Final Report to Florida Sea Grant

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PROJECT TITLE: Habitat-Mediated Predator-Prey Interactions: Implications for Sustainable Production of Gag Grouper in the Eastern Gulf of Mexico

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INTRODUCTION AND RATIONALE:

The strength and sustainability of a fishery depends on the quality and quantity of habitat. This was highlighted by the Essential Fish Habitat (EFH) amendment to the federal Magnuson-Stevens Fishery Conservation and Management Act that established guidelines to assist fishery managers in the description and identification of essential fish habitat. EFH is defined as "*... those waters and substrate necessary to fish for spawning, breeding, feeding, or growth to maturity. ...waters includes aquatic areas and their associated physical, chemical, and biological properties that are used by fish, and may include areas historically used by fish where appropriate; "substrate" includes sediment, hard bottom, structures underlying the waters, and associated biological communities; "necessary" means the habitat required to support a sustainable fishery and a healthy ecosystem;...*" That is, the amendment provided the framework for using ecosystem concepts in the management of fisheries. However, habitat is often described only by the habitat variables that can be measured most easily, e.g., water temperature, salinity, turbidity, bottom type, etc. This simplification, i.e., lack of biological properties and lack of emphasis on biological communities, may have profound implications in all but the most extreme situations (massive destruction of wetlands, eutrophication and low DO, modification of freshwater flow).

Small changes or differences in physical habitat characteristics may have little, if any, direct effects on the species being studied, but may have profound effects mediated through indirect food web interactions. For example, small changes in the spatial and thermal distribution of pelagic planktivorous fishes can have profound affects on the growth and production of predators (Mason et al. 1995), and commercial trawling may decrease substrate complexity and available refugia, and thereby increase predator induced mortality of juvenile cod, *Gadus morhua* (Auster and Langton 1999; Langton and Auster 1999). Ultimately, the issue that must be addressed is what makes a habitat "essential"? Obviously, it is the interactive effect of physical, chemical and biological components that must be examined to provide the scientific basis for essential fish habitat. Thus, to know the habitat requirements of fishes, we must also know those factors controlling the spatial distribution, local abundance and temporal-spatial variability of their prey resource and predators. The need to understand the spatial-temporal dynamics of pelagic food resources for reef-associated predators is thus fundamental to quantifying the essential habitat of certain reef fishes.

We conceptualized this notion of habitat-mediated food web effects in the context of a single predator population and a single prey population (Fig. 1). The underlying premise was that a particular habitat (e.g., warm-temperate reefs) can be essential for both the predator and the prey, with each responding to specific habitat characteristics as well as to each other. Thus, the habitat itself determines the local abundance of both predator and prey (Fig. 1A,B). Predator-prey interactions, leading to predator consumption of prey, occur as a result of the habitat bringing predator and prey into close proximity to one another (Fig. 1C). In addition, since habitat acts to concentrate predators, density-dependent effects may be realized through competition for prey and/or elevated activity levels that reduce the energy allocation to growth (Fig. 1C,D,E). Such an example may occur with gag grouper (*Mycteroperca microlepis*) and pelagic planktivorous fishes, where gag grouper require reef structure as a refuge (Lindberg and Loftin 1998) and pelagic planktivorous fishes aggregate in great numbers above these reefs.

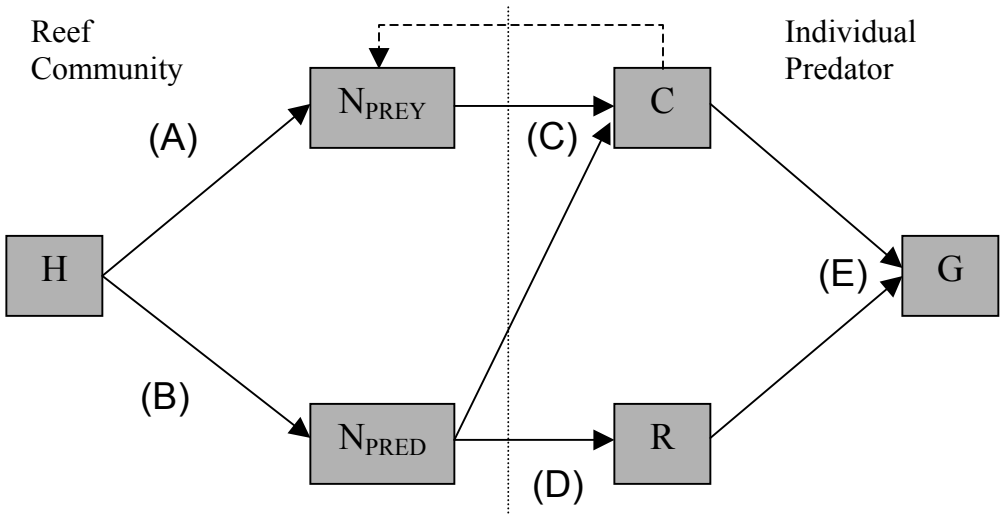

Fig 1. Conceptual model of habitat-mediated predator-prey interactions and predator growth rate where H is non-consumable habitat (patch reef size), N_{PREY} is prey density (pelagic planktivorous fishes), N_{PRED} is predator density (gag grouper), C is predator consumption rate, R is predator energetic costs and G is predator growth rate. Parentheses represent functions (hypotheses) that define linkages between boxes. (A) Prey density as a function of habitat $N_{PREY} = N_{PREY}(H)$ (note- N_{PREY} may also be dependent on predator consumption). (B) Predator density as a function of habitat $N_{PRED} = N_{PRED}(H)$. (C) Predator-prey interactions, resulting in predator consumption, as a function of prey density and predator density $C = C(N_{PREY}(H), N_{PRED}(H))$. (D) Predator density dependent energy expenditure $R = R(N_{PRED}(H))$. (E) Predator growth rate as a function of prey density and predator density as determined through predator consumption and predator energetic costs and mediated through habitat- $G = G(C(N_{PREY}(H),N_{PRED}(H)), R(N_{PRED}(H))$.

Application of the Conceptual Model to Gag Grouper

We took our conceptualization of essential fish habitat and applied it to gag grouper. Reef habitats were considered essential for gag. Experimental studies of reef habitat (Lindberg and

Loftin 1998) led to the following conclusions and questions. Gag preferred relatively large patch reefs to small ones, with large patch reefs attracting and holding more gag for longer periods of time. Gag remained resident on patch reefs for an average of almost 10 months, long enough for local habitat differences to affect growth and condition. Gag growth rates and condition (i.e., relative weights), however, were significantly greater on small (4-cube) patch reefs than on large (16-cube) patch reefs, despite their preference for large patch reefs – but why? Is it simply a supply-demand issue (e.g., per capita prey availability) mediated by habitat or is it complicated by density-dependent interactions (e.g., interference, social behavior) that either decrease consumption rates (i.e., decrease foraging efficiency) or increase metabolic costs at high gag densities? Answers to such questions are essential if we are to predict the effects of management options involving essential fish habitat.

The primary prey of gag grouper is what can be collectively referred to as pelagic planktivorous fishes. Pelagic planktivorous fishes are often made up of several species with the most common groups being sardines, herring, anchovy and scad. Planktivorous fishes are abundant on shallow coastal reefs in the eastern Gulf of Mexico during the summer and fall, are often observed above reef structures during daylight hours and appear to act as intermediaries funneling pelagic-derived energy into the reefs and directly into gag. The ability of reefs to attract and retain, and perhaps even to enhance regional abundance of pelagic planktivorous fishes are important for the growth and production of gag grouper. Thus, predictable patterns in the spatial distribution and abundance of pelagic planktivorous fishes may be a critical component of reef fish production.

Preliminary results from a fisheries acoustics feasibility study conducted in October 1998 on the experimental Suwannee Regional Reef System (SRRS) in the eastern Gulf of Mexico showed that pelagic planktivorous fishes have a strong association with reef structure and this association deteriorates with decreasing light levels.

The SRRS thus provided a unique opportunity to quantify the role of habitat in mediating predator-prey interactions, and the direct implications for gag growth and production. The basic relationship between a predator's food consumption relative to its growth can be viewed as a balanced bioenergetic budget (Winberg 1956, Warren and Davis 1967):

$$[1] \qquad C = W + M + G$$

where: C = energy consumption (gross energy intake); W = energy losses due to egestion (feces) and excretion (urine); M = metabolic energy losses due to standard metabolic rate (SMR) (M_R), metabolic rate increases (above SMR) due to activity (M_A), and metabolic increases due to specific dynamic action (SDA) (e.g., deamination of proteins) (M_D), where $M = M_R + M_A + M_D$; and G = production in the form of somatic growth (G_S), e.g. protein synthesis, and production due to reproduction (gametes) (G_R). For gag grouper inhabiting coastal reefs in the SRRS, G_R = 0 because the fish are all pre-reproductive females (i.e., protogynous hermaphrodites) and therefore not actively shedding gametes. In this instance, G represents solely somatic tissue production.

If we assume that W is a fixed fraction (k_1) of consumption, $W = k_1 C$ (e.g., $k_1 = 0.1$ or less for piscivores, Brett and Groves 1979) and M_D is a fixed fraction (k_2) of consumption, $M_D = k_2 C$ (e.g., $k_2 = 0.15$ for carnivorous fish, Brett and Groves 1979, Hanson et al. 1997) and define $M_T = M_R + M_A$, equation [1] reduces to:

$$[2] \qquad G = \phi C - M_T$$

where the constant $\phi = (1 - k_1 - k_2) = 0.75$. Thus, growth ($G$) is proportional to food consumption (C) and to metabolic activities (M_T) related to standard metabolism (M_R) and activity levels (M_A).

For gag on the SRRS, it has been shown that $G_{4\text{-cube reefs}} > G_{16\text{-cube reefs}}$ (Lindberg and Loftin 1998). Based on a balanced energy budget, such a difference in growth must either occur as a difference in consumption (C) (an input) or a difference in metabolic costs, specifically M_T, between the 4-cube and 16-cube reefs. However, all reefs within the SRRS occur at the same depth (13-m) and have the same representation of environmental characteristics, especially with regard to temperature regimes (i.e., different sized reefs were interspersed by restricted randomization along the 13-m contour sites). In addition, gag grouper on these two sizes of reefs are within the same relative range of body size, all gag being juvenile-to-young-adult females (Lindberg and Loftin 1998). Since metabolic expenditures related to standard metabolic rate (M_R) are primarily affected by temperature in poikilothermic animals, it is reasonable to assume that M_R for gag in this particular experiment will be equivalent for equivalent-sized gag on both 4-cube and 16-cube reefs. We are then left with either consumption or the metabolic costs associated with activity levels, or their interaction, as the major force in balancing the energy budget of gag between a 4-cube system and a 16-cube system. Given that both of these parameters will be variable, which parameter do we believe to be more accurately quantifiable in a field situation? Two major factors were considered in this decision: 1) The SRRS provides a relatively unique opportunity because of its controlled habitat characteristics in relation to a very specific prey base (small pelagic fishes) and a very specific piscivorous predator (gag grouper). The general belief is that if this experimental system does not allow us to quantify adequately the *in situ* consumption rates of a piscivore, then there is little hope for other field studies that attempt to quantify daily consumption in piscivorous fish under usually much less ideal conditions! And 2) measuring M_A, such as metabolic costs of swimming, requires not only observations on activity and swimming patterns and speeds from the field but also laboratory studies measuring respiration rates of gag at specified temperatures and swimming velocities so that oxygen consumption can be converted to energy equivalents. For the purposes of the present study, we therefore believed it was more explicit to quantify consumption rates of gag living on 4-cube versus 16-cube reefs rather than estimate a realistic *in situ* value of M_A for gag living on these different sized reefs.

