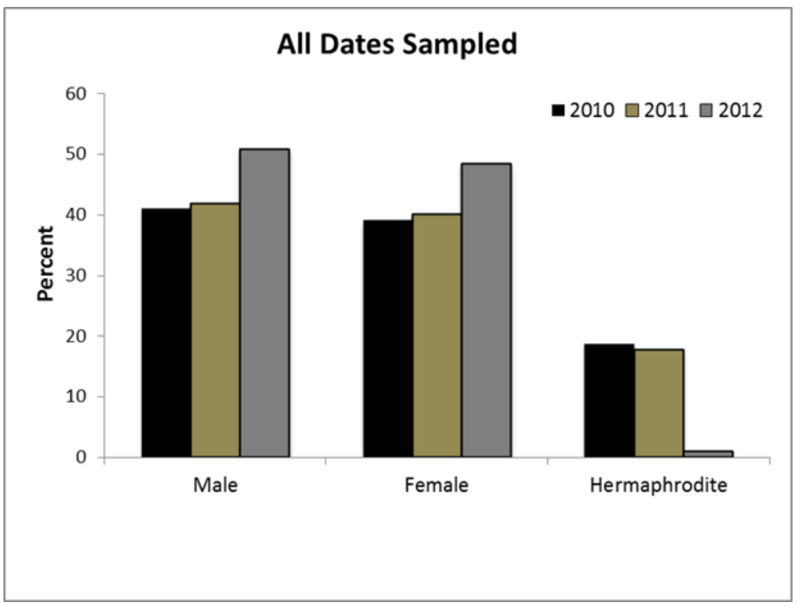

Figure 32. Percent males, females, and hermaphrodites in year-round catches of goliath grouper in 2010 (N = 28 (M), 27 (F), 13 (H)), 2011 (N = 33 (M), 32 (F), 14 (H)), and 2012 (N = 107 (M), 102 (F), 2 (H)) off Jupiter, FL.

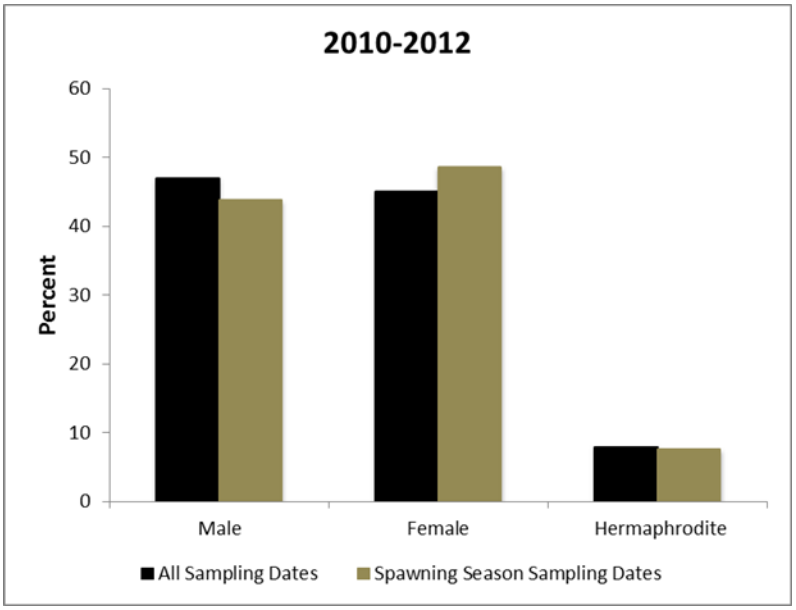

Figure 33. Percent males, females, and hermaphrodites for catches made on all dates (N = 168 (M), 161 (F), 29 (H)) and only during the spawning seasons on spawning sites (N = 127 (M), 141 (F), 22 (H)) of 2010 to 2012 off Jupiter, FL.

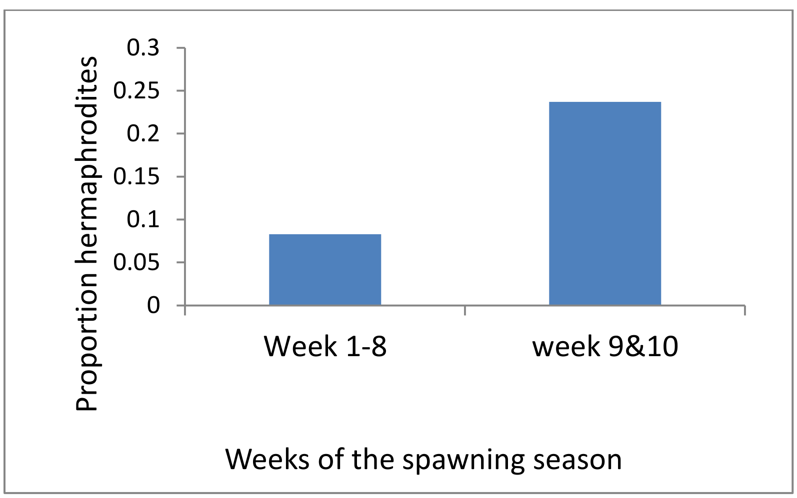

Figure 34. Proportion hermaphrodites in catches of goliath grouper from off Jupiter, FL in 2010-2012. The frequency of hermaphrodites was significantly lower in the early weeks (30 July – 23 Sept; N=145) of spawning than they were in the later weeks (24 Sept – 7 Oct; N=38; χ^2, $p<0.05$).

Discussion

Gonad histology

We compared the size frequency of biopsied goliath grouper with the size frequency of the catch and found no significant difference. This comparison is important because it supports the overall use of the biopsy method to consistently sample goliath grouper throughout their size range. Goliath grouper less than 1.0 m TL (juveniles) are under-represented in the samples. This is because we are dealing with mostly adults on the spawning aggregations; juveniles less than 1.0 m TL occupy mangrove forests, the essential juvenile habitat (Koenig et al. 2007).

In the most recent stock assessment for goliath grouper (SEDAR23 2010), goliath grouper were considered to be gonochorists (separate sexes). This is clearly not the case based on the reproductive biopsies obtained from fish sampled from spawning aggregations off the east coast of Florida. Goliath grouper are obviously hermaphrodites, with 16% of the biopsied fish having both female and male tissue occurring simultaneously in the sample.

Bullock et al. (1992) found little evidence of hermaphroditism in their samples, although one male had remnants of oocytes in the testes. This may be due to the timing and/or location of sampling—all our samples were taken during the spawning season on spawning sites. Although a female-biased sex ratio would be typical of a protogynous hermaphrodite, the fact that males and females occur over the same size distributions is perplexing. There is some evidence to suggest that females should transition to males at smaller sizes under conditions of over-exploitation or in areas of heavier mortality, which does fit the fishery history of goliath grouper.

The complete overlap in the size distributions of goliath grouper that were female, transitional, male, and “bisexual” is at odds for classifying goliath grouper unambiguously as strictly “protogynous” hermaphrodites because the modal size of males was not larger than the modal size for females. There may be some interpretational limitations because the reproductive samples were obtained through biopsies of live individuals, such as not being able to view the gonad wall in the histological section and difficulty in obtaining samples from immature individuals. However, despite these limitations, the occurrence of hermaphroditism in goliath grouper is now confirmed.

Spawning sounds

Previous research on goliath and other grouper species has demonstrated that patterns of fish sound production are positively correlated with seasonal patterns of reproduction (Mann et al., 2009; Mann et al. 2010, Nelson et al. 2011, Rowell et al. 2011, Rowell, 2012, Scharer et al. 2012, Scharer et al. 2013). Long-term acoustic recording systems may therefore be used as a cost-effective way to monitor (by proxy) spawning behavior and/or density of sound-producing fishes on aggregation sites. When coupled with histological structures in the ovary (ePOFs and hydrated eggs) or fertilized egg collections downstream from spawning sites, the qualities and timing of sounds produced may be understood more clearly. Significant correlations between gonad histology patterns, movement patterns, and sound production gives meaning to sound production, and also provides a practical approach to evaluating regional spawning. For example, suspect spawning sites could be verified through the deployment of acoustic receivers on new-moon nights, but they could also be evaluated in terms of spawning intensity or spawner intensity by measuring either frequency of "booms" or the energy level of sound per time.

Pattern of hermaphrodite occurrence

Transition from female to male in protogynous fishes is typically initiated within the time period when the fish are together in a social or spawning group (Warner 1988, Munday et al. 2006). This is logical because sex change is, in the vast majority of cases, socially mediated (Warner 1988). Some assessment of the reproductive value (i.e., predicted future reproductive success) must be made by the individual in the group. Goliath grouper may travel many miles from their home reefs to attend the spawning event (see Part III on movements), so unless goliath grouper form tight social groups during non-spawning times, it is unlikely that sex change is initiated at times other than the spawning time. Social cues would likely be available only when males and females are together. Because sex change is not instantaneous, one would expect a higher frequency of transitional fish on spawning sites late in the spawning season or after the spawning season than early in the spawning season, which is what we observed (Figure 34). It may be that individuals change sex slowly and still function as females while changing sex, which could account for the bisexual pattern (mature oocytes and sperm present in the biopsies). But the frequency of these transitionals is too low to derive any clear conclusion.

The pattern of transitionals appearing after or late in the spawning season occurs in gag, *Mycteroperca microlepis* (Koenig and Coleman 2011). A similar pattern can be seen with goliath grouper for late in the spawning season (Figure 34). But goliath grouper does not show the typical pattern for protogynous species where males are generally larger and older than females—goliath grouper transitionals occur at all mature sizes and ages. And typical protogenous species have a female-biased sex ratio, not 1:1, as in goliath grouper. The reason for this difference is not clear—possibly individual assessments of reproductive value are made within sub-groups on the spawning grounds.

Lunar spawning

Among the biopsied fish, new moon ePOFs were significantly higher in frequency than full moon ePOFs indicating a decline in spawning during full moons—a pattern that matches the pattern of night-time sound production. Because of limited sampling (low N) on quarter moons, the statistical power of our comparisons was too low to discern possible differences between quarter moon phases and either new moon or full moon phases. These data support the contention that night-time chorusing is related to spawning and that these sounds can be used to determine if a site is a spawning site or not. A remaining question is whether or not night-time sound patterns (booms per hour or band level per hour) reflect the number of spawning fish on a site or the intensity of spawning by the fish present. If acoustic patterns do reflect density of spawners, then acousitic monitoring alone could be used efficiently to determine spawner density or, by proxy, if used over the entire spawning areas of SW and SE Florida, spawning stock biomass. A study showing the possibilities of passive acoustics in determining spawning site densities was recently done by Rowell et al. (2012). They showed a relationship between red hind

(*Epinephelus guttatus*) spawner density on spawning sites and number of calls and mean band level per time.

Transmitter-tagged goliath grouper show a strong aggregating behavior on sites they were tagged during the new moon phases of August and September (see Part III for details). This behavior suggests that there is strong spawning site fidelity—i.e., the same fish aggregate to spawn on the same sites year after year, but select new moons to do so. Thus, the most parsimonious view is that new moon phases are the dominant times of goliath grouper spawning and spawning-site fidelity is very high.

Drawing a connection between goliath grouper night-time chorusing and lunar spawning patterns (frequency of ePOFs and hydrated eggs in ovary biopsies) allows a straight forward and rapid evaluation of spawning sites off southwest and southeast Florida. Receivers may be deployed on suspect sites on new moon phases (say, + and – 4 days around the new moon). If there is night-time chorusing, then the site is a spawning site, otherwise not. From this "acoustic sampling" of suspect sites, the frequency of spawning sites in a selected region could be determined. Then, as described above, the abundance on spawning sites could serve as an estimate of spawning stock abundance.

Hydrated oocytes are also indicative of recent or imminent spawning; however, with the biopsy method in which tissue in the lumen of the ovary is sampled, unshed hydrated oocytes may remain within the lumen for extended periods of time and therefore not be as certain as the occurrence of ePOFs.

Sex ratio

The overall sex ratio for all catches of goliath grouper off Jupiter, FL from 2010 to 2012 was 1:1, and all catches during the spawning season were not significantly different from 1:1 (X^2, p>0.05). Bullock et al. (1992) obtained a sex ratio of 1.75:1, which is strongly female biased. However, his catches were mostly obtained opportunistically, so the circumstances about catch locations and times are not clear. We sampled mostly on multiple spawning sites and focused most of our catches in the spawning season. The fact that Bullock et al. (1992) did not find clear evidence of hermaphroditism in their samples may be due to the timing and location of sampling—all our samples were taken during the spawning season on spawning sites. However, we did experience a dramatic and significant drop in the frequency of hermaphrodites in 2012 relative to 2010 and 2011. So, it is uncertain why such changes in the frequency of hermaphroditism occurs.

Part III. Movements, geographical range of spawning sites, and site fidelity.

Introduction

Goliath grouper exhibit a restricted home range and high site fidelity (Koenig et al. 2007, Koenig and Coleman 2009), but they also form spawning aggregations (Koenig et al. 2007). It is therefore important to determine movement patterns showing how these fish get from their home sites to spawning sites and what their behaviors are when they get there. It is also important to know how large the spawning populations and areas are, how far the spawning fish travel to get to spawning sites, the timing (seasonal, lunar, diel) of spawning, and the size and age structure of the spawners (SEDAR 2004).

We realized that we had a rare opportunity to monitor patterns of behavior related to reproduction in great detail by becoming involved with the Florida Atlantic Coast Telemetry (FACT)-array of receivers which is run by Florida Fish and Wildlife Conservation Commission (FWC). This cooperative effort allows us to monitor movements of goliath grouper over a very large area of the east coast of Florida, from Palm Beach County to the Florida-Georgia border. To become involved we purchased and deployed 10 - VR2W Vemco receivers on suspect goliath grouper spawning sites. Researchers with VR2W receivers on sites along the shelf contact us through the FACT management group and report detections of transmitter-tagged goliath grouper to us--when we detect transmitter-tagged fish of species, we report the detections to them. The cooperative provides a tremendous advantage for all involved. A similar array would be productive on the Gulf coast, but it does not yet exist.

Materials and Methods

Acoustic telemetry

We tagged goliath groupers intraperitoneally with VEMCO V16 coded acoustic (69 kHz) transmitter tags with an 8 year battery life. Tags were recorded by VEMCO VR2W-69 kHz receivers which were mounted on a 3/8" (9.5 mm) diam stainless cable anchored to the bottom at known and suspected spawning sites (Figure 35). Additional VR2W receivers maintained by the Florida Atlantic Coast Telemetry (FACT) Array group added to our sampling area. The FACT group makes use of compatible telemetry receiver hardware and a commitment to coordinate receiver spacing and coordinate detection data to allow member researchers to track study animals over longer durations and over greater distances. As of early 2013, coordinating FACT group members maintained 201 VEMCO VR2 and VR2W receivers over a 500-km span of Florida's Atlantic coast from Cumberland Sound (30°42'N) on the Florida-Georgia border to Delray Beach (26°29'N) in south Florida. Receivers are deployed along a continuum of coastal habitats from freshwater estuaries (e.g. Indian River Lagoon) to marine waters of the adjacent continental shelf.

Figure 35. Photograph of goliath grouper near the VR2W mooring structure (brake drum anchor, 3/8 in (9.5 mm) diam stainless steel cable, VR2W, and hard float buoys) at the Sun Tug spawning-aggregation site.

During the course of the study (2010 – 2012), we deployed ten VR2W receivers at 14 different locations. Two of our receivers were lost: one likely due to structural failure of the mount (Zion Train, deployed fall 2012) and the other likely due to theft (Castor wreck, deployed spring 2012). We deployed VR2W receivers at known and suspected spawning aggregation sites and on other natural and artificial reefs known to hold goliath groupers. Monitored sites were visited twice annually (March and August) to download data, replace batteries (done in August only), and check on the integrity of the mooring system. Six of the sites we monitored over the course of the study off Jupiter were confirmed as spawning aggregation sites: Hole-in-the-wall, Zion Train, 3-Holes, Sun Tug, MG-111, and Gary's Greys.

Data were downloaded into the VEMCO VUE program and exported into Excel (Microsoft, 2007, Redmond, Washington). All detections were first scanned for false detections using a 2-detection within 20-minute filter criteria. False detections were eliminated, and the remaining detection data were entered into the database.

We calculated two different distance metrics using the tag data from 2011 and 2012: maximum distance (MD) and cumulative distance (CD). We define MD as the maximum distance moved by a transmitter-tagged fish between any two stations during the study. MD is analogous to metrics calculated from mark and recapture studies, which usually underestimate the actual distance that an individual moved in a given time. CD is the sum of fine-scale movements by a transmitter-tagged fish. Because of the many sites monitored by the FACT group, we are able to determine fine-scale movements when transmitter-tagged fish move among the monitored sites. Because all sites in the region were not monitored, CD also somewhat underestimates total movements, but to a much lesser degree than MD. Because none of the transmitter-tagged fish were continuously recorded, they must have visited some unmonitored sites. We calculated the CD moved by a transmitter-tagged fish as the sum of all distances moved among monitored sites during a given time period (e.g., month, year). We also calculated a proxy for activity by subtracting MD from CD for each transmitter-tagged fish. Large values of this proxy indicate tagged fish moved often between sites, while small values suggest more sedentary behavior.

Another advantage to using acoustic telemetry on protected species is the ability to estimate natural mortality (this estimate also includes illegal fishing and incidental catch mortality) using the Kaplan-Meier method (Krebs 1999). This methodology allows for a staggered entry design, where new individuals are added to the cohort of tagged animals, and also allows for censoring of animals lost to the study for reasons other than death. The survival estimate for our population of tagged goliath groupers, and associated variance was calculated using Ecological Methodology v.7.2 (Krebs, 1999, Exeter Software, Setauket, NY).

Results

Geographic distribution of spawning

Between 4 September 2010 and 16 September 2012 we tagged 45 goliath groupers with VEMCO V16-P coded transmitters. Most fish were implanted with transmitters during fall 2010 (38 of 45 fish), with the remaining transmitters implanted in fish during May 2011 (2 fish) and September 2012 (5 fish). Results presented here only consider the 40 individuals tagged in 2010 and 2011; not enough data is yet available for the 5 individuals tagged in late 2012, so they are excluded from the analyses presented below. Tagging was conducted at three suspected spawning aggregation sites off Jupiter, FL (Figure 36), two of which have since been confirmed as spawning sites: Zion Train (artificial reef; 30 fish tagged) and Three-Holes (natural reef complex; 5 fish tagged). Ten fish were tagged at the Gulfland wreck (artificial reef), which has been confirmed as a non-spawning site. Tagged goliath groupers ranged in size from 104 to 205 cm TL (mean TL = 159.1 cm). Sex distribution of tagged fish (as determined visually at time of capture, or from histology in the case of transitional individuals) was as follows: female = 17; male = 13; transitional = 6; immature = 3; unknown = 1 (this individual is excluded from analyses which compare movement patters of males and females).

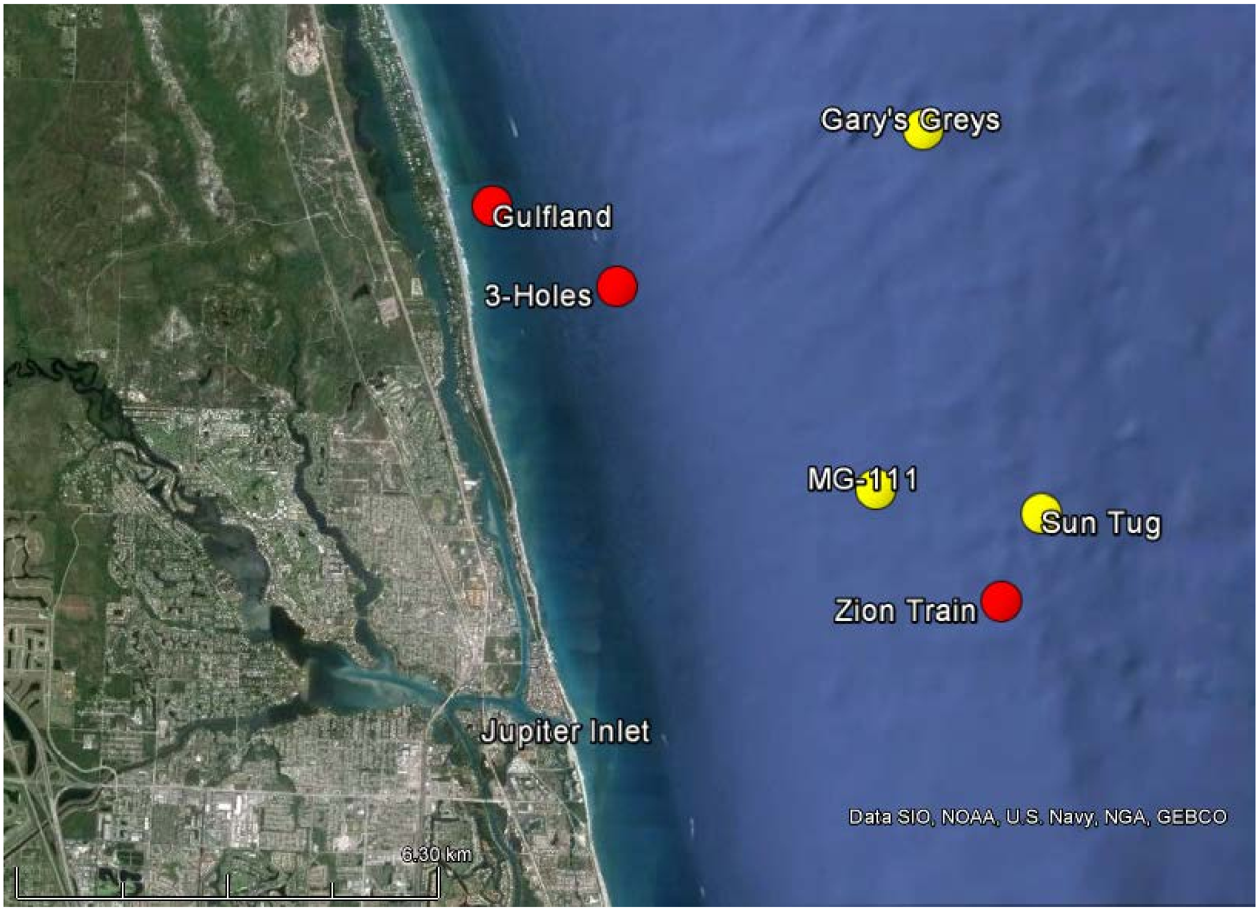

Figure 36. Map of the main study area offshore of Jupiter, FL. All sites except for Gulfland were confirmed as spawning aggregation sites during the course of this study. Red markers indicate sites where fish were implanted with transmitter tags.

Between 1 January 2011 and 31 December 2012, transmitter-tagged goliath groupers were detected at 43-- VR2W-monitored stations within the FACT array (Figure 37). In 2011, 37 of the 40 tagged goliath groupers (92.5%) were recorded within the array. In 2012, 35 of the 40 tagged goliath groupers (87.5%) were recorded within the array. Two individuals were never detected in the array after being tagged. However, one of these individuals was recaptured during sampling approximately 4 months after being tagged; a VR2W deployed at the site of recapture failed to detect the fish indicating a malfunctioning transmitter. (We had another non-functioning transmitter in that batch of 40, but we detected it before implanting it in a fish and sent it back to Vemco for a replacement). The majority of detections occurred within 10-km of the tagging site, but tagged goliaths were detected at sites that spanned the entire range of the FACT array, a total distance of approximately 500 km.