On the basis of this logic, we predicted that if:

$G_{4\text{-cube reefs}} > G_{16\text{-cube reefs}}$ for gag grouper (Lindberg and Loftin 1998), and

$M_{T(4\text{-cube reefs})} = M_{T(16\text{-cube reefs})}$, then

$C_{4\text{-cube reefs}} > C_{16\text{-cube reefs}}$

This predicted difference in prey (energy) consumption by gag may be due to increased per capita availability of prey fishes on the 4-cube reefs (see below). If the prediction of $C_{4\text{-cube reefs}} >$

$C_{16\text{-cube reefs}}$ is falsified, or if the difference is inadequate to explain the difference in growth, then the assumption of $M_{T(4\text{-cube reefs})} = M_{T(16\text{-cube reefs})}$ must be further tested.

A Need as well as an Opportunity

As emphasized by the National Marine Fisheries Service (NMFS) (*Federal Register* Vol. 63, No. 208, p. 57660), "The ecology of reef fish makes them vulnerable to overfishing, because they tend to concentrate over specific types of habitat with patchy distribution. This behavior pattern can make traditional fishery statistics misleading." Therefore, scientific knowledge of how reef fish use patchy habitat and the effects on individual growth dynamics is essential for effectively planning and evaluating proposed actions to rebuild or maintain reef fishery stocks (e.g., changes in size limits, marine reserves, or selective use of artificial reefs).

How and why motile reef fish distribute themselves among habitat patches is of the utmost importance, and is likely mediated in larger species by the process of density-dependent habitat selection (Lindberg and Loftin 1998 and references therein). The management implication is that motile reef fishes could experience significant density-dependent effects on growth, survival, or reproduction despite reduced stock sizes as a consequence of fishing. As one example of the relevance to management, the Gulf of Mexico Fisheries Management Council increased the legal size of gag from 20 to 24 inches. Density-dependent growth rates could affect the availability of legal-sized fish for recreational fishermen on the shallow continental shelf. As another example, Koenig (1998) emphasized that many fisheries species in the Gulf of Mexico and South Atlantic have spatially structured populations needing networks of marine reserves. At the same symposium, however, St. Mary et al. (1998) pointed out that effective reserve strategies for such fisheries would depend on knowing if and where density-dependence actually occurs.

Among groupers, gag is second only to red grouper (*Epinephelus morio*) in the Gulf of Mexico fishery, and is dominant in the U.S. South Atlantic fishery. Gag is among the most valuable finfishes in the southeastern United States (1998 commercial ex-vessel value = $4 million; commercial landings = 1.76 million lbs., recreational landings = 3.8 million lbs.). In addition to increasing the minimum size limit for gag, the Gulf of Mexico Fishery Management Council also created two Marine Protected Areas (no-take zones) along the 40-fathom contour of the northeastern Gulf of Mexico. Those areas had been identified as spawning habitat for gag and subject to intense commercial fishing. One can expect commercial harvest to be re-directed inshore where pre-reproductive females predominate (gag are protogynous hermaphrodites), and where recreational fishers already take 78% of all gag harvested. Understandably, gag has been a priority species for fisheries research with respect to age and growth, stock structure (including movement patterns), and habitat requirements and limitations.

The remainder of this report contains the methods and results for the first three of five objectives originally proposed. These were the most essential objectives in this project and were given priority for completion. For the status of Objectives 4 and 5 the reader is referred to the Sea Grant Summary Form that serves as the cover pages for this report.

OBJECTIVES, METHODS AND RESULTS:

OBJECTIVE 1. To test the hypothesis that availability of pelagic prey fish to gag grouper, in total or per capita, differs as a function of patch reef size.

Methods

Planktivorous Fish Abundance

Data collection*:* Mobile hydroacoustics surveys were used to estimate pelagic planktivorous fish abundance as a function of patch reef size. We used state-of-the-art 120 kHz split-beam echosounders to measure the abundance of pelagic planktivorous fishes on 4 and 16 cube patch reefs. Due to changes in equipment availability, two different echosounders were used during this project; a BioSonics DT6000 echosounder was used in the summer of 2000 and a Simrad EY500 echosounder was used in the summer of 2001. These two systems provide comparable results (Mason and Schaner 2001) so there is no reason to believe that the switch between the BioSonics and Simrad systems altered our results. For both acoustic systems, the transducer was mounted on a stable, 1.2-m towbody and towed alongside the research vessel at speeds of 2.5-3.5 m s^{-1}. The transducer was aimed in a down-looking configuration and the tow body was towed at a depth of approximately 1-m. Acoustic transects consisted of a crisscross pattern over top of individual patch reefs (Figure 1.1). This transect configuration was designed to ensure that approximately 5 passes over top of a patch reef was achieved and to minimize the time spent per patch reef. A patch reef was typically assessed in less than 10 minutes.

Equipment performance was monitored in the field using an oscilloscope and a digital echogram recorder. Raw digitized acoustic signals were time-marked and geocoded using a Differential GPS (Ashtech BR2G GPS Beacon Receiver) with submeter accuracy. Raw acoustic and DGPS data were saved directly to computer files and later copied to CDR disks for data processing and archiving. Routine calibrations were performed using a tungsten carbide reference sphere (Foote 1987; Foote 1990).

Sampling design targeted collecting acoustics data on four 4-cube reefs and four 16 cube reefs at a minimum of once a month beginning in July and ending in October. In the summer of 2000, severe weather and mechanical problems with research boats limited the collection of hydroacoustics data; only one complete set of acoustic data was collected, i.e., four 4-cube and four 16-cube reef arrays (Table 1.1). Acoustic transects were completed from early to mid-August and from mid to late September of 2000. In the summer of 2001, acoustic transects were completed for 7 reef arrays in July, all 8 reef arrays in early August, all 8 reef arrays in late August, and 5 reef arrays in October. Acoustics data were not collected in September of 2001, due to severe weather and boat scheduling conflicts.

Limited biological samples of the pelagic planktivorous fishes were collected for species identification. Daylight sampling with a small midwater trawl (4.8 m mouth opening with 25 mm stretched mesh body and 20 mm stretched mesh cod end) was ineffective at capturing pelagic planktivorous fish. Use of a larger trawl was logistically impossible from our 23' research vessel. We also found that a 100' x 20' purse seine was both difficult to deploy from our research vessels and ineffective at capturing pelagic fishes. In approximate 10 different attempts at deploying the purse seine, we were unable to capture any fish. Diver deployed cast nets were able to capture pelagic fish, however the numbers were small and likely not representative of the populations present due to divers selecting for schools easy to capture. As a

result of our poor capturing efficiency, and time and personnel constraints, it was determined that biological sampling would be omitted in order to ensure adequate acoustic data could be collected. Thus, forage fish are presented as an aggregation of species and not by species.

***Data Analysis**:* Acoustic data collected from the BioSonics echosounder were processed using BioSonics Visual Analyzer 4.0 (http://www.biosonicsinc.com/), and acoustic data collected from the Simrad echosounder were processed using Digital Echo Visualization and Information System (DEVIS) (Jech and Luo, 2000). Both of these software packages use echo-squared integration (Powell and Stanton 1983; Thorne 1983) and split-beam analyses (Ehrenberg 1983) to estimate absolute fish density. Echo-squared integration provides a quantitative relative measure of fish density that can be scaled to absolute fish density with system parameters obtained from equipment calibration and measures of the mean backscattering cross-section of the fish obtained from split-beam analyses. Split-beam analysis was used to determine the depth distribution of fish backscattering coefficients (σ_{bs}) and fish target strengths (TS), i.e., acoustic size. Acoustic size is the fraction of incident sound energy that is reflected by a fish back toward the transducer. Fish density (number m^{-3}) was determined by dividing the corrected sums of squared voltages by σ_{bs}. Acoustic data were inspected for noise and bottom contamination before applying the mean backscattering cross-section to the echo-squared integration.

Acoustic data in a 50-m radius of any given patch reef were selected for the analyses (Figure 1.3). This ensured that only fish associated with the patch reef were included in the analysis and that we could use density as an index of abundance. Once the appropriate acoustic data were selected for each patch reef, a mean density of pelagic fish was determined. This typically provided a density estimate for each patch reef within a given reef array (N=6). Density estimates were not normally distributed, so all density values were $\log_{10}$ transformed. In addition, forage fish density estimates were standardized by dividing by the number of gag grouper estimated on the same patch reef providing a per-capita index of food availability to the gag. Per capita estimates were not normally distributed, so these data were $\log_{10}$ transformed. We attempted to use gag grouper data collected at approximately the same time as the acoustics data (Table 1.2). For August, two acoustic data sets are available for each of the 8 relevant reef arrays during the month, but only one census count was done for each array. Therefore, only the acoustic data for each reef array taken near the time of the gag grouper census for that array was used in the per capita analysis. Analysis of variance (ANOVA) was used to test for the affect of patch reef size, month, and year on pelagic fish density and per capita index of forage fish availability. An individual sample for the ANOVA consisted of a single pelagic density estimate over a patch reef for each time that patch reef was sampled. In doing this we assumed that each patch reef is an independent sample and that the pelagic fish of interest randomly disperse at night, making the probability of the same fish reoccurring in the same school over the same patch reef from day to day very low. An alpha level of 0.05 was assumed significant.

Gag Grouper Abundance.

SEE METHODS FOR OBJECTIVE 3.

Results:

Pelagic fish density

When all density estimates were combined, there was a significant year (P=0.0006) and month (P=0.005) affect, but patch reef size (4-cube vs 16 cube) was not significant (P=0.780). There was no apparent interaction between year, month and patch reef size (P>0.10).

A lack of acoustic data during the summer of 2000 made determining the effect of reef size on pelagic fish density difficult. During the summer of 2000, pelagic fish densities ranged from 0.2 to 6.8 fish m^{-2}. Densities did not differ between reef arrays (P=0.698, Fig. 1.4), between 4-cube and 16-cube reefs (P=0.962, Fig. 1.5), or between months (P=0.058, Fig. 1.6).

In the summer of 2001, mean aerial density of pelagic fish ranged from 0-10.7 fish m^{-2}. As in 2000, density estimates did not differ between reef arrays (P=0.426, Fig 1.7) or between 4 and 16 cube reefs (P=0.75, Fig 1.8), but densities did differ between months (P=0.01, Fig. 1.9). When 4 and 16 cube reefs were considered separately, there was no apparent difference between months for 4-cube reefs (P=0.750, Fig. 1.10), but a statistical difference was observed between months for the 16-cube reefs with highest density estimates occurring in July (P=0.003, Fig 1.11). When July and August-October (combined) was analyzed separately (Fig. 1.12), density differed between patch reefs in July (P=0.0503) but not in the combined months of August and October (P=0.316).

Density estimates differed between years (Fig. 1.13) for all reefs combined (P<0.001) and when 4-cube (P=0.030) and 16-cube (P=0.010) reefs were analyzed separately. Mean density in 2000 was higher than mean density estimated for 2001.

Pelagic fish density per capita of gag

There were insufficient acoustic data with corresponding census data to examine the effect of reef size on pelagic fish density per capita of gag for the summer of 2000. There were, however, sufficient data to do so for the summer of 2001. We collected acoustic data with corresponding gag census data in July and August of 2001, but none in October due to weather and vessel restrictions.