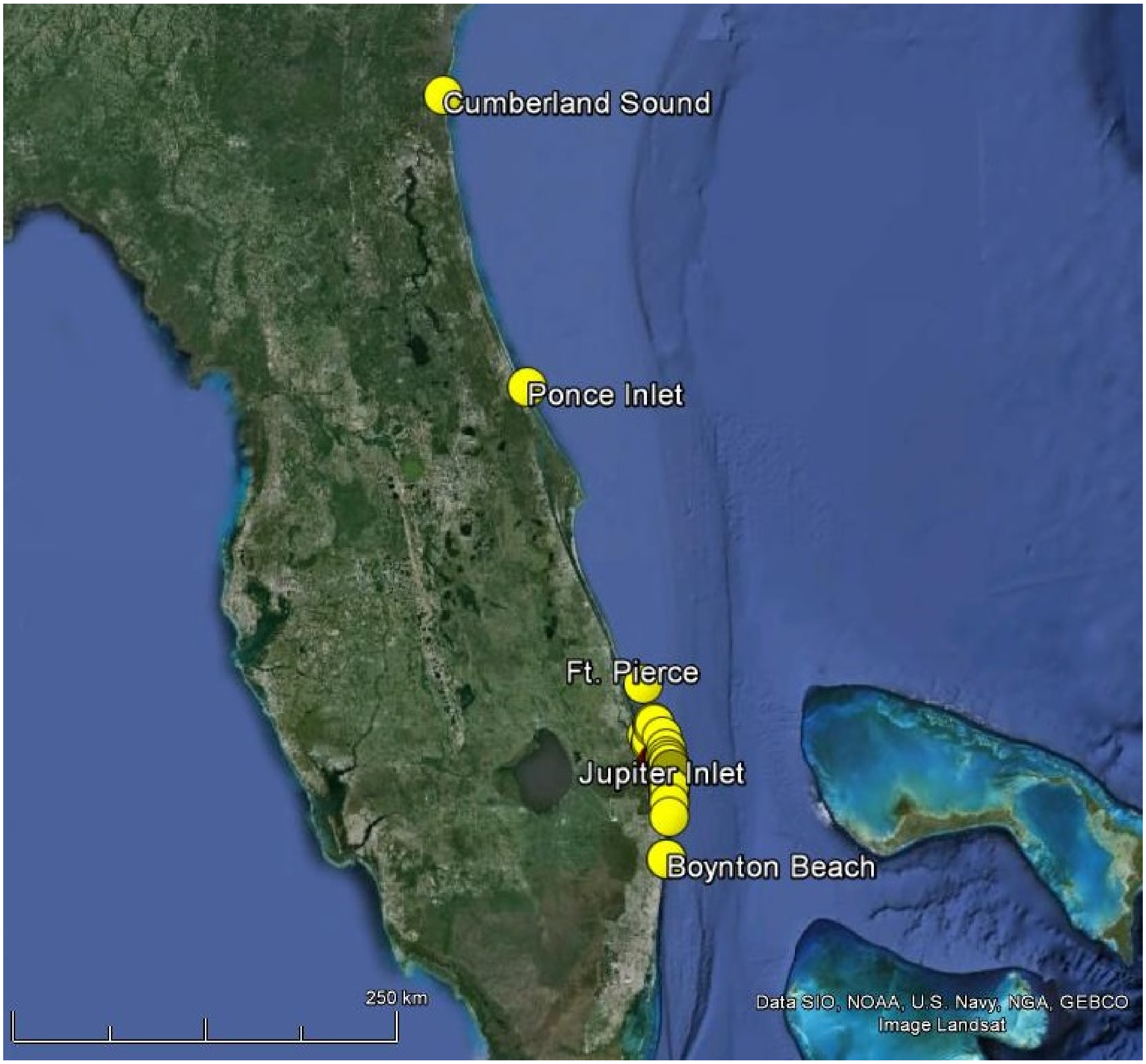

Figure 37. FACT Array of VR2W stations on the Atlantic coast of Florida where transmitter-tagged goliath groupers were recorded during 2011 and 2012.

Spawning site fidelity & movement among spawning sites

Spawning site fidelity of transmitter-tagged fish was high: 75% of all fish tagged in 2010 and 2011 (30 fish) returned to the site where they were tagged within one year; 65.8% of tagged fish returned to the tagging site in both 2011 and 2012 (25 fish). (We only have one year of data for the two fish that were tagged in 2011, so cannot evaluate site fidelity.)

Of all transmitter-tagged goliath grouper, 95% (38 fish) were detected at a confirmed spawning aggregation site at some point during the study. Within a given year the number of tagged fish detected at spawning sites was the same for 2011 and 2012: 85% or 34 tagged fish detected each year. Two transmitter-tagged fish were never recorded at any FACT site after tagging, thus were never detected at a monitored spawning site. All transmitter-tagged fish that were not detected at a monitored spawning site in 2011 were detected at a spawning site in 2012, and vice versa.

Transmitter-tagged fish visited an average of 1.78 ± 0.141 spawning sites over the course of the study. Tagged fish were detected at slightly more spawning sites in 2012 relative to 2011 (1.88 [± 0.212] vs. 1.70 [± 0.187]). The maximum number of spawning sites visited by a single individual in 2011 was four; in 2012 a single individual was detected at all five confirmed spawning sites. All five of the confirmed spawning sites were visited by at least one transmitter-tagged fish each year of the study.

The most frequently visited spawning site was Zion Train (ZT); it was also the site where the most fish were tagged. In 2011, 28 of 40 (70%) tagged goliath groupers were detected at ZT; in 2012, 21 of 40 (52.5%) of tagged goliath grouper were detected at ZT. Over both years, 29 of the 40 (72.5%) tagged goliath groupers were detected at the ZT site, followed by Sun Tug (26 tagged fish), MG-111 (17 tagged fish), 3-Holes (12 tagged fish), and Gary's Greys (12 tagged fish).

The number of VR2W-monitored stations visited by transmitter-tagged goliath grouper varied seasonally (Figure 38). The number of stations visited peaked in July – September indicating increased activity during the spawning season. Most individuals moved little during the year, remaining at one or few nearby reefs while a few individuals were detected at multiple sites each month. The mean number of sites visited each month for all tagged fish ranged from 0.2 (± 0.07) sites in January 2011 to 2.65 (± 0.44) sites in September 2012. Tagged fish visited more sites during July, August, and September compared to the rest of the year. Over the entire study, the average transmitter-tagged fish was detected at just over 5 unique stations (5.03 [± 0.441]). Tagged fish were detected at slightly more stations in 2012 compared to 2011 (5.53 [± 0.661] vs. 4.53 [± 0.582]). The maximum number of monitored stations visited by any tagged fish over the course of the study was 20.

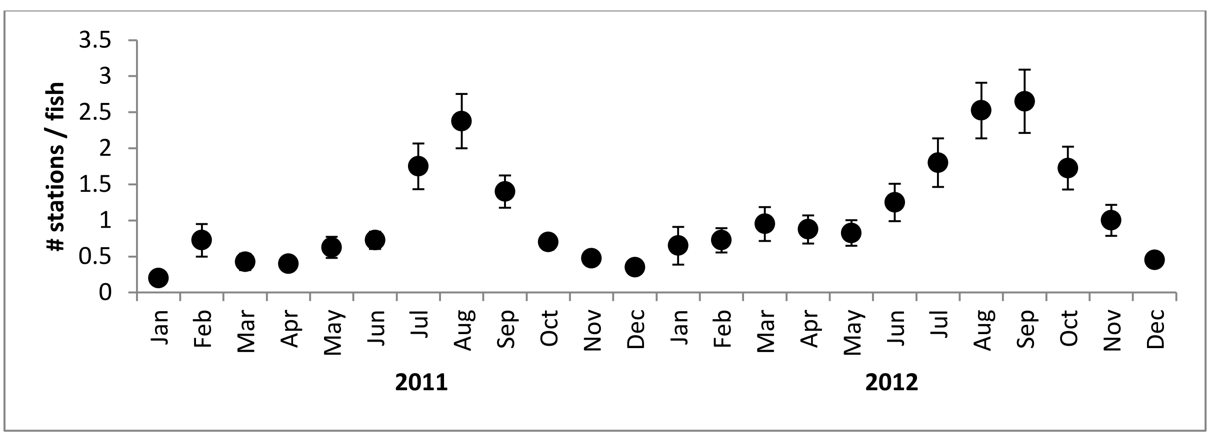

Figure 38. Number of FACT VR2W-monitored stations (mean no. stations per fish ± SE) visited per month by transmitter-tagged goliath groupers (N = 40) throughout 2011 and 2012.

Movement Patterns

Fish moved more often and farther during months associated with spawning activity compared to the rest of the year (Figure 39). There were slight differences between the two years of the study: fish moved farther in July 2011, while the highest values for both distance metrics calculated (CD and MD) occurred in August 2012. Our proxy for activity (CD – MD) showed that fish were most active during the spawning season (Figure 40). The highest activity was observed at different points each year: in 2011 activity peaked in July and was above average through September; in 2012 activity was highest in September but was above average from July through November. Activity of fish was above average during March and April of 2012, and elevated (yet below average) during February 2011.

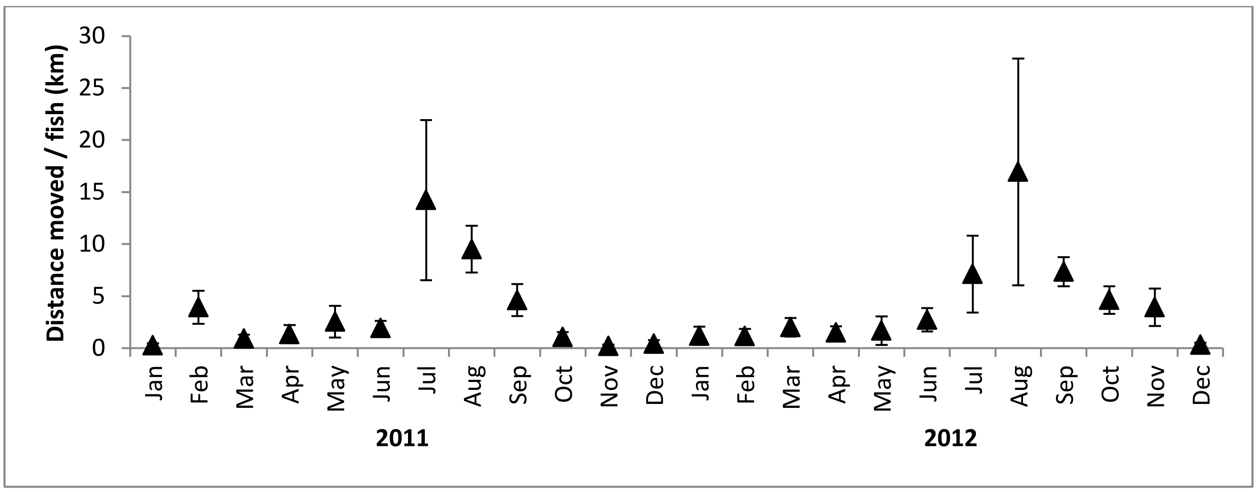

Figure 39. Distance moved, calculated as the maximum distance (km) among FACT-monitored stations detecting transmitter-tagged goliath groupers (N = 40) per month of the study (mean ± SE).

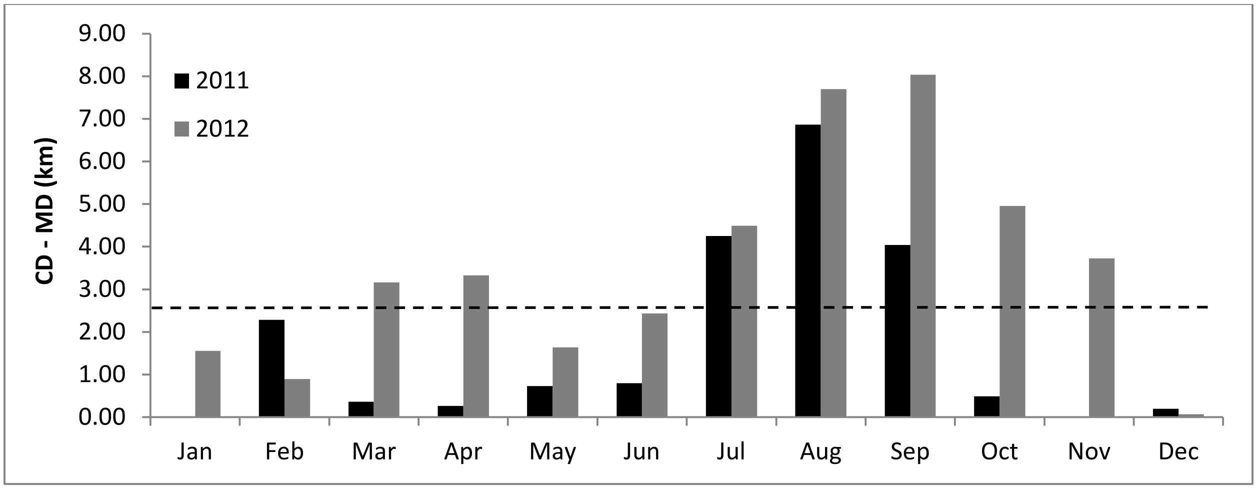

Figure 40. Activity of transmitter-tagged goliath groupers each month, measured as the difference between the mean cumulative distance moved per fish and the mean maximum distance moved per fish. Dashed line indicates the mean activity value for the entire study (CD – MD = 2.60).

The maximum distance moved between consecutive detections by any tagged fish in the study occurred between 11 and 21 August 2012—it was between Cumberland Sound and the spawning site MG-111, a straight-line distance of 437.8-km (43.7 km/day). This same individual accounted for the second longest movement of 252.3-km in July 2011 between Ponce Inlet and Tunnels, a natural reef site near Jupiter, FL, over a period of 22 days (11.5 km/day). Also in July 2011 another tagged fish moved 222.1 km between Ponce Inlet and an artificial reef near Port St. Lucie, FL in 9 days (24.7 km/day). These long distance migrations contrast to the average distance between consecutive detections for all fish in 2012 of just 6.74 km.

Size and Sex Differences

Movement data were analyzed by both fish size and sex to determine if any patterns could be attributed to either factor. In general, larger fish visited more FACT-monitored stations during the study (Fig. 41) and moved farther (calculated as maximum distance; Figure 42) compared to smaller individuals. Simple linear regressions performed on both of these metrics showed that both patterns are significant and positively correlated (number stations visited: $df = 39$, $F = 20.4$, $p < 0.001$; maximum distance moved: $df = 39$, $F = 4.28$, $p = 0.0453$). The regression of maximum-distance traveled relative to total length was re-calculated discarding the three individuals that moved more than 150-km, as each of these values resulted in standardized residual values > 2 and were considered outliers. The resulting regression was still significant and positively correlated ($df = 36$, $F = 4.15$, $p < 0.0492$). Unsurprisingly, the number of stations visited was positively correlated with maximum distance moved (multiple-R = 0.245), because visiting more stations requires an individual to move a greater distance.

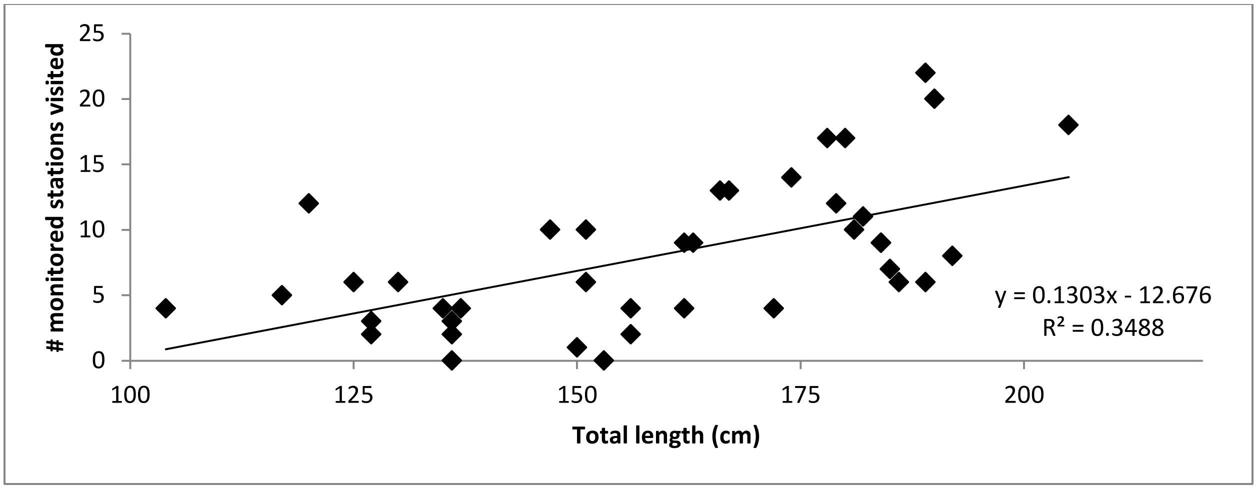

Figure 41. Number of distinct FACT-monitored stations visited during 2011 and 2012 by transmitter-tagged goliath groupers (N = 40) plotted against fish size measured as total length (cm) at time of tagging. Linear regression slope is significantly positive (*df* = 39, F = 20.4, $p < 0.001$).

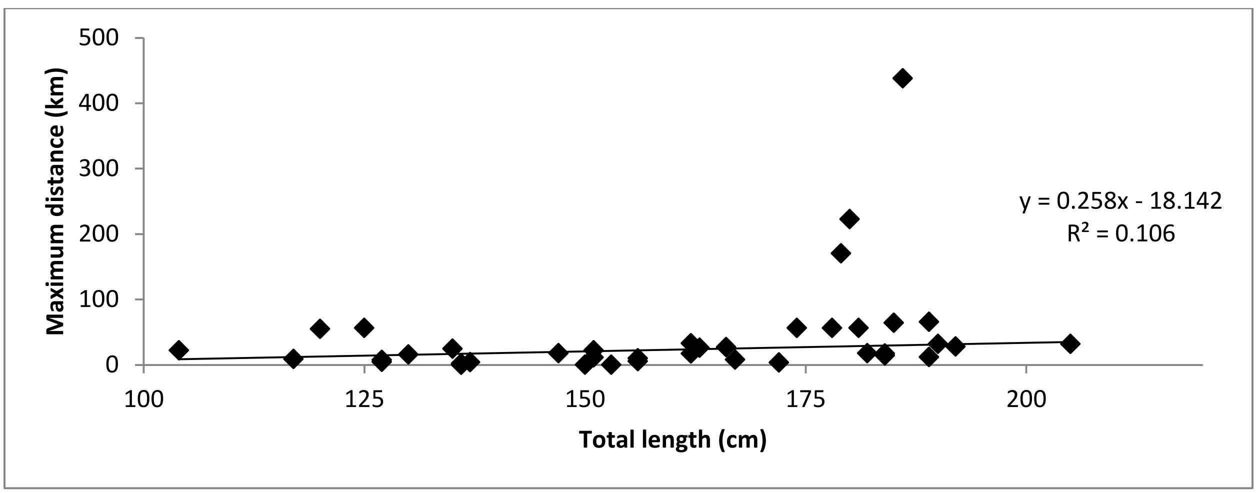

Figure 42. Maximum distance moved in kilometers during 2011 and 2012 by transmitter-tagged goliath grouper (N = 40) plotted against total length (cm) at time of tagging. Linear regression is significantly positive (*df* = 36, F = 4.15, $p = 0.0492$), and was calculated without including the three fish with movements > 150-km (considered outliers).

We found no differences between sexes in terms of the number of stations visited (Figure 43). The maximum distance moved by females was significantly greater than either the distance moved by males or transitional fish (Figure 44). However, a *t*-test performed on maximum distance moved by sex (transitional animals were excluded due to low sample size), showed that the result was only marginally significant (*df* = 28, *T* = 1.63, $p = 0.0572$).

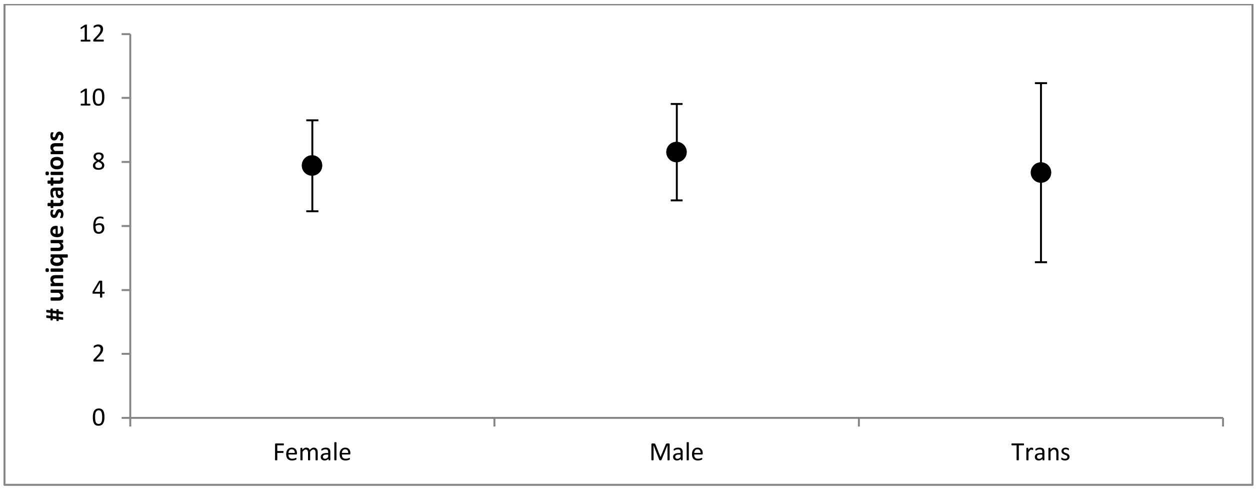

Figure 43. Total number of unique FACT-monitored stations visited during 2011 and 2012 by transmitter-tagged goliath grouper (N = 40) separated by sex as determined by histological examination of gonads or visually at time of tagging. “Trans” indicates a transitional individual that had both female and male gonads present in histological examination. Error bars are ± 1 S.E.

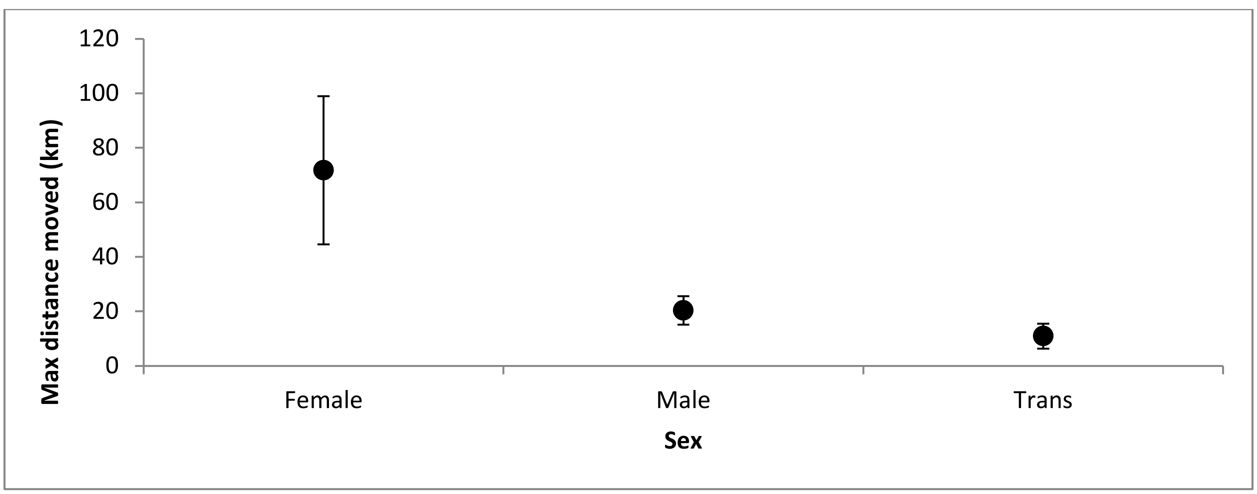

Figure 44. Maximum distance moved in kilometers during 2011 and 2012 by transmitter-tagged goliath grouper (N = 40) separated by sex. “Trans” indicates a hermaphroditic (transitional) individual with both female and male tissue in gonad biopsies. Error bars are ± 1 S.E.

Aggregating Behavior

Analysis of transmitter-tagged fish visiting the Zion Train site during the spawning seasons of 2011 and 2012 (Figure 45) suggests strong lunar control of spawning site fidelity. In both years the number of transmitter-tagged goliaths recorded at the site peaked around the new moons of August and September (but not October). In both years a total of 27 of the 40 tagged goliath groupers visited the

Zion Train aggregation during the spawning season, approximately the same number of transmitter-tagged goliath groupers visited the Zion Train site each year (N_{2011} = 26; N_{2012} = 25). Of these there was 74% overlap in the identity of recorded individuals (20 of 27 visited both years, only 7 visited during a single year (Table 2).