Mean pelagic fish density per capita of gag during the entire summer 2001 varied significantly (P=0.010) across all 8 reef arrays, with little apparent pattern in the distribution (Figure 1.14). Highest mean density per capita was found on array 16 (0.22 fish/m^2/gag) and the lowest mean density per capita was found on array 7 (0.02 fish/m^2/gag). There was no significant difference (P=0.320) in mean density per capita between July and August. Both July and August showed the highest density on array 16 (0.23 fish/m^2/gag and 0.22 fish/m^2/gag, respectively), but the lowest density in July was on array 7 (0.03 fish/m^2/gag) and the lowest density in August was on array 13 (0.01 fish/m^2/gag).

There was a significant patch reef size affect (P=0.02, Fig. 1.15) but no month (P=0.370, Fig. 1.16) or patch reef size × month affect (P=0.582). There was a higher mean pelagic density per capita of gag on 4-cube reefs (0.11 fish/m^2/gag) than on 16 cube reefs (0.05 fish/m^2/gag).

Frequency of Occurrence

Pelagic fish were found to occur consistently over the patches of the 8 relevant reef arrays in this study for both the summer of 2000 and 2001. In the summer of 2000 pelagic fish densities were acoustically recorded over every sampled patch for all reef arrays. This resulted in a 100% frequency of occurrence of pelagic fish over the sampled arrays for the summer of 2000. The summer of 2001 showed a frequency of occurrence of 98% for pelagic fish over patches in the

sampled reef arrays. The two sampled reef array sizes showed similar frequencies of occurrence, with 4-cube reef arrays having a 98% frequency of occurrence and 16-cube reefs having a 99% frequency of occurrence for the summer of 2001.

Summary:

We found no consistent differences in pelagic fish density as a function of patch reef size, suggesting that reef habitat, of the size and complexity used in this study, does not determine the density of forage fish at reef sites. However, there was a significant year and month affect reflecting the inter-annual and inter-seasonal variability in pelagic fish abundance. Prey fish availability, as measured by the density of forage fish at a reef divided by the number of gag grouper on the reef (prey fish m^{-1} gag^{-1}) differed by reef size with the 4-cube reefs having greater prey availability than the 16 cube reef.

Table 1.1. Acoustic data collected during the summers 2000 and 2001 for each of the 8 reef arrays (Fig. 1.2) by month.

	2000			2001			
Array	**July**	**Aug**	**Sept**	**July**	**Early Aug**	**Late Aug**	**Oct**
0			X	X	X	X	
4			X	X	X	X	
7		X		X	X	X	
11		X		X	X	X	X
13		X		X	X	X	X
16			X	X	X	X	X
20			X		X	X	X
21		X		X	X	X	X

Table 1.2. Day of the month when acoustic data for pelagic fishes and census data for gag grouper were collected in the summer of 2000 for each month.

	July		August		October	
Array	**Acoustic**	**Census**	**Acoustic**	**Census**	**Acoustic**	**Census**
0	26	30	8	8		
4	27	28	20	21		
7	27	30	8	10		
11	26	30	10	8	2	
13	27	19	10	13	2	
16	27	30	29	21	2	
20			10	16	2	
21	18	20	10	16	2	

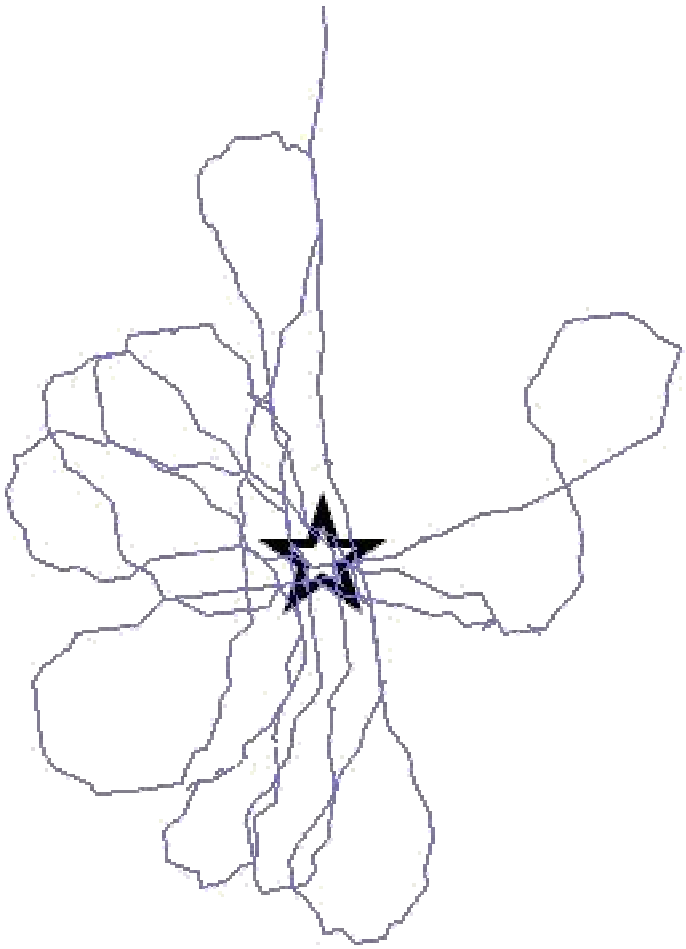

Figure 1.1. Transect pattern over an individual patch reef, where the line represents the continuous acoustic transect and the star represents the patch reef.

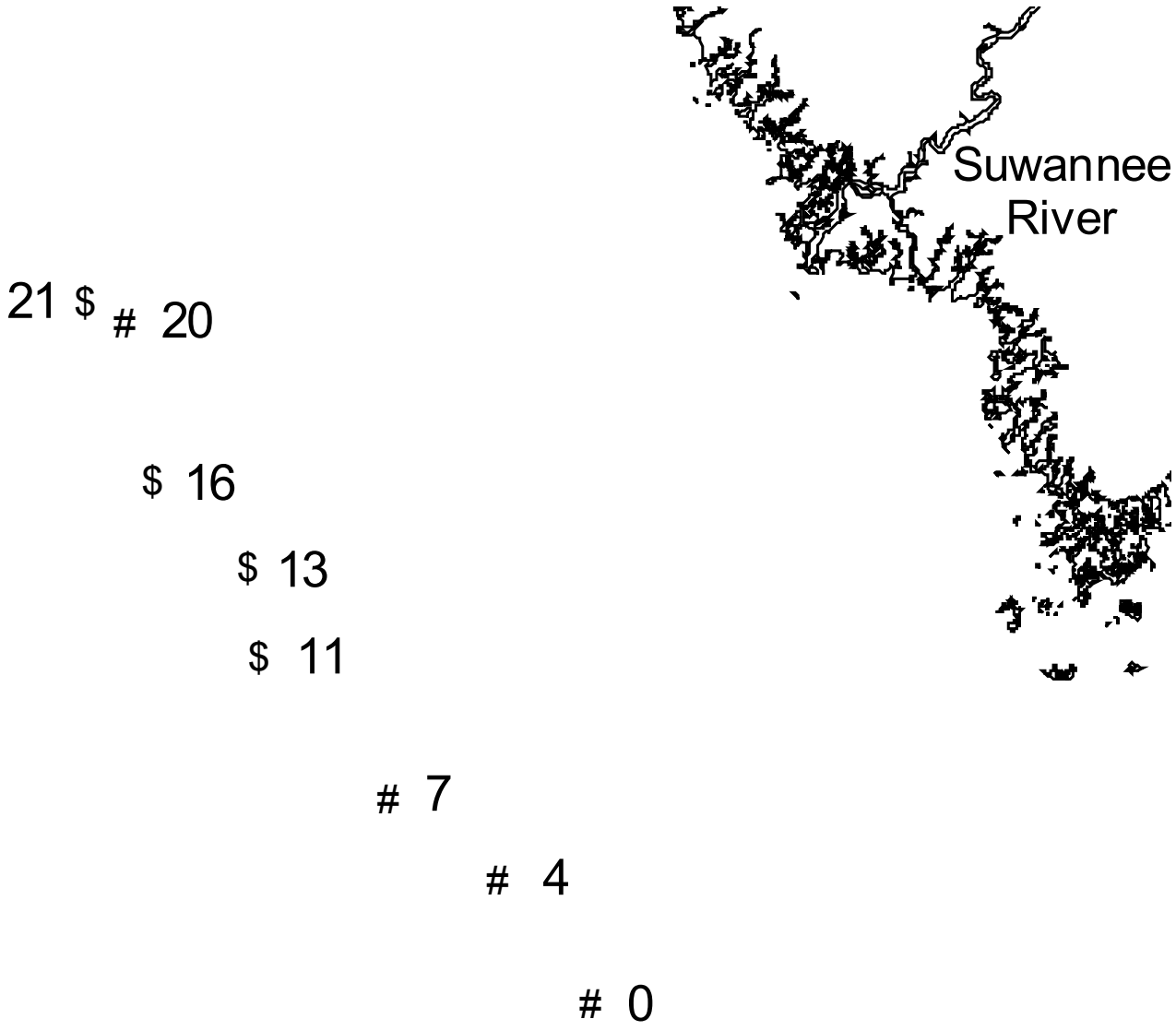

Figure 1.2: Map of the big bend, Florida showing approximate locations for the 8 relevant reef arrays used in this study, where triangles represent unpublished reef arrays and circles represent published reef arrays. (Bill, symbols in the graph are not consistent with symbols referenced in the figure caption. I'll send Brian an email to fix this.)