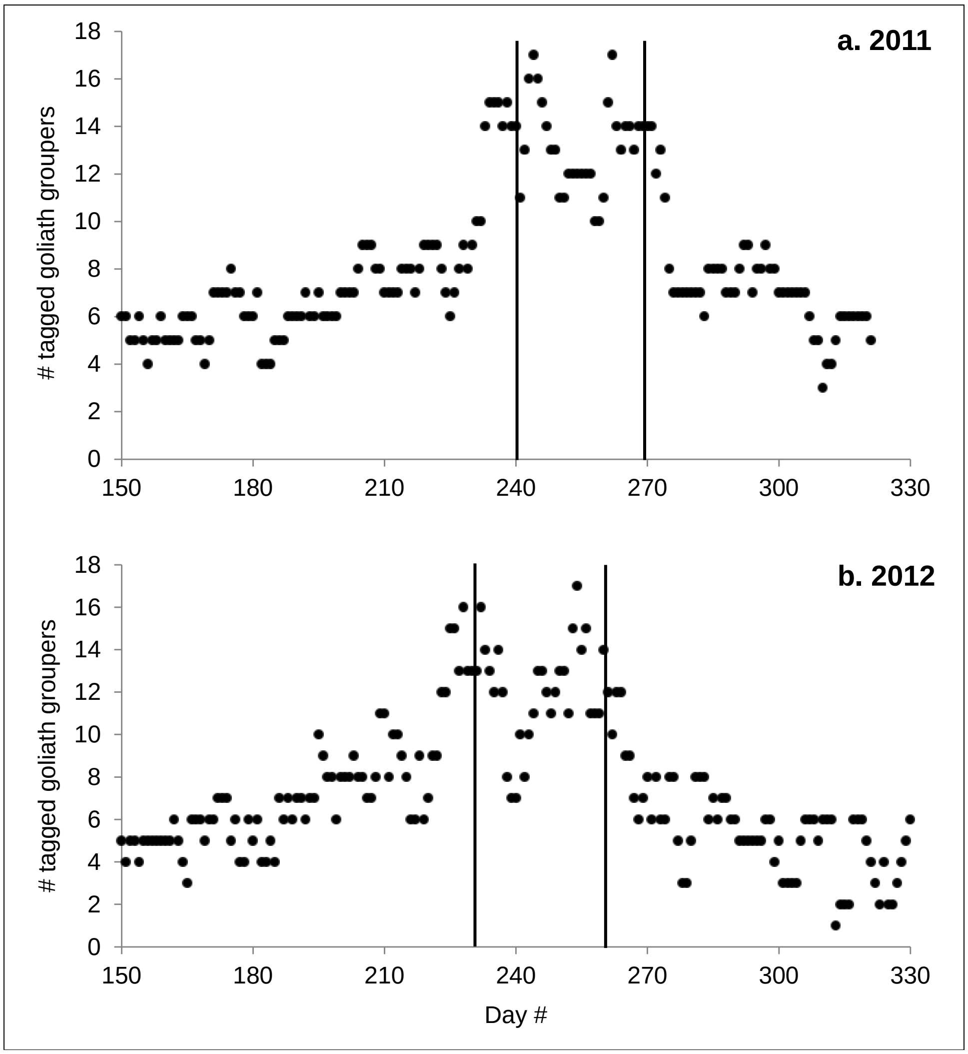

Figure 45. Total number of transmitter tagged goliath groupers detected at Zion Train from June through November in 2011 (a) and 2012 (b). Vertical lines correspond to the approximate dates of the new moon for August and September. Note that aggregation times were followed new moon times, not dates.

Aggregation of transmitter-tagged fish was clearly evident from the tag detection data from 2011 (Figure 46). During non-spawning months fish were generally spread out across numerous sites and few sites averaged multiple fish per day. However, during spawning season fish were detected at fewer sites and sites averaging multiple individuals per site per day were more common. The pattern was similar in 2012 (Figure 46). In each figure, the size of circles represents the average number of daily detections.

The aggregation of transmitter-tagged individuals is clear in Figures 46 and 47, especially for three of the confirmed spawning aggregation sites: Zion Train (ZT), Three-Holes (TH), and MG-111 barge (MGW).

Table 2. Identity of transmitter-tagged goliath groupers that visited the Zion Train spawning aggregation in 2011 and 2012.

Transmitter tag no.	Visited in 2011	Visited in 2012
46522	X	X
46523		
46524		
46525	X	
46526	X	
46527		
46528		
46529		
46530		
46531	X	X
46532		
46533	X	X
46534	X	X
46535	X	X
46536	X	X
46537	X	X
46538	X	X
46539	X	X
46540	X	X
46541		
46542		
46543	X	
46544		
46545		X
46546	X	
46547	X	X
46548		
46549	X	X
46550	X	X
46551	X	X
46552	X	X
46553		
46554	X	X
46555	X	
46556	X	
46557	X	X
46558	X	X
46559	X	X
46560		
46561	X	X
Total	26	25

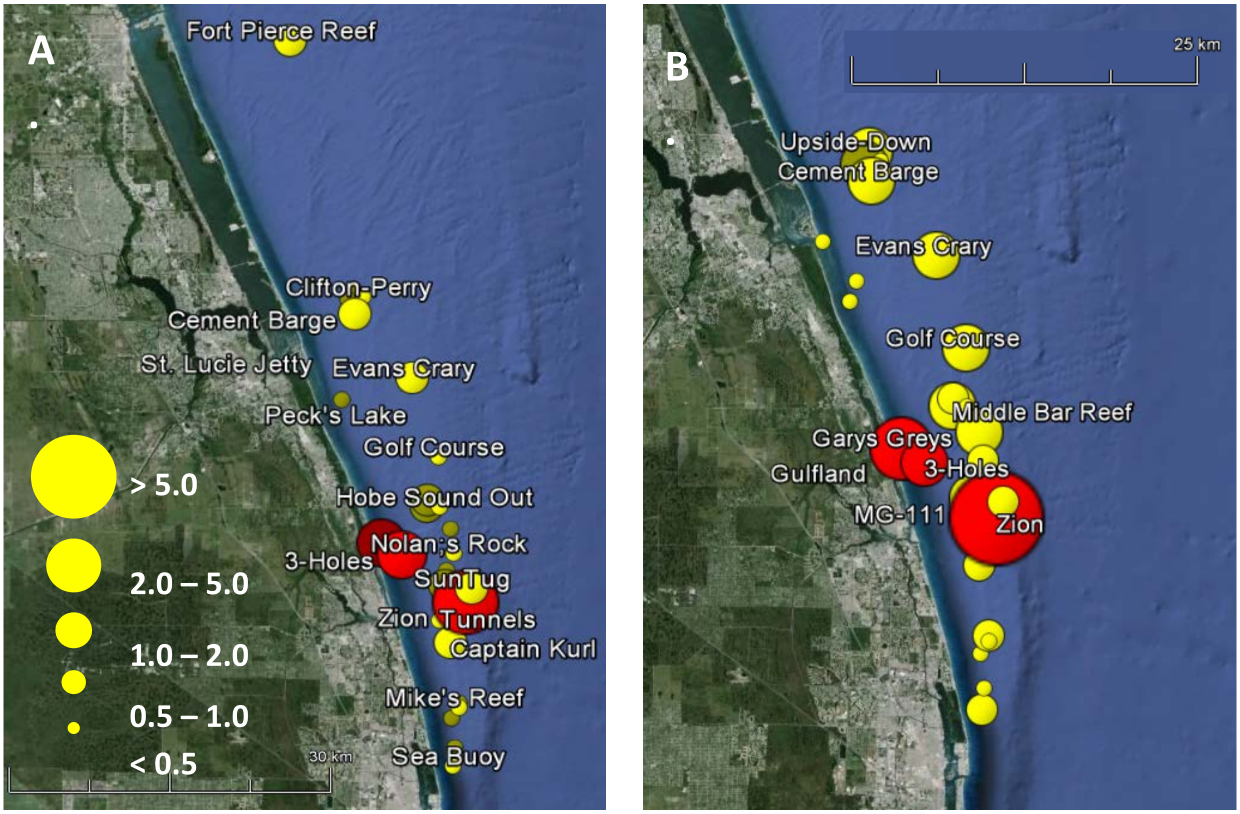

Figure 46. Mean number of tagged goliath groupers detected daily in 2011 during non-spawning months (A) and spawning months (B). For spawning months only, those stations recording > 1.0 fish per day are labeled. Red markers indicate sites where fish were tagged with transmitters.

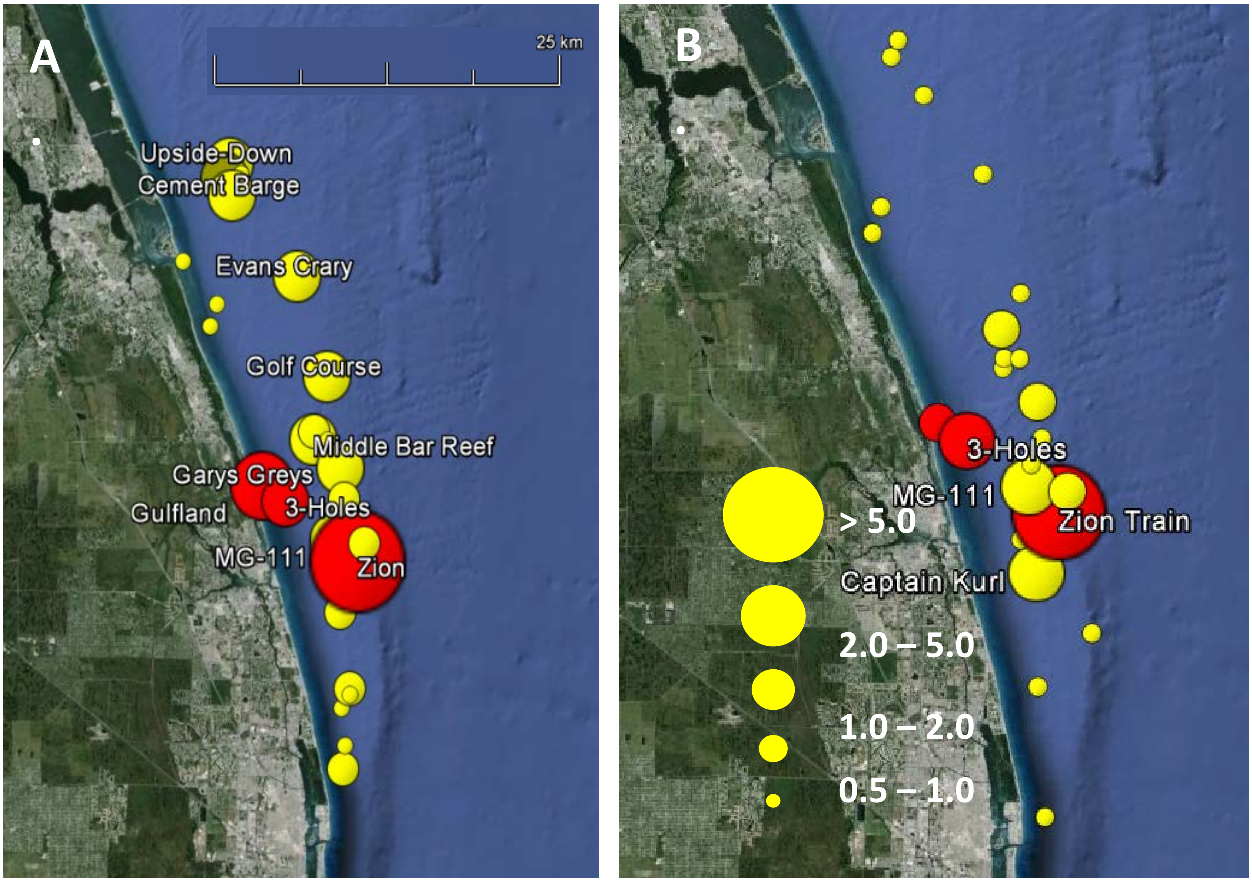

Figure 47. Mean number of tagged goliath groupers detected daily during spawning season in 2011 (A) and 2012 (B); only stations averaging > 1 fish per day are labeled. Red markers indicate sites where fish were tagged with transmitters.

We examined the aggregating behavior of transmitter-tagged goliath grouper by comparing the number of sites that at least one tagged goliath grouper was detected during 2011 (Figure 48) with the number of tagged goliath groupers detected at the Zion Train site (Figure 49) over the same time period. The number of sites where a tagged goliath grouper was detected was relatively stable during the first half of 2011 until mid-July, around the time of the July full moon on day #196 (Figure 48). Starting mid-July the number of sites where a tagged goliath grouper was detected increased and stayed high until mid-August. Following the August full moon (day #225; Figure 48) the number of sites with a tagged goliath grouper decreased again. At the same time, the number of transmitter-tagged goliaths detected daily at the Zion Train site increased until it peaked at the time of the August new moon (day #241; Figure 49).

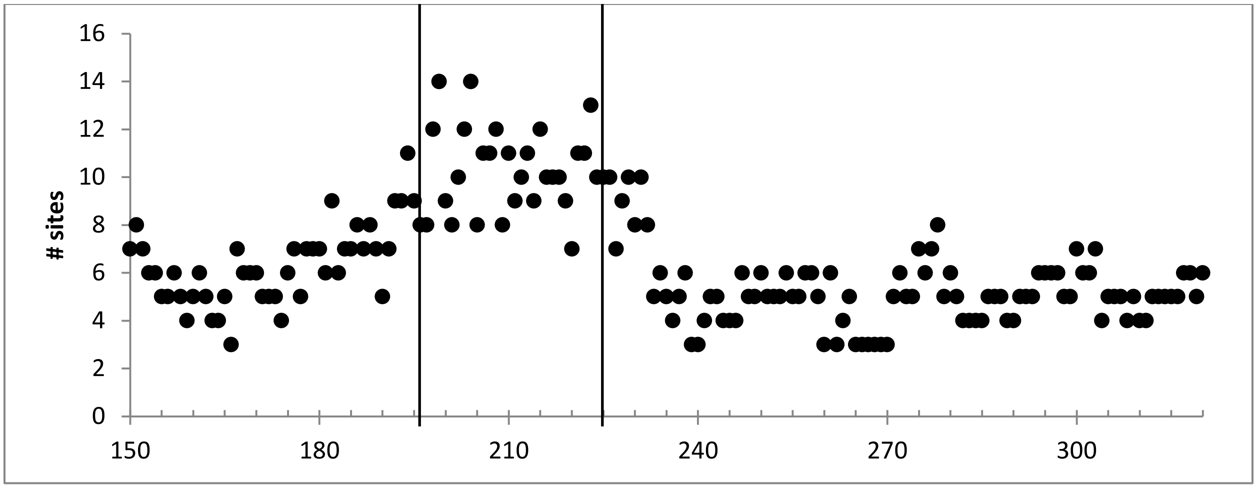

Figure 48. Number of FACT-monitored sites where a transmitter-tagged goliath grouper was detected each day between 30 May and 16 November, 2011. Vertical lines indicate the approximate dates of the July and August full moons.

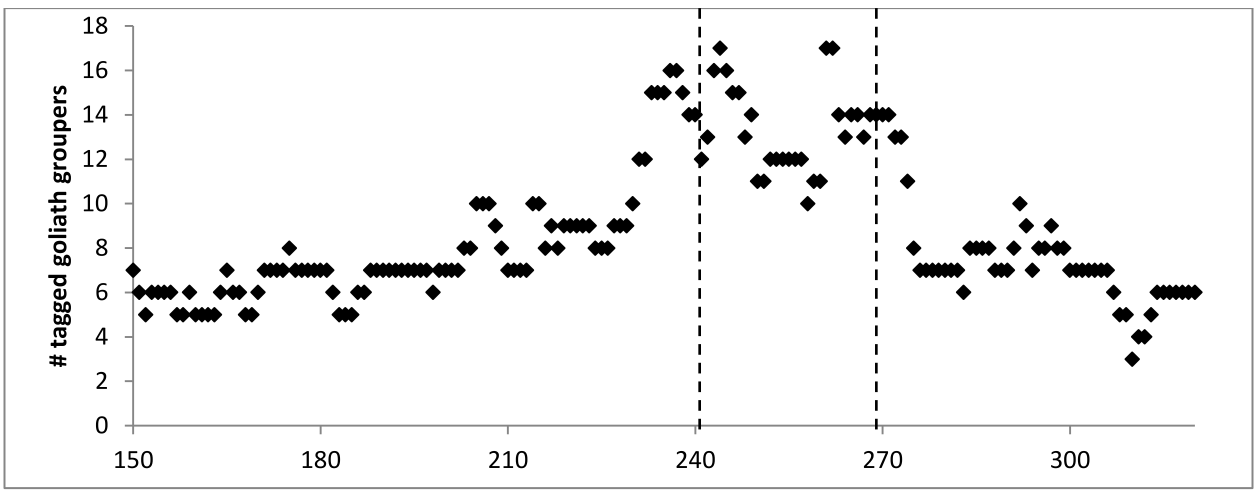

Figure 49. Number of transmitter-tagged goliath groupers recorded per day at the Zion Train spawning site between 30 May, 2011 and 16 November, 2011; vertical dashed lines indicate approximate dates of August and September new moons.

Identification of spawning sites

During the course of this study we have determined that there are multiple criteria for identifying spawning sites. These include: (1) increased site abundance in August through September, (2) ePOFs in gonad biopsies, (3) night-time chorusing during new moon phases, and (4) the collection of fertilized eggs downstream from the aggregation site. In Table 3 we have listed known and suspect spawning sites off Palm Beach and Martin Counties.

Table 3. Suspect and verified goliath grouper spawning sites off SE Florida.

***Hole in the wall	N26 53.640 W079 59.170
***Zion Train	N26 57.770 W080 00.450
***MG111	N26 58.670 W080 01.490
***Three holes	N27 00.210 W080 03.990
***Sun Tug	N26 58.477 W080 00.914
***South FAD	N26 54.979 W080 02.827
***Gary's Greys	N27 01.543 W080 01.301
***208 wreck	N27 00.732 W080 02.677
**Warrior Reef	N26 58.679 W080 01.516
**Upside down	N27 13.944 W080 06.702
**Chip's reef	N27 32.268 W080 10.769
**Pipe Barge	N27 13.368 W080 06.966
*Halsey WWII wreck	N27 20.155 W080 04.574
*Evans Crary	N27 09.342 W080 03.378
*Esso Bonaire	N26 57.850 W080 00.480
*Miss Jenny Barge	N26 57.830 W080 00.438
*Mike's Reef	N26 53.077 W080 01.220
*Juno High Reef Ledge	N26 52.230 W080 01.130
*Mizpah	N26 47.160 W080 00.998
*Budbar wreck	N26 28.788 W080 01.830
*Castor	N26 28.730 W080 02.230

*Suspect site based on REEF data and/or reports of spawning-season build-up

**Suspect site based on the occurrence of transmitter-tagged fish

***Confirmed spawning site based on ovary biopsy ePOFs, night chorusing and/or egg collection

The frequency of ePOFs in biopsied goliath grouper provides evidence for spawning on sites of capture (Figure 50). Of course, because the fish may move among sites during the spawning season, the occurrence of ePOFs as an indicator of a spawning site is never certain. Intense night-time chorusing on those sites increases the certainty that those sites are spawning sites.

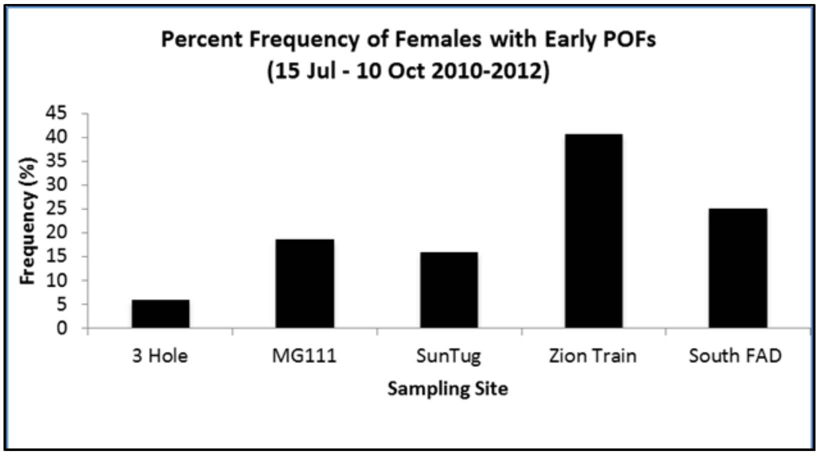

Figure 50. Percent frequency of ePOFs on sites off Jupiter, FL indicating that the sites are goliath grouper spawning sites.

Discussion:

Acoustic telemetry

Our data indicate a connection between reproductive behavior of goliath grouper and the lunar cycle. Increased activity of the fish was observed to begin in July around the full moon. Fish were active during this time, moving between sites and into the spawning area around Jupiter, FL. While aggregating behavior appears to be triggered by the full moon, spawning is apparently centered around the new moon phase. This conclusion is supported by a high frequency of ePOFs and hydrated oocytes in ovarian biopsies on the new moons of August and September, but not full moons of either month. It is also supported by sound production peaking on the new moons and the aggregating behavior of transmitter-tagged goliath grouper on home spawning sites (Zion Train) on the new moons of August and September (Figures 45 and 49).

Monitoring of transmitter-tagged goliath grouper revealed that they do not, on average, move very far or very often, except around spawning time, a point suggested by Koenig et al. (2011) from tagging data, but confirmed by this study. By transmitter-tagging fish caught during the aggregation in 2010, our study tracked these fish as they moved back to home sites and then returned to the spawning aggregation area in 2011 and 2012. Some fish did not leave the aggregation area. An average of 4 to 6 tagged individuals was detected daily on the Zion Train spawning site throughout the year. Likewise, detection data from the 3-Holes, MG-111, and Sun Tug spawning sites all showed that a few resident individuals remained at these sites year-round. Another group of tagged goliath groupers (7 individuals) was detected year-round at a group of artificial reefs offshore of St. Lucie Inlet, approximately 25 km north of the spawning area. Individuals from this group made multiple movements between their residence reefs and the spawning reefs before and during the spawning season. These repeated movements between St. Lucie and Jupiter, in part, account for the increase in the distance metrics calculated for July and August, and seen in Figures 39 and 40. Post-spawning fish returned to the St. Lucie reefs and did not return to Jupiter until the following spawning season.

Most of our tagging effort was conducted at the Zion Train site: in 2010 and 2011, 25 of the 40 transmitters were implanted in fish caught there primarily because this was the site of the largest aggregation in the area and a presumed spawning site when the study started in 2010. Site fidelity estimates suggest that most fish return to the same sites year after year. However, with multiple aggregations in relatively close proximity, fish not only visited their "home" aggregation but others as well. In fact, 6 of the 15 fish tagged at other sites visited the Zion Train site at some point during the study.

By calculating multiple movement metrics (MD and CD) we were able to arrive at a deeper understanding of fish movement and behavior relative to a mark-recapture study. The tagging data suggest that goliath groupers move from residence or home sites to spawning aggregation sites beginning in July and remain highly active during the spawning period. The ability to estimate activity of tagged fish highlights the importance of using continuously monitored sites, like those maintained by the FACT-array group. Not only can we determine some maximum distance that a fish may have moved over a given time period, but we can also estimate relative activity levels by summing all movements made over the same period. This activity proxy allows us to show graphically the different behaviors that goliath grouper exhibit during the spawning time when they move between spawning and non-spawning sites more often than during non-spawning periods (Figure 40). It may also reveal environmentally forced movements, such as those induced by cold-water upwelling events. In February 2011 and again in March-April 2012 the activity proxy shows an increase over the baseline mean value, at a time when such movements would not be expected to be spawning induced. It is possible that these elevated values represent fish moving in response to environmental variables. Unfortunately, we do not have corresponding temperature records to confirm this hypothesis. Since fall 2012 all VR2W receiver deployments have included temperature loggers in order to rectify this data gap.

Detection range was not tested for this study. However, other studies using these tags report detection ranges between 50 to 750 m, with peak efficiency between 250 – 500 m (Humston et al. 2005, Whitty et al. 2009). Based on diver observations, goliath groupers tend to stay close to structure, well within the detectable range of the transmitters. Furthermore, the detection range is much less than the distance between sites, allowing us to assume that single individuals cannot be detected at multiple sites simultaneously. The detection filter we used to eliminate false detections (2 detections within 20-minutes) is designed to eliminate false detections that can occur due to code "collisions" and it follows the recommendations of the transmitter manufacturer.

Identification of spawning sites

The collection of fertilized eggs downstream from a goliath grouper aggregation provides the most definitive evidence for the identification of a spawning site, but it is the most difficult to obtain. Conditions must be right for the collection of eggs—current speed and direction must be constant; spawning must take place directly upstream from the nets (i.e., the fish must remain where initially observed and do not spawn away from the site); concentrations of plankton, including jellyfish and ctenophores, must be low so that the nets can fish for extended periods of time without becoming heavily fouled. In addition, eggs must be allowed to develop to the neurula stage so that there is enough DNA to confirm the identity of the species.

A straight-forward method of identifying a spawning site is through the use of hydrophones and sound recorders deployed on suspect sites on new moon nights of August and September. Our research shows that night-time chorusing occurs most intensely on new moons and is nearly absent on full moons, so monitoring night-time sounds on new moons (± 4 days) would provide strong evidence that the site is a spawning site. Goliath grouper night-time sound production intensity (number of calls or sound energy per time) and abundance of spawners may be positively correlated. This will be tested most intensely in the spawning season of 2014. If the number of goliath grouper calls per time is positively related to the number of spawners on spawning sites during August and September new moons, it may be possible to estimate the size of the spawning population simply by monitoring night-time sounds. A similar approach to estimating the density of a spawning aggregation of red hind (*Epinephelus guttatus*) was done by Rowell et al. (2012).

Part IV. Miscellaneous topics not in the objectives of the project: Egg sampling, Diet and stabile isotopes, genetic tests.