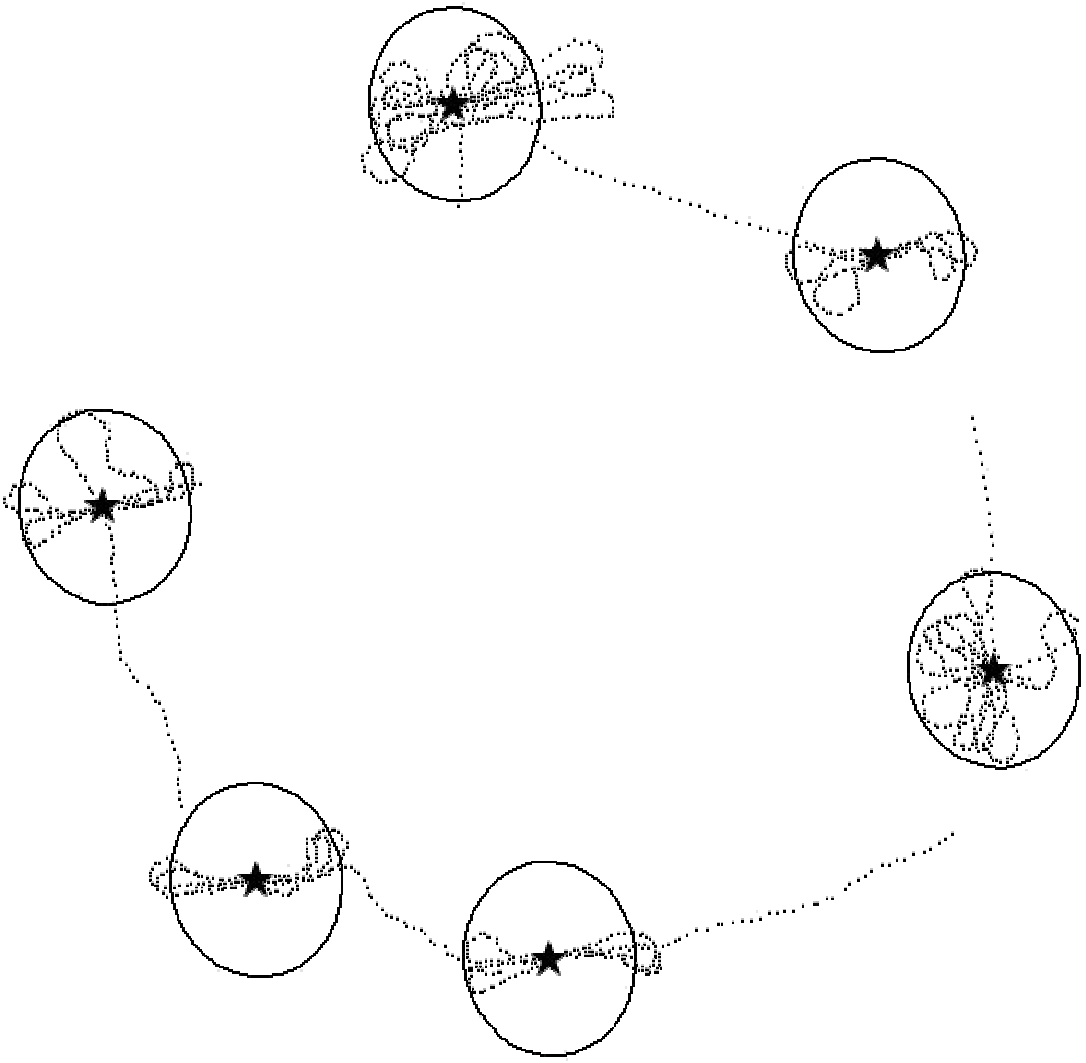

Figure 1.3: A reef array showing acoustic transects (dotted line) and 50-m radius buffers (large circles) over top of individual patch reefs (star) used for data collection for data selection.

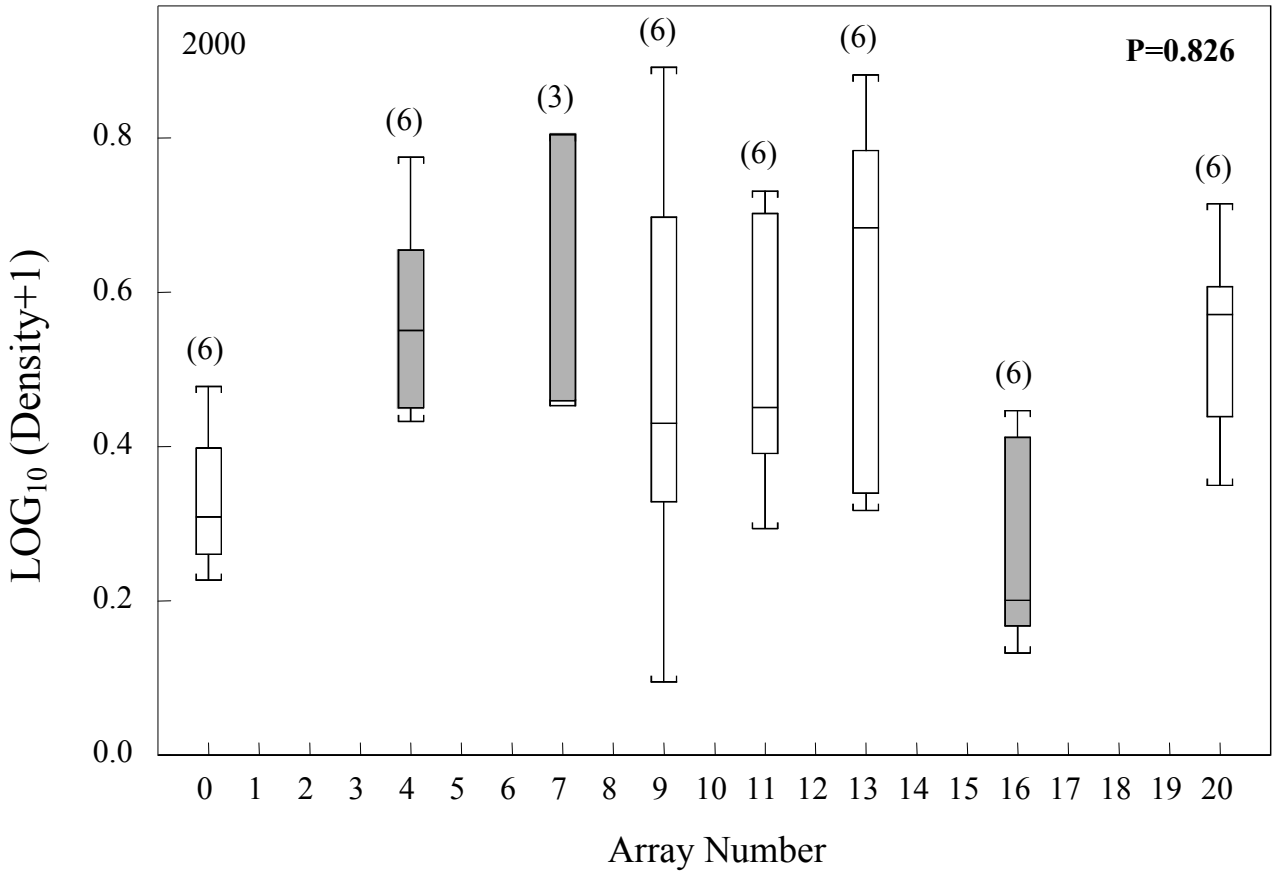

Figure 1.4. Box and whisker plot (median, 25% quartiles, 75% quartiles, and range) of the log transformed fish density (fish m^{-2}) with respect to patch reef number (fig. 2) for the summer of 2000. Fish density data has been combined for August and September. Solid gray boxes are 4-cube reef arrays and open boxes are 16-cube reef arrays. Number of patch reefs sampled is in parenthesis.

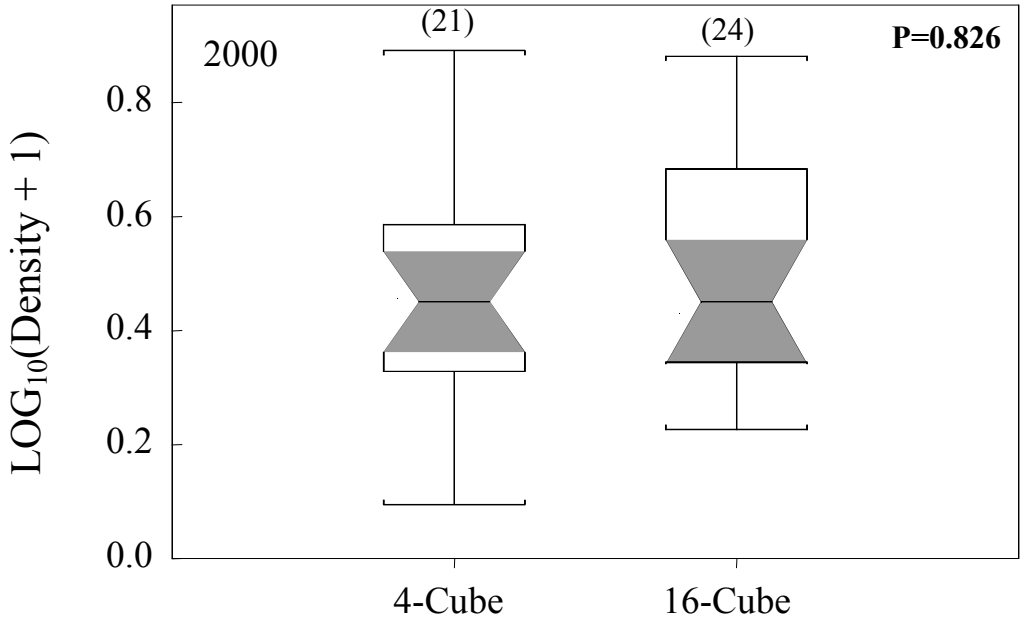

Figure 1.5. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] for all months as a function of patch reef size (4 cube vs. 16 cube) in 2000. Number of patch reefs sampled is in parenthesis.

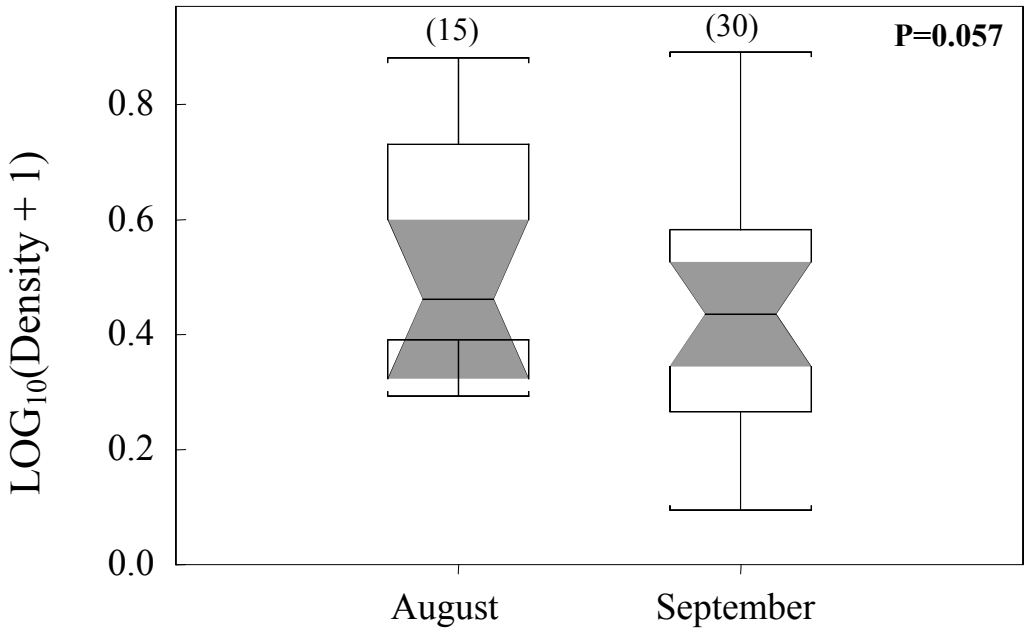

Figure 1.6. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] for all patch reefs combined as a function of months in 2000. Number of patch reefs sampled is in parenthesis.