Introduction

When we sample goliath grouper on their spawning sites, we maximize that amount of information we can gain from them. So, starting in 2010 we have been collecting stomach contents, muscle tissue (for stable isotope analysis), dorsal fin rays (for aging), and fin tips (for genetic stock ID). We have also been attempting to collect fertilized eggs at night, downstream from the spawning fish. In this section of our report we present results from those ancillary studies.

Egg collections using downstream plankton nets were initially attempted to verify spawning sites, but after the difficulty of this endeavor was realized, we attempted it only for rearing eggs primarily for the description of the developmental stages, as we did with red grouper (Colin et al. 1996) . To date we have been successful at collecting, but not rearing.

Diet through the evaluation of stomach contents has always been part of our investigations of goliath grouper. Our earlier study described the diet of juvenile goliath grouper (Koenig and Coleman 2009), but in this present study we have expanded that work to include adult diets, especially on spawning aggregations. Diet of goliath grouper is such a contentious issue among fishers, that it is important to gain an understanding of their food and feeding habitats.

We also have the opportunity to collect goliath grouper DNA samples to evaluate genetic stock structure. This work is being done by researchers at the Fish and Wildlife Research Institute (FWRI), but is not yet complete.

We are also offering goliath grouper samples (muscle, ovarian tissue, etc.) to Doug Adams (FWRI) for tissue analysis of mercury contamination and histopathology which would extend his work on mercury contamination in goliath grouper (Adams and Sonne 2013). We expect to provide samples to that work in 2014.

Materials and Methods

Egg collection

Eggs were sampled from spawning sites through the use of downstream (50 - 100 m) plankton nets which were clipped to mooring lines with an anchor at the bottom and floats at the surface (Figure 51). Each of three mooring lines had three--0.75 m diam plankton nets. Plankton nets were attached at equal intervals from just below mid-depth to the surface. We arranged three mooring lines in a line perpendicular to the current and separated by equal intervals of about 10 to 15 m. The aggregation of goliath grouper on a spawning site was located late in the afternoon with the ship's echosounder. A drogue (a weighted 1-m diam sea anchor attached to a float) was lowered to mid-depth and allowed to drift freely from the point where the aggregation was located to about 50 to 100 m downstream. Coordinates were taken at the end point and a mooring line with nets attached was anchored there. The two other mooring lines with their nets were lowered about 10 – 15 m on either side of the first mooring line so that there were three lines, each with three nets downstream from the aggregation and perpendicular to the current (Figure 51 shows more plankton nets than we used).

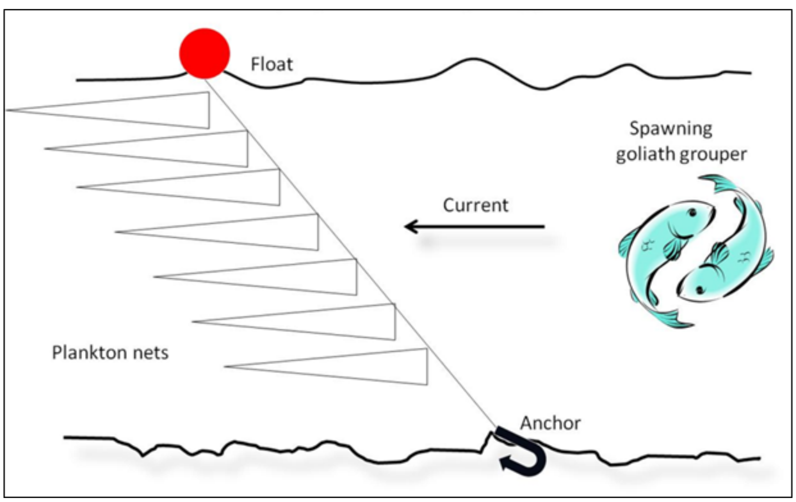

Figure 51. Cartoon of setup for sampling fertilized eggs from goliath grouper off Jupiter, Florida.

After we retrieved the nets, we gently washed them down taking care not to dislodge plankton and small jellyfish embedded in the mesh of the net. Wash-down samples were put through 2 sieves affixed to the top of the buckets, first a 3mm mesh, then a 1.5 mm mesh and finally eggs were collected on a 0.5 mm mesh (eggs are about 0.95 mm diam). Sieved samples with eggs were washed off the 0.5 mm mesh into a 5 gallon bucket with clean aerated seawater. Eggs float at 35 ppt, so after initial aeration, we removed the air stone and allowed the eggs to float to the surface (dead plankton and eggs would sink). After about 20 min we swept a small fine-mesh aquarium net (0.5 mm mesh) around the inner edge of the buckets and transferred the net contents to another bucket with clean seawater. When we returned to shore, we again swept the small net around the inner edge of the bucket and transferred the eggs to a plastic bag lining a cooler. The bag was closed except for a small hole at the top into which we pumped 100% oxygen, then sealed the bag. The eggs were then transferred to the mariculture lab at Rosensteil School of Marine and Atmospheric Science (RSMAS University of Miami) for rearing. Hatching occurs in about 18 hrs at 28 ^{o}C. Hatched larvae are extremely delicate, so all the travel must be done within that time period.

Dietary and stabile isotope analysis

Stomach contents are removed by hand—we first insert a 15 cm diam aluminum or PVC tube into the fish's mouth taking care to keep the tongue out of the way. We then reach through the tube with a gloved hand and reached as far back into the stomach as possible to remove all of the contents. If fish hooks were present in the stomach, gills, or mouth, we remove them, or if we couldn't remove them, we cut them off with bolt cutters and remove any leader material. All stomach contents, including fishing tackle, were saved in labeled plastic bags and put on ice, then later frozen.

At the lab we thaw the samples and sort them. Contents are identified to the lowest taxon practicable, weighed, and enumerated. Fishing tackle is also included in the analyses.

Data are graphed using percent occurrence, mass, and number of prey items. Stable isotopes are used as an indication of long-term dietary intake.

We sample goliath grouper during the spawning season because they represent individuals drawn from a broad geographical area. In the case of the spawning aggregations off Jupiter, the fish have been shown to come from a wide geographical area—the maximum migration to spawning sites off Jupiter was nearly 500 km (see Part III).

We sample several grams of muscle at the base of the fin rays for stabile isotope analyses, mainly to identify trophic level. For 13C and 15N analysis, fish muscle tissue is subsampled into approximately 1.5 g (wet weight) batches. Samples are oven dried for 48 hrs in drying oven at 60 ^{o}C and then homogenized using a ball mill grinder (SPEX CertiPrep 5100 ball mixer/milling unit, Spex CertiPrep, Metuchen, New Jersey). Lipids are removed prior to stable isotope analysis using a modified version of the lipid extraction method outlined by Bligh and Dyer (1959). Ten ml of 2:1 chloroform-methanol mixture is added to the homogenized tissue; samples are then shaken for 20 min on an orbital shaker, centrifuged, and the supernatant decanted. The remaining tissue is then rinsed with a 1:1 methanol-deionized water mixture, vortexed for 2 minutes, centrifuged and decanted and a final deionized water rinse was performed on the samples to get rid of any contaminants. Samples are dried again in the oven for 48 hrs at 60 ^{o}C. Between 0.5-0.8 mg of tissue is weighed into tin capsules at 13 ^{o}C and 15N isotopic ratios are determined using a Carlo-Erba Elemental Analyzer (EA) connected to a Finnigan MAT Delta Plus XP stable isotope ratio mass spectrometer (IRMS) through a Conflo III interface at the Florida State

University High Magnetic Field Laboratory. Levels of 15N in goliath grouper muscle tissue is then compared with known trophic patterns in species in the diet and in the environment of goliath grouper.

All dietary and stable isotope information has recently been sent to Dr. Paul Richards (NOAA/NMFS, Miami Lab) who is using it and other data we sent him earlier on juvenile diet to develop a bioenergetics model for goliath grouper.

Genetic evaluation of stock structure

All genetic samples (tips of fin rays) are preserved in 95% EtOH and sent to Florida Wildlife Research Institute (FWRI) in St. Petersburg where samples are processed using microsatellite techniques (see Seyoum et al. (2013) for detailed methods) to determine stock structure in Florida. Thus far we have sent over 300 samples to FWRI, mostly from off Jupiter, Florida, as part of a collaborative effort with Angela Collins and others at FWRI to identify any genetic differentiation between goliath grouper in the Atlantic and the Gulf of Mexico. These samples are currently being processed and the results will be available in the near future.

Results

Egg collection

Initially we attempted sampling fertilized eggs downstream from the goliath grouper aggregation site for the purposes of verifying spawning activity on those sites. We ran into multiple difficulties using this approach to spawning site verification, but were successful.

On 19 Aug. 2012 we were successful in collecting thousands of goliath grouper eggs downstream from a spawning site (MG111) off Jupiter, FL. Eggs were transferred to Dr. Refik Orhun (NMFS-Miami and RSMAS) who attempted to rear them in their mariculture facility. However, attempts were unsuccessful—eggs were reared to the feeding stage (Figure 52), but apparently the rotifers reared for food at first feeding were too large for the grouper larvae, so all were lost. The culture conditions were apparently not a problem because Dr. Orhun was successful in rearing spadefish (*Chaetodipterus faber*) eggs to the juvenile stage. Spadefish were seen spawning in small groups near the goliath grouper aggregation (Koenig, personal observation), so their eggs were accidentally included with the goliath grouper eggs.

Figure 52. Photograph of a 6-day old goliath grouper larva (total length = 3.94 mm). The eggs were captured downstream from a spawning site (MG111) off Jupiter, FL on 19 Aug 2012.

Diet and stabile isotope analysis

The diet of adult goliath grouper is similar to that of juveniles (Koenig and Coleman 2009) in that crabs dominate the diet by both occurrence and percent weight (Figures 53, 54 and 55). However, scad (mostly *Decapterus punctatus*) numerically dominate the diet, but are a relatively minor prey item in mass. Large items like whole lobster contribute much mass, but were uncommonly found in the stomachs. We classified lobster parts separately from “whole lobster” on the basis of presence of the edible tail, or pieces of it. Some stomachs contained fresh terminal pieces of antennae, but no other parts of the lobster; others contained head but no tails (as if the tail had been wrung off and the head discarded—these too were counted as “lobster parts”: rather than “whole lobster”.) We found one freshly eaten green sea turtle (*Chelonia mydas*) in the diet that was 30 cm long and contributed considerable mass to the overall diet. Several newly hatched loggerhead turtles (*Caretta caretta*) were also found in goliath grouper stomachs, which is not surprising considering that Jupiter island, just inshore from our sampling sites, is a major loggerhead turtle nesting area. Cutlass fish became relatively abundant in the diet during a time in 2012 after considerable rain flushed estuarine waters onto the shelf. Nearly every snapper found in the stomach had a hook in its mouth suggesting that the goliath grouper took it while it was on a fishing line. Fishing gear was sometimes abundant in mouth, gills and stomach (Figure 56). In the fish pictured in Figure 56, it is amazing that the fish could feed at all, but it did not look sick and it did not appear to be weakened.

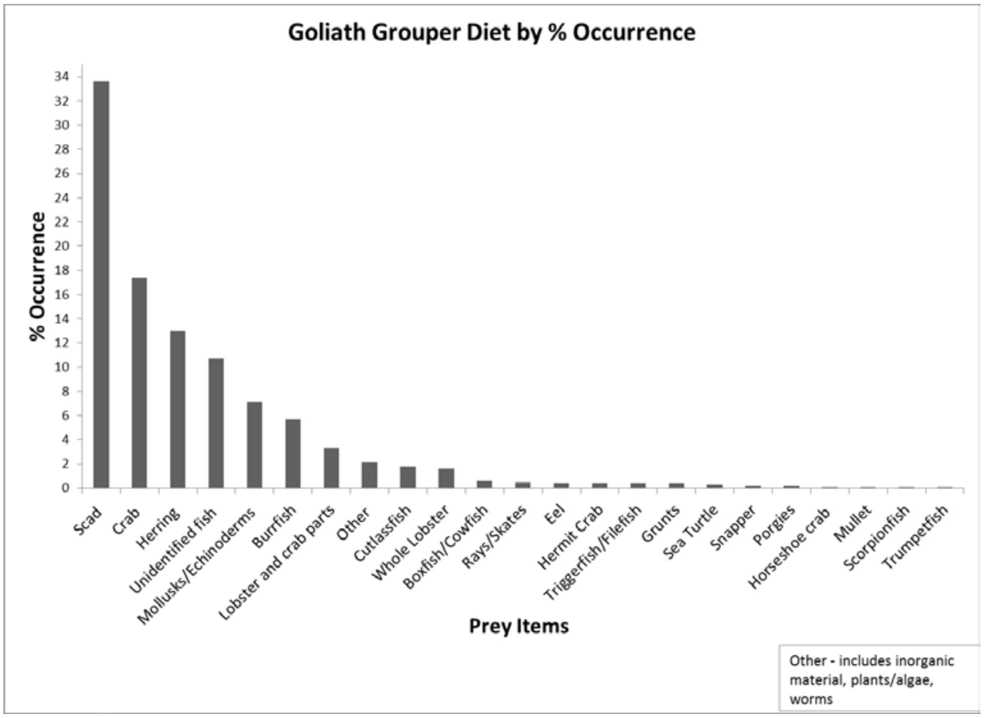

Figure 53. Diet (% occurrence) of adult goliath grouper caught off Jupiter, FL from 2010 to 2012.

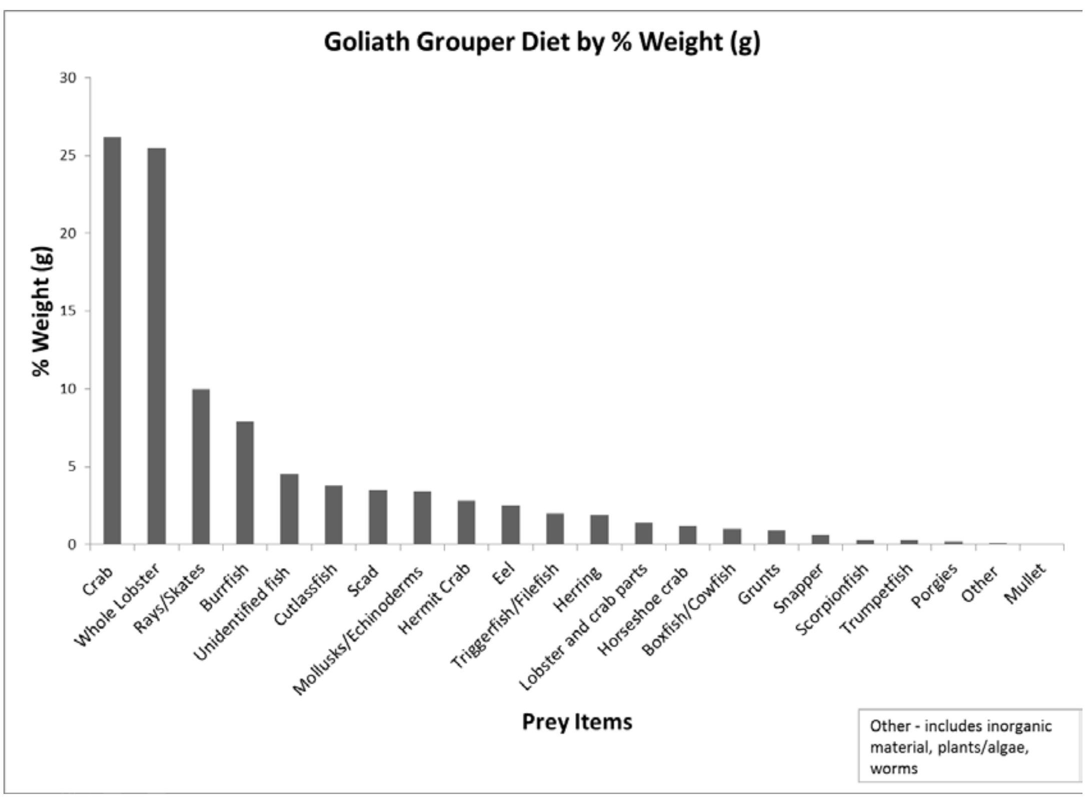

Figure 54. Diet (% by weight) of adult goliath grouper caught off Jupiter, FL from 2010 to 2012.

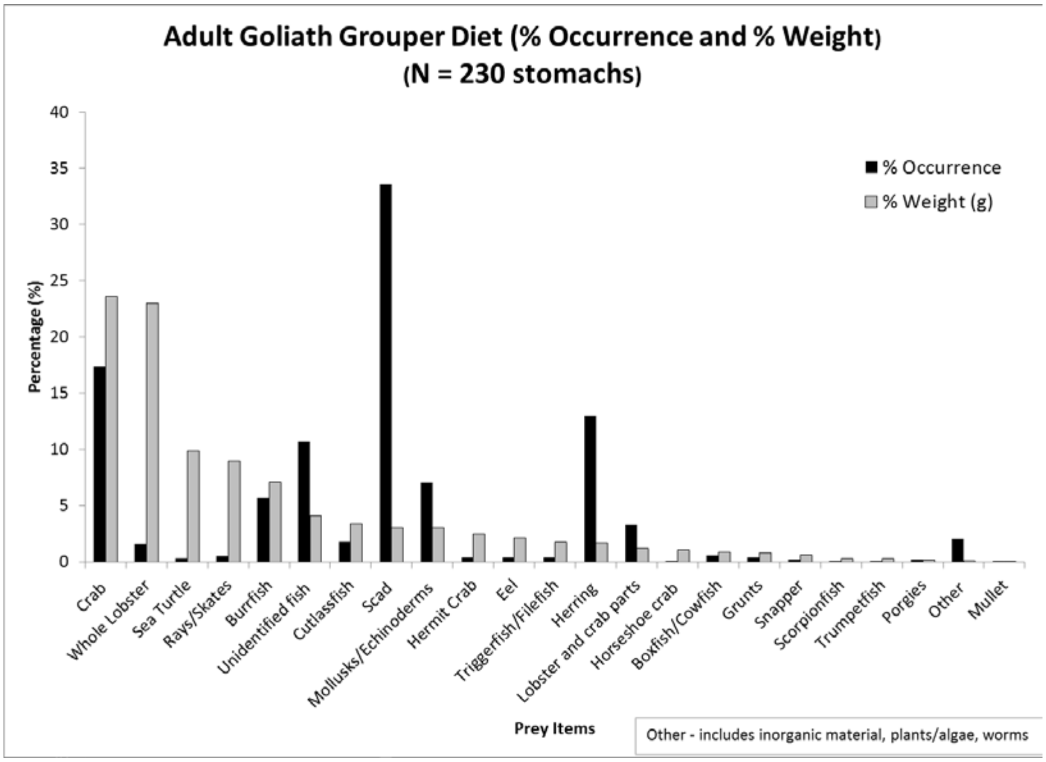

Figure 55. Diet of goliath grouper showing both percent by weight and by occurrence for catches off Jupiter, Florida from 2010 to 2012.

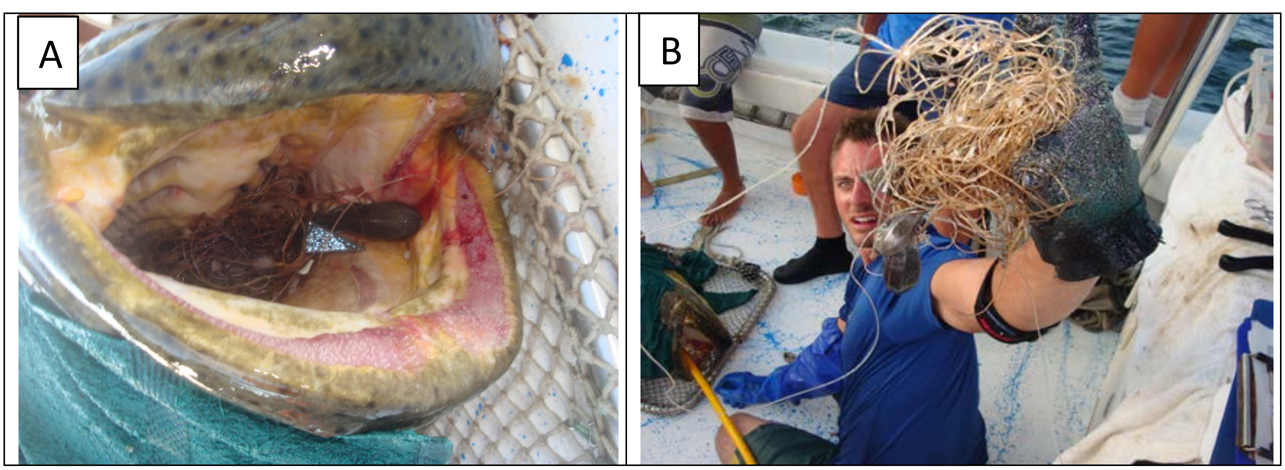

Figure 56. Fishing gear in the mouth, gills and stomach of a goliath grouper caught off Jupiter, FL A— mouth had 5 sinkers, 5 hooks and a mass of monofilament leader, B—Fishing tackle removed.

Genetic identification of stock structure

There are no results yet available from this work. FWRI is doing the genetic analysis.

Discussion

Egg collection

Our initial objective for verifying spawning sites was to use passive nets set up downstream from the suspect spawning site. The collection and verification of the eggs genetically would unequivocally confirm that the suspect site was a spawning site. The collected eggs were to be reared and a series of the stages were to be described. We assumed that this was straight-forward because of our success in collecting fertilized goliath grouper eggs on the Hole-in-the-Wall site on the first try in 2008 (Koenig and Coleman 2009). Even though, we selected a new moon night on a suspicion that the night-time chorusing was tightly linked to spawning. But, we came to find out that there were many obstacles to the successful downstream sampling of goliath grouper eggs passively.

Goliath grouper spawn at night, and we discovered that they can spawn at any time during the night and that new moon phases are, indeed, the times when peak spawning is occurring. Problems encountered were: first, in order to monitor our nets and surface floats we had to remain out all night. This was a problem because we had worked all day catching and sampling goliath grouper and would have to do so again the following day. Another problem was that leaving the nets out all night caused them to foul and lose capture efficiency. We tried retrieving the nets every few hours, but that was exhausting and dangerous on dark new-moon nights, so, we opted to soak the nets until about 2200 hrs, then retrieve the nets and process the samples. But even the abbreviated night-sampling protocol was problematic. We found that current speed and direction were not highly predictable in the area where we worked. Even though the Florida Current runs north, many of the sites are on the western edge of the Current and are therefore subject to multiple variations in current structure, such as slowing and nearly stopping, and moving south. A chronic problem was poor water quality conditions—for example, in September and August new moons of 2013, the water column was filled with moon jellyfish (*Aurilia aurita*) and debris, apparently from runoff from the extensive rains that occurred throughout Florida during the summer of 2013. So, for the purposes of spawning site verification, the passive plankton net method of collecting fertilized goliath grouper eggs has limited applicability. Nevertheless, we will continue to try to collect eggs for a second round of rearing trials so that we can describe larval development. Egg collection would be feasible under very good conditions: the water must be clear and clean and the current must be moving at 1.5 to 2.0 kts to the north and there should be not any jellyfish in the water (one moon jellyfish can completely foul a net).

Diet and stabile isotopes

The relatively large number of scad found in the stomachs of goliath grouper is probably a pattern only found around spawning aggregations. Scad and herring were very abundant around goliath grouper on spawning sites during the spawning season. We assume that these small fish are egg predators benefiting from the availability of goliath grouper eggs. They crowd around the goliaths very closely (see picture in Koenig et al. 2011) and often go into their mouths, apparently to evade other fish chasing them, like little tunny (*Euthynnus alletteratus*). In so doing, the goliath grouper needs only to swallow to ingest them, no active chasing or seeking is necessary.

Most of the crabs in the goliath grouper diet are calapid or shame-face crabs (family, Calappidae). These crabs spend most of their time under sand, so they are usually not seen by divers. In fact, we have never seen one except in goliath grouper stomachs. Fish in their diets consist mostly of slow-moving fish that defend themselves with spines, such as sting rays, burrfish, triggerfish, etc. Burrfish are a very common dietary item, but we have never seen one on the reef, so goliaths probably find them in sand habitat as well.

Atlantic cutlassfish (*Trichurus lepturus*) were common dietary items only after heavy rains of 2012 apparently flushed them from the estuary to the open waters of the shelf. We found several moribund, floating at the surface. Goliaths apparently took advantage of this prey windfall.

We assume that the scad and herring feed on goliath grouper eggs, so are therefore abundant around them when they are spawning. One observer who witnessed a goliath grouper spawning event in the early evening (Jen Hays, National Geographic Society) said that the scad crowded even closer around the presumed female goliath just before she was about to make her spawning ascent. If the scad and herring are indeed egg predators, then they likely depend on sight for feeding. Avoiding very numerous egg predators could be a problem for goliath grouper and it might explain why they spawn at night, especially the darkest nights of the new moons.

Genetic identification of stock structure

We will report on the genetic aspects of the recovering goliath grouper population when the data become available.

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