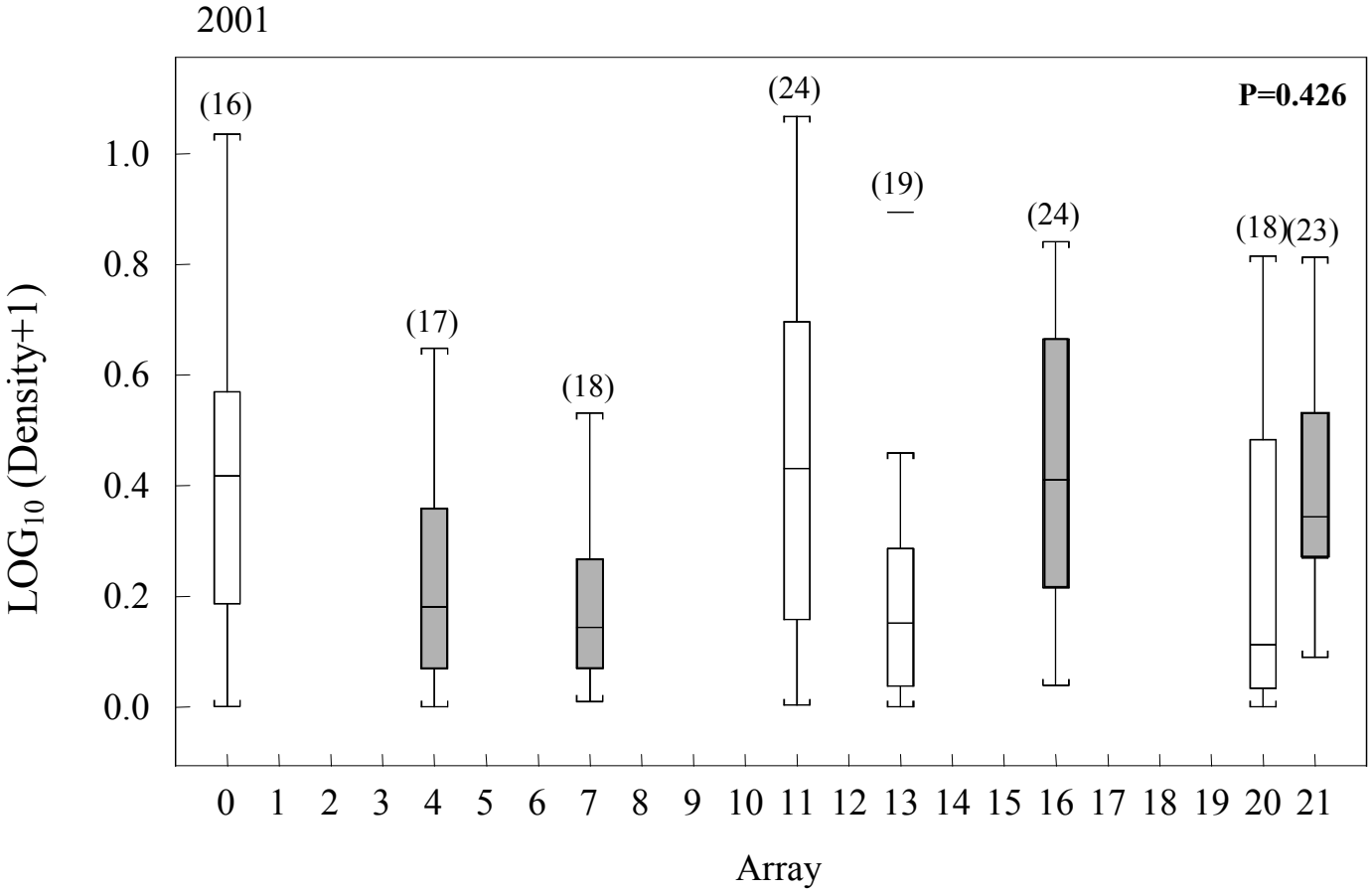

Figure 1.7. Box and whisker plot (median, 25% quartiles, 75% quartiles, and range) of the log transformed fish density (fish m^{-2}) with respect to patch reef number (fig. 2) for the summer of 2001. Fish density data has been combined for August and September. Solid gray boxes are 4-cube reef arrays and open boxes are 16-cube reef arrays. Number of patch reefs sampled is in parenthesis.

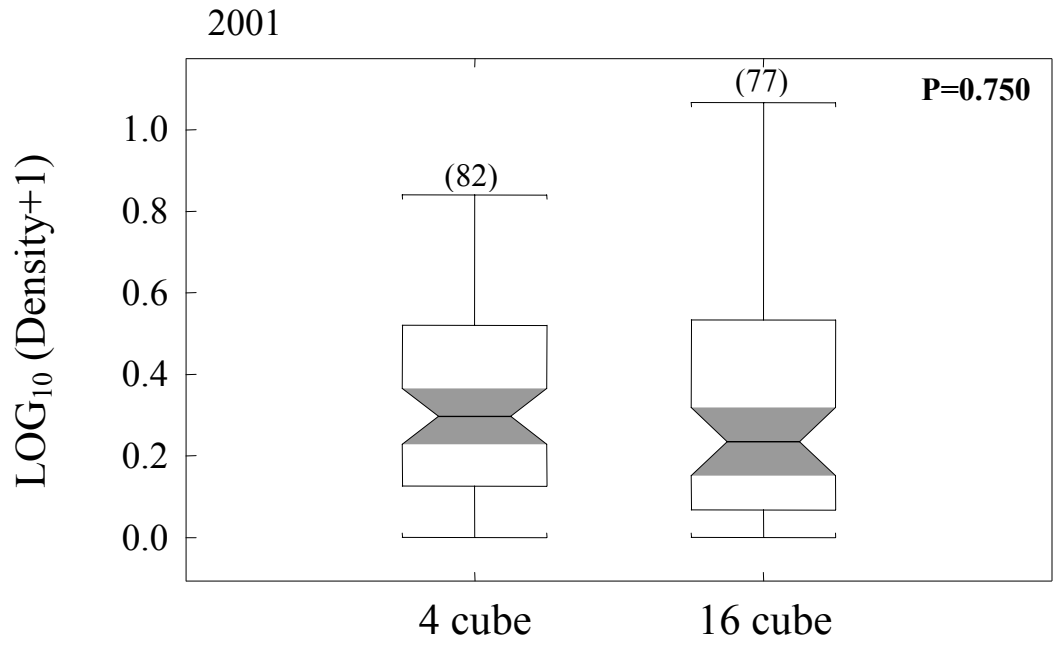

Figure 1.8. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] for all months as a function of patch reef size (4 cube vs. 16 cube) in 2001. Number of patch reefs sampled is in parenthesis.

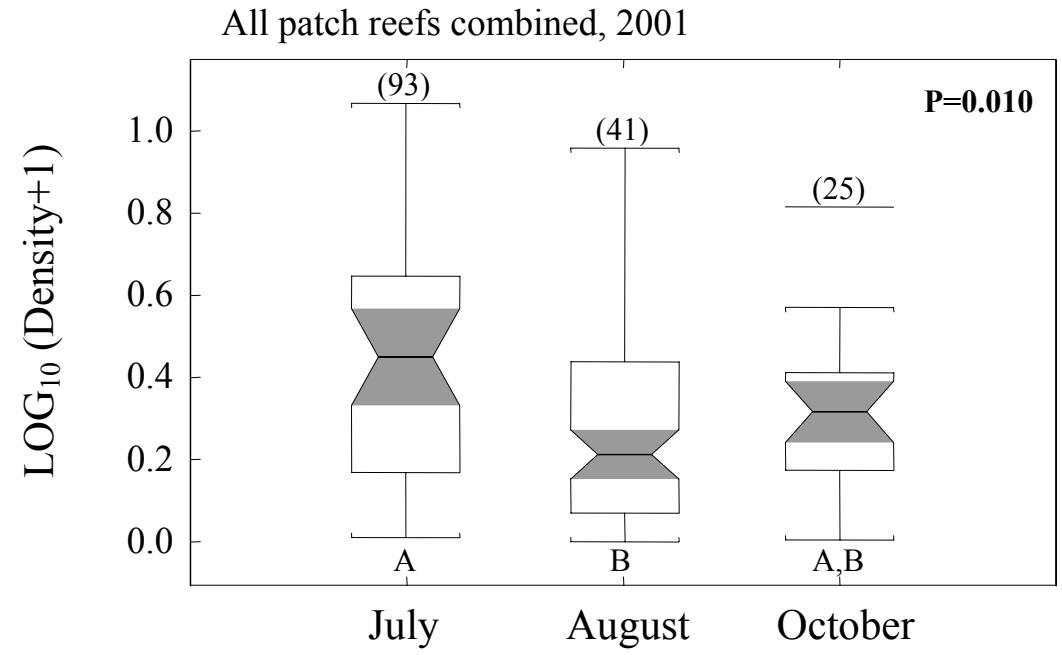

Figure 1.9. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] for all patch reefs combined as a function of months in 2001. Isolated line for October represents an outlier. Number of patch reefs sampled is in parenthesis. Letters that are the same are not significantly different based on a Tukey's multiple comparisons test with significance set at P=0.05.

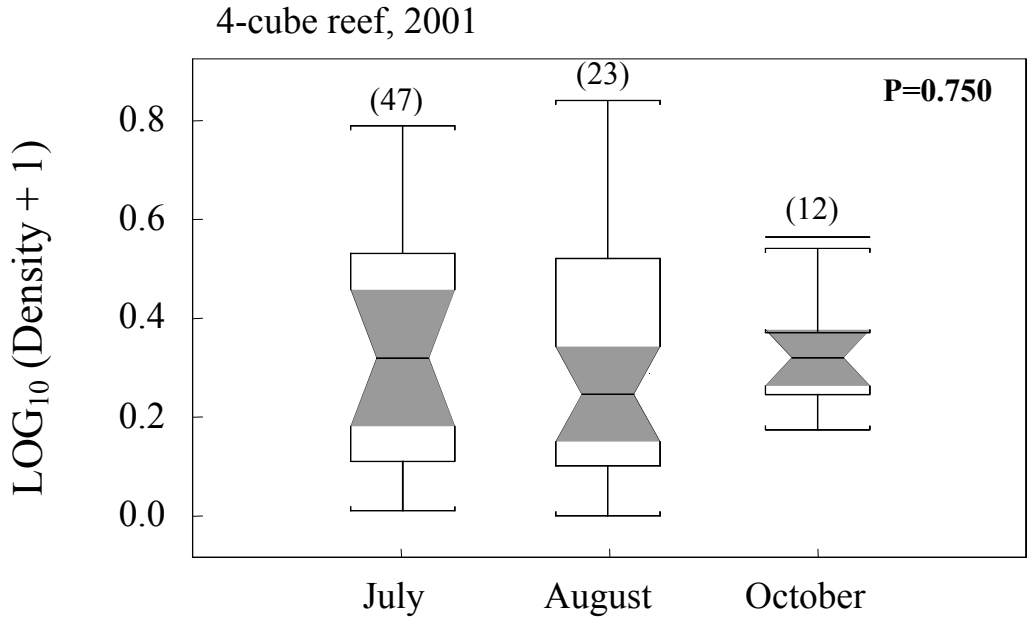

Figure 1.10. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] for 4-cube patch reefs as a function of month in 2001. Isolated line for October represents an outlier. Number of patch reefs sampled is in parenthesis.

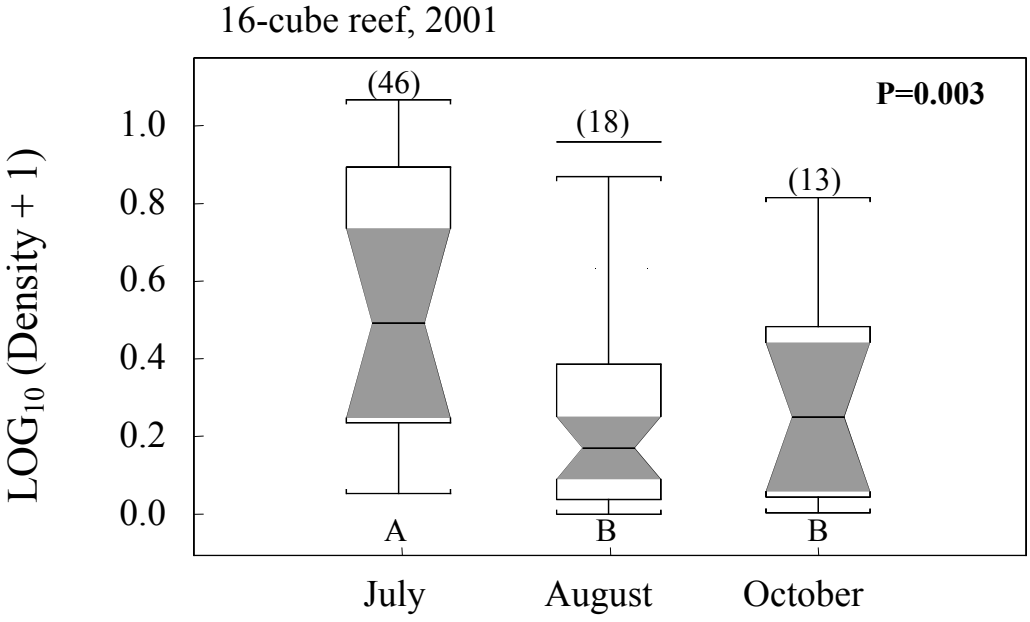

Figure 1.11. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] for 16-cube patch reefs as a function of months in 2001. Isolated line for August represents an outlier. Number of patch reefs sampled is in parenthesis. Letters that are the same are not significantly different based on a Tukey's multiple comparisons test with significance set at P=0.05.

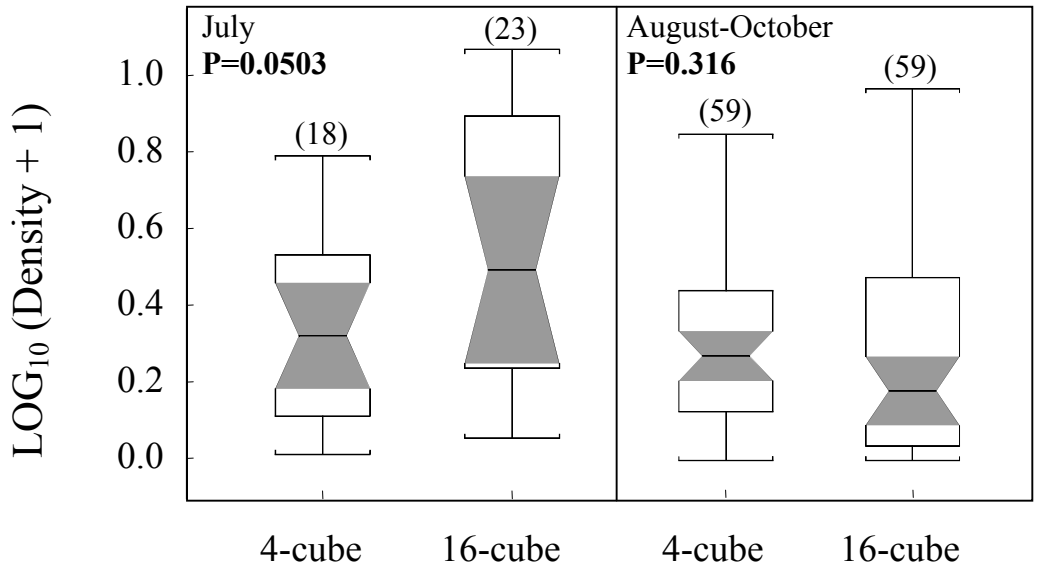

Figure 1.12. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] Comparing differences in pelagic fish density between cube size within the months of July and August-October (combined). Number of patch reefs sampled is in parenthesis.

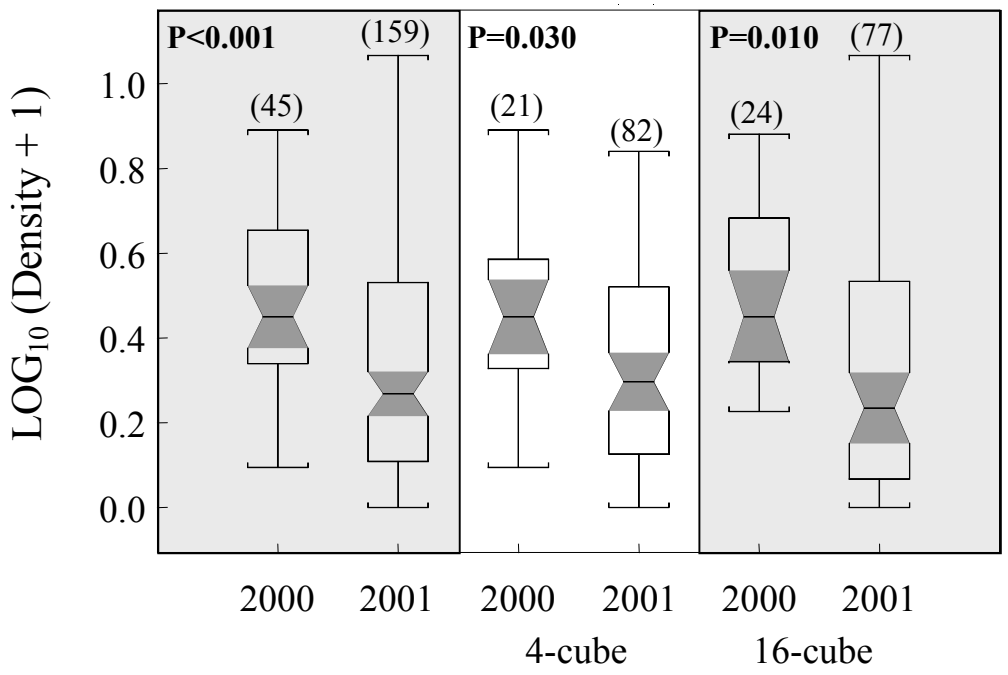

Figure 1.13. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed fish density (fish m^{-2}), LOG_{10} [density+1] Comparing differences in pelagic fish density between years. Data are displayed for 4-cube and 16-cube reef combined and separated. Number of patch reefs sampled is in parenthesis.

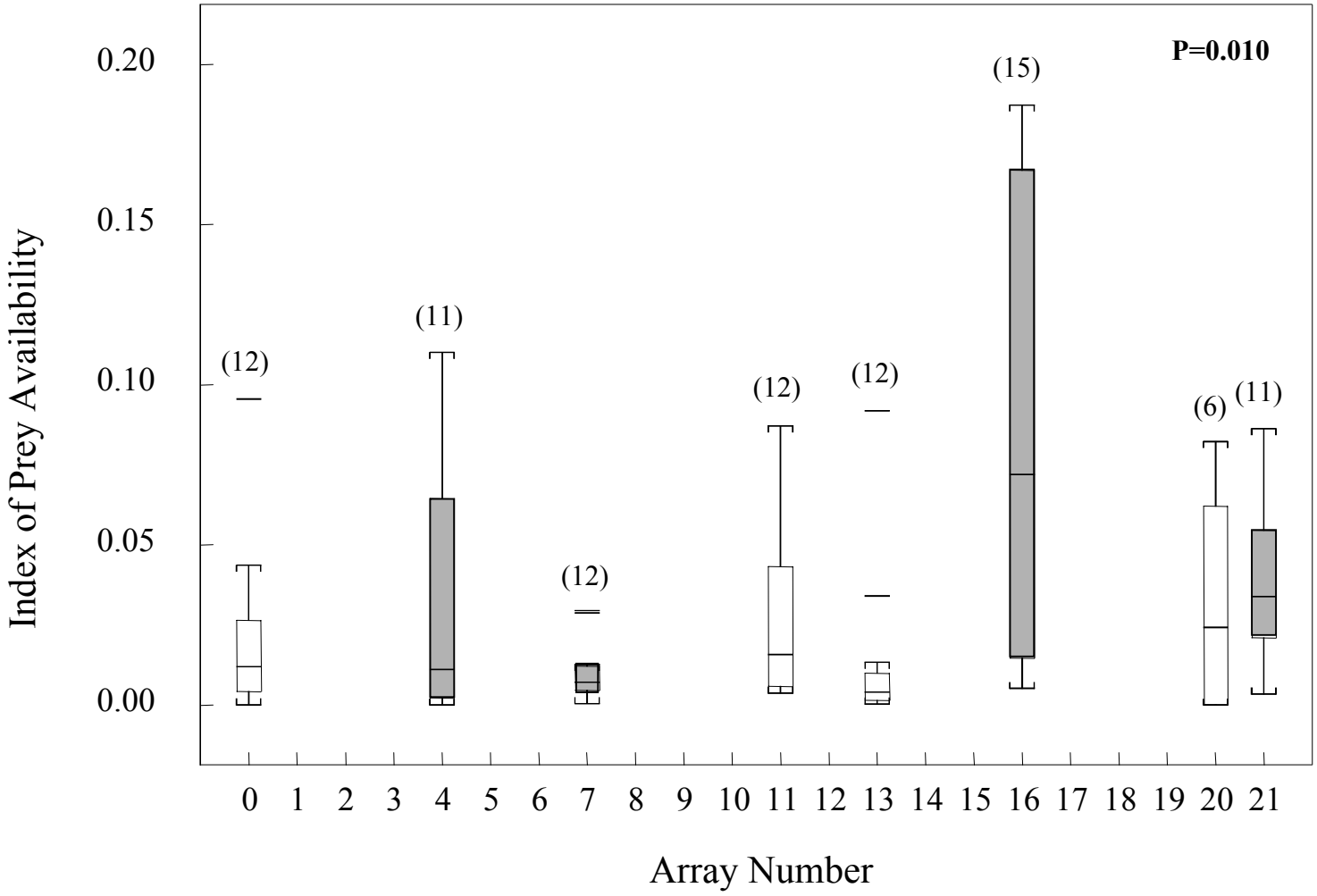

Figure 1.14. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed index of prey availability, LOG_{10} [(fish density/gag numbers) + 1] for all reef arrays in 2001. Isolated lines represent outliers. Number of patch reefs sampled is in parenthesis.

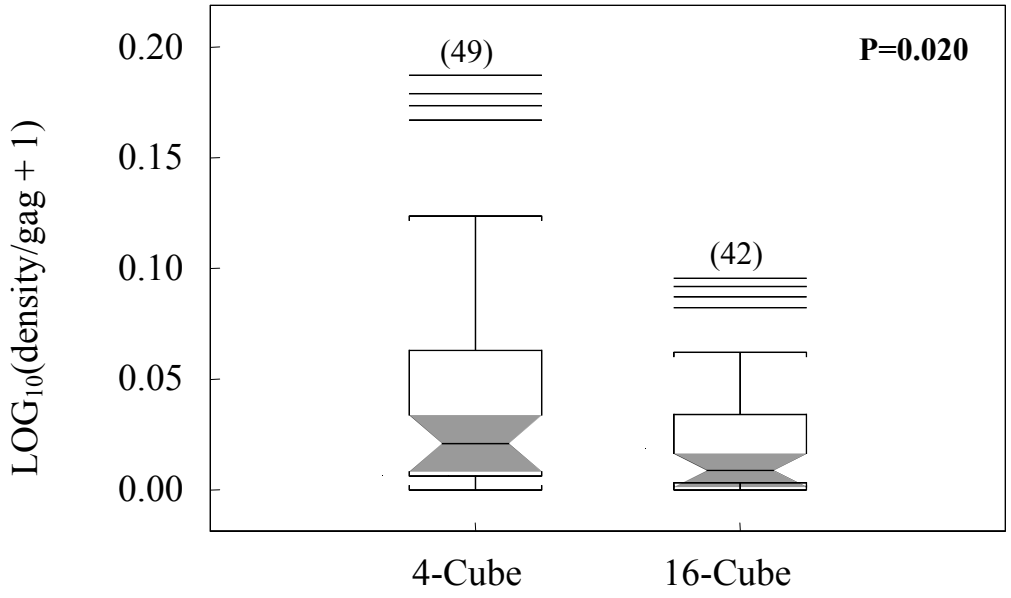

Figure 1.15. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed prey availability, LOG_{10} [(fish density/gag numbers) + 1] for all months as a function of patch reef size (4 cube vs. 16 cube) in 2001. Isolated lines represent outliers. Number of patch reefs sampled is in parenthesis.

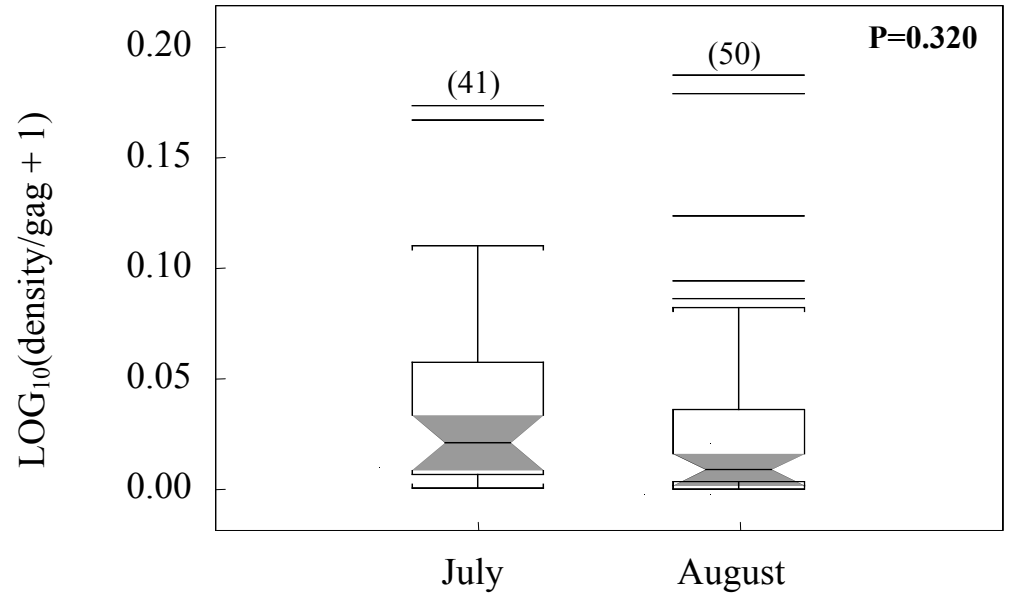

Figure 1.16. Box and whisker plot (median, 25% quartiles, 75% quartiles, range, and shaded area is the ±95% CL) of the log transformed prey availability, LOG_{10} [(fish density/gag numbers) + 1] for all patch reefs combined as a function of months in 2001. Isolated lines represent outliers. Number of patch reefs sampled is in parenthesis.

OBJECTIVE 2: Comparison of Prey Consumption by Gag Grouper between Patch Reefs of Contrasting Size

Methods

Daily food consumption was quantified on a wet weight and gross energy basis for gag grouper on replicate 4-cube and 16-cube reef sites during summer months (June-September 2002) when pelagic prey fishes were present in the vicinity of the SRRS. Food consumption on a daily gross energy intake basis was determined by using a consumption model that integrated components of the gag's diet composition, weight of ingested prey, conversion of weight of prey consumed to gross energy of prey consumed, diel feeding periodicity, and an estimate of food evacuation rate.

Sampling of Gag

To determine prey consumption of gag from 4-cube and 16-cube reef arrays, gag were sampled from each of three replicate patch reefs from four 4-cube arrays and from four 16-cube arrays. A randomized design blocked on time was used to sample over the four summer months to ensure that sampling of gag from the patch reefs was not biased by clumping of stomach samples during any one time period (i.e., an attempt was made to collect gag from all patch reefs sampled in random order over three blocks of time throughout the summer). Two of the 4-cube patch reefs sampled for the present study had half of their cavity holes closed as part of another ongoing study on the effect of habitat limitation on gag density. These two manipulated reefs, however, did not have a significantly different density of gag when compared to 4-cube reefs that had not been manipulated (Hart 2002), and were considered hereafter to be representative of 4-cube reefs without regard to the habitat manipulation.

Gag were collected by SCUBA divers lowering traps underwater, positioning the traps around the entrances to the reef blocks, and then herding grouper into the traps. Gag were then lifted to the surface and immediately placed in insulated coolers supplied with air. A portion of gag to be retained for sampling in conjunction with Objective 3 were speared rather than trapped. Each gag was measured for maximum total length and fork length (Anderson and Gutreuter 1983). Fish were also measured for girth, which was the circumference of the fish immediately behind its pectoral fins, over its pelvic fins and over the anterior portion of its dorsal fin. While not maximum girth, measuring girth at this position allowed for landmarks to be used in a consistent measuring position. Each gag was then lavaged to recover its stomach contents. Lavage methodology followed that of Murie and Parkyn (2000). Any prey items regurgitated in the cooler were added to the lavaged stomach contents for individual fish. Gag sampled from each patch reef were lavaged, with a subsample of fish from each patch reef retained and sacrificed to obtain condition and measure otoliths for growth analysis (Objective 3) and to check for the completeness of the lavage of stomach contents. Lavaged gag were fin-clipped in the posterior portion of their dorsal fin to avoid recaptures. Retained gag were weighed to the nearest 0.1 g in the laboratory. Measuring length and girth of each gag was later used in regression analysis to predict gag weight for those gag that were lavaged and released at sea.

Composition of the Diet

Stomach contents were identified to species whenever possible using identification keys (Abele and Kim 1986, Robins and Ray 1986, Hoese and Moore 1998) and unique anatomical features (i.e., sagittal otoliths, beaks of squid). Due to digestion, most fish recovered in the stomach contents were identified using sagittal otoliths. An otolith reference collection was assembled from identified, whole fish collected from the Gulf of Mexico during the summers of 2000 and 2001. Sagittal otoliths were extracted from each reference fish, washed, measured for total length and width, weighed, and digitally imaged. Each reference fish was also measured for maximum total length, fork length, standard length, vertebral column length (base of skull to hypural plate), and weight.

Relative importance of food items in the diet of gag grouper from 4-cube and 16-cube arrays was assessed by determining: 1) the percent occurrence of food types in stomachs (number of stomachs in which prey type occurred/total number of stomachs sampled that contained food items x 100); 2) the percent numerical abundance (number of items of each prey type/total number of items for all prey types combined x 100); 3) the percent wet weight (estimated wet weight of each prey type/wet weight of all prey types combined x 100) (Hyslop 1980); and 4) the percent contribution to gross energy (estimated gross energy for each prey type/total gross energy for all prey types combined x 100) (Murie and Lavigne 1991).

Weight of Ingested Prey

All components of the stomach contents were weighed (wet weight) after first being separated into major prey categories of fish, crustacean, mollusk, and miscellaneous. Size and weight of each prey item consumed was then measured directly if the prey item was undigested. For prey that were partially digested, length and weight of individuals were estimated from back-calculations using predictive regressions from the reference collections. Predictive regressions used in back-calculations were based on measurements of vertebral column length or otoliths in relation to the length and weight of the fish, or carapace length and width relative to whole crab carapace width and crab weight. Average size and weight of relatively undigested specimens from stomach contents were used to estimated size and weight of prey items consumed when predictive regressions could not be formulated due to a lack of reference specimens.

Calorific Conversion

A sub-sample of 5-10 individuals of each reference prey species used for back-calculation regressions were also used to determine caloric density of prey species. Each specimen was homogenized in a blender and up to 50 g wet weight was then freeze-dried to determine % moisture. The freeze-dried sample was then re-ground for homogenization before a 0.05-1 g sub-sample was analyzed for caloric density using an adiabatic bomb calorimeter (Parr Instruments) with appropriate corrections made for fuse wire burn and acid production (Paine 1971). Caloric densities on a dry weight basis were then transformed to kcal/g wet weight using percent moisture determinations. Regressions of caloric density as a function of fish size were

estimated for each prey species when a size range of prey was available. These regressions were used to estimate the caloric density of specific sizes of specific prey species. If regressions were non-significant, or the size range of prey was narrow, then a mean caloric density for the prey species was used in calculations.

Diel Feeding Periodicity

Feeding periodicity was estimated for gag grouper to determine whether they were feeding throughout the day or during discrete periods of time. The proportion of empty stomachs based on 2-hr time blocks was plotted as a function of time of day to determine if there were periods in the day when the majority of gag grouper had empty stomachs. A stomach fullness index was calculated for each gag based on the weight of its stomach contents as a percentage of its estimated body weight. The mean stomach fullness index for adjacent 2-hr time blocks was plotted as a function of time of day to determine if any modes in feeding frequency were observed.

Weight of each gag was estimated for those gag that were measured but released and was based on a pooled predictive regression of weight (W) as a function of maximum total length (MTL) using gag that were retained from both 4-cube and 16-cube arrays for Objectives 2 and 3. Girth was not included with length as a predictor variable for estimating weight of gag because it did not add significantly to the fit of the overall regression. Weight and MTL were $\log_{10}$-transformed prior to regression analysis to correct for heteroscedasticity and to linearize. Data were pooled for gag collected off 4-cube and 16-cube arrays because the tests for differences in relative weight between gag from the two reef sizes were statistically inconclusive (see Objective 3).

A stomach fullness index was also calculated for fish prey only, as the primary prey group (Prey Fish Stomach Fullness Index) and expressed as a mean index as a function of 2-hr time blocks throughout the day. In addition, a fish digestion index (% Digested Prey Fish) was calculated based on the weight of digested fish in a gag's stomach expressed as a percentage of the estimated back-calculated weight of the fish prey prior to any digestion (i.e., at ingestion), subtracted from 100%. This index gave the percentage of fish that had already been digested and evacuated from the stomach.

An index of recent feeding was also determined by using the occurrence of relatively undigested prey in the stomach contents. Each prey item recovered was initially assigned a qualitative digestion index of 0 (<5% digestion), 1 (5-10% digested), 2 (10-25% digested), 3 (25-50%), 4 (50-75%), 5 (75-90%), 6 (90-99%), or 9 (>99%). These digestion codes were teamed with qualitative assessment of the condition of the prey item. For example, fish prey given a code=1 would have had intact bodies with eyes, skulls, skin, and gut tracts (as in code = 0) but may have some small amount of skin and a small number of fin rays removed by digestion. For crab prey, individuals given a code = 1 would have intact bodies with eyes as well as have all legs and chelipeds (as in code = 0) but may have one or two legs or a cheliped macerated and/or broken open and the exoskeleton softened from digestion. The index of recent feeding was the percentage of gag that had recently consumed (digestion code of 0, 1, or 2) a prey item. This

index was calculated for three prey categories: summer baitfish (Spanish sardine, scaled sardine, or scad), tomtate (as a resident baitfish), and crab.

In addition to gag sampled from 4-cube and 16-cube arrays used in the overall diet study, gag were also lavaged from other 4-cube and 16-cube arrays in an attempt to determine their feeding periodicity over consecutive blocks of time throughout the day and night of a 24-hr period (Culp 1989, Adams and Breck 1990). Initially, a 24-hr block of time was partitioned into six 4-hr blocks and 10-15 gag were to be collected in each of the blocks. All stomach contents were weighed and expressed as an index of stomach fullness. This 24-hr sampling regime was conducted once during late June of 2001 and, due to sample size, stomach fullness indices for gag sampled in this 24-hr period were pooled with stomach fullness indices for all gag sampled from 4-cube and 16-cube arrays to determine an overall feeding periodicity (Elliott and Persson 1978, Booth 1990). Future sampling over contiguous time blocks in a 24-hr period of time for feeding periodicity will involve at least three to five separate sampling events.

Food Evacuation Rate

Evacuation rates of gag grouper were to be determined in the field using a serial-slaughter technique (after Windell 1967). This method required not only that gag feed in a relatively discrete period of time, but that the time period when gag have just recently fed is known, which was to be estimated by the diel feeding periodicity. Food evacuation rate was not determined in the field using the serial-slaughter technique because the 24-hr diel sampling regime could not be replicated an adequate number of times. However, an approximate estimation of the evacuation rate for fish from gag stomachs could be calculated from analyzing the stomach contents directly, given that specific assumptions were stated. These assumptions remain to be validated in experimental feeding trials in the summer of 2002 and 2003. Specifically: 1) the % fish remaining in a gag's stomach could be estimated from the difference between 100% and the % of fish already evacuated from the stomach (i.e., 100% - % Digested Prey Fish); and 2) the evacuation rate is linear and the relative rate therefore does not change throughout the digestion period. This latter assumption was the most critical and was subject to validation. However, this assumption was reasonable given that a linear evacuation model has been used previously for piscine predators consuming relatively large, high energy prey items (Persson 1986, Hopkins and Larson 1990). On this basis, a linear evacuation rate for gag could be modeled if: 1) the greatest observed percentage of fish remaining in gag stomachs could be standardized to 100% and assumed to represent 0-hr post-prandial time; and 2) the % fish remaining in the gag stomachs could be estimated over a long enough period of time to provide for an adequate model fit.

Future studies in 2002 will determine food evacuation rates and prey digestion rates for gag using controlled, experimental feeding trials of gag in captivity under simulated field conditions. These feeding trials will also be used to validate food evacuation rates determined from field sampling.

Food Consumption Estimates

Daily food consumption estimates were made using the Diana (1979) consumption model under specific assumptions. This model was deemed the most appropriate because it does not make

any assumptions about the characteristics of the rate of gastric evacuation (i.e., linear versus exponential), which was a critical assumption that was necessary until more detailed experiments on food evacuation rates of gag were undertaken. The model is:

$$C = 24 \cdot \frac{M \cdot S}{B' \cdot N}$$

where: M is the back-calculated average meal size; S is the number of gag in the population that had food in their stomachs; B' is the number of hours post-prandial when empty stomachs first appear; and N is the total number of stomachs examined. Since this model does not take into account the size of gag relative to an average meal size, gag were divided into two size classes (<50 cm and >50 cm MTL). The size division at 50 m MTL was chosen because it corresponds to the minimum legal size for gag and most gag captured off both the 4-cube and 16-cube arrays were less than 50 cm in length. The average size of gag in these two size categories were compared among the 4-cube and 16-cube arrays using a Wilcoxon two-sample nonparametric test (NPAR1WAY) (SAS 1996).

For average weight of food consumed during one meal event (M), the total weight of prey consumed by each gag was estimated by summing the estimated wet weight of all individual prey items in the stomach for each gag and then estimating the average weight of food consumed on a per array basis (three reefs per array or ~12-15 fish). B' was determined from the food evacuation rate analysis and was assumed to be the same regardless of whether the gag was from a 4-cube or a 16-cube reef. Similarly, average daily gross energy intake for each gag was estimated by multiplying the wet weight of individual, back-calculated prey items by their estimated caloric density, and then summing over all prey items for each gag. An average gross energy intake was then calculated on a per array basis for 4-cube versus 16-cube gag. Differences between the food consumption and gross energy consumption of gag on 4-cube versus 16-cube arrays was determined for gag <50 cm MTL and for gag >50 cm MTL using a nonparametric Wilcoxon two-sample test (SAS 1996).

Results and Discussion

Sampling of Gag

In total, 139 gag were captured from four 4-cube arrays (12 patch reefs in total) from June to October 2001 for food habit analysis. Of these 139 fish, 88 were measured, lavaged and then released back on to the patch reef of capture whereas 36 gag were captured, lavaged, and retained for growth and condition processing in the laboratory. A further 15 of the 139 gag were captured and retained without being lavaged.

A total of 45 out of 80 gag that were captured from four 16-cube arrays (12 patch reefs in total) were lavaged and retained for processing, with an additional 6 fish captured and retained without being lavaged. In total, 29 out of the 80 gag captured were lavaged and then released back on to the reef of capture.

For the sampling over a 24-hr block of time for diel feeding periodicity, a total of 53 gag were captured, measured, lavaged, and returned to the reef of capture. A number of these gag were sampled from the 4-cube and 16-cube arrays used in the main study and are included above in total number of gag sampled for food habit analysis. These fish were used in the determination of diel feeding periodicity, as well as diet composition and food consumption. However, gag sampled in the 24-hr period that were not sampled from the main study arrays (n=26) were only used to aid in the determination of diel feeding periodicity and were not used in the consumption estimates.

Diet Composition

Presence of Stomach Contents: Of all gag sampled off of 4-cube arrays, 71% (99 of 139) had stomach contents and 29% had empty stomachs. Sixty-three percent of the 80 gag sampled from 16-cube arrays had stomach contents present whereas 30 (37%) had empty stomachs. The proportion of gag on 4-cube and 16-cube arrays that had stomach contents was not significantly different ($\chi^2 = 0.183$).

Regurgitation of Stomach Contents: Of 94 gag with stomach contents captured from 4-cube arrays and checked for regurgitated prey items when brought to the surface, 33% showed signs of regurgitation as indicated by the presence of food items or pieces of food caught in the teeth or gill rakers. Of the 50 gag possessing stomach contents that were captured from 16-cube arrays, 48% showed signs of regurgitation.

The occurrence of 33-48% of gag captured at depth and brought to the surface for lavaging showing signs of regurgitation is problematic for accurately estimating their food consumption. Unfortunately, it was only possible to remove a small number of gag from each array in order to insure that density of gag on the arrays was not compromised, since our overall study was based on a density-dependent process. It will be necessary in future food consumption studies of gag to capture gag off of the study arrays (and from other similar array structures), and place them in a sealed bag for transport to the surface. The proportion of stomach contents regurgitated (in the bag) relative to the amount lavaged or collected from the stomach itself can then be determined. This would allow a correction factor to be used in estimating total food consumption from lavaged fish.

Completeness of Lavage: Twenty-six gag that were lavaged and observed to have stomach contents were retained from 4-cube arrays to check if the lavaging had removed all of the stomach contents. Of these gag, 65% were completely lavaged whereas 35% showed remnants of prey items in their stomachs following the lavage. Of 31 gag retained for growth and condition processing after being captured and lavaged from 16-cube arrays, and which were determined to have lavaged contents, 77% were completely lavaged. Prey remnants not removed from stomachs by the lavage method were primarily whole crabs or pieces of crab exoskeleton, whole large fish (not baitfish), or small pieces of fish vertebrae and spines. In the case of whole crabs and whole large fish (which were not baitfish), the presence of stomach contents was also apparent visually from the exterior surface of the gag.

The original intent of checking for incomplete lavaging was to provide a correction factor for lavaged stomach contents to correct for an underestimation bias. In processing of stomach contents, however, lavaged contents and food remnants left in the stomachs were inadvertently scored together and, for most cases, could not be distinguished from one another. It will be important in future studies to determine whether the food remnants left in the stomachs after lavaging provide a significant bias in the measure of total food consumed.

Composition: Overall, the number of prey species consumed by gag on 4-cube arrays was higher than gag sampled from 16-cube arrays (17 versus 8 prey species, excluding mixed species of baitfish) (Tables 2.1 and 2.2). Mixed baitfish was excluded because it was a category that included prey fish that could be ascribed to being one of either Spanish sardine, scaled sardine, round scad and tomtate (primarily based on vertebral column and skull case size and structure) but not specifically identified to only one of the species. Because gag had consumed up to three of these different baitfish species at one time, prey fish from the same stomach contents that could not be positively identified to the species-level were labeled only as "baitfish", without the usual proportional allocation made on the basis of the proportion of identified species in the stomach contents. The trend in diversity of prey species was still apparent when the number of gag sampled from 4-cube arrays was randomly reduced to be comparable to the smaller number of gag sampled off of 16-cube arrays (15 versus 8 prey species, excluding mixed species of baitfish). The increased diversity of prey consumed by gag from 4-cube reefs, relative to gag from 16-cube reefs, merits further study. The abundance and availability of prey other than baitfish on or near the arrays, relative to their occurrence in the stomach contents of gag, may be important because of the seasonal and ephemeral nature of baitfish.

On a percent occurrence basis, the majority of gag from both 4-cube and 16-cube arrays had consumed fish (88% and 94%, respectively). Of gag consuming fish, most had fed on tomtate (*Haemulon aurolineatum*) (37% and 42% of the gag from both 4-cube versus16-cube arrays, respectively), followed by round scad (*Decapterus punctatus*) (25% versus 20%), scaled sardine (*Harengula jaguana*) (28% versus 12%), and Spanish sardine (*Sardinella aurita*) (9% versus 16%). A substantial number of gag had also consumed crustaceans (25% and 26% for 4-cube and 16-cube, respectively) and to a lesser extent, molluscs (i.e., squid, *Loligo pleii*) (2% and 10%, respectively). The majority of gag feeding on crustaceans had consumed portunid crabs (*Portunus floridanus*) (21% and 26%, respectively).

On a numerical abundance basis, the food habits of gag from both the 4-cube and 16-cube arrays consisted primarily of pelagic baitfishes (79% and 84%, respectively) (Tables 2.1 and 2.2). These baitfishes were predominantly scaled sardine, Spanish sardine, round scad, and tomtate. Crabs, primarily *Portunus floridanus*, also contributed a significant amount to the diet of gag from both 4-cube and 16-cube arrays (13% and 14%, respectively). Squid was of relatively minor occurrence in the diet (≤ 2% for both arrays).

Size of Ingested Prey: Predictive regressions relating partial dimensions of non-digested prey (e.g., otolith length) to their size (e.g., maximum total length, MTL) were significant (Table 2.3). These predictive regressions were therefore used to estimate the length of fish or the width of crabs ingested by gag.

Gag from both 4-cube and 16-cube arrays had consumed relatively small prey (Table 2.4), with typical baitfish species approximately 4.5 to 7 cm MTL. There was no significant difference between the size of baitfishes consumed by 4-cube versus 16-cube gag (Wilcoxon: $P > 0.1294$). Only fish other than baitfishes, consumed primarily by gag on 4-cube arrays, were relatively larger (e.g., *Centropristis striata*, 14 cm MTL) (Table 2.4).

Portunid crabs (*Portunus floridanus*) consumed by gag were also relatively small but, in contrast to baitfishes, gag on 16-cube arrays had consumed larger (wider) crabs, which were also heavier, than gag on 4-cube arrays (Wilcoxon: $P < 0.0001$ and $P < 0.0001$ for carapace width and total weight, respectively). Gag from 16-cube arrays had ingested crabs that were on average 33.1 mm (± 0.1.5 mm) carapace width and 4.8 g (± 0.44 g) total weight compared to gag from 4-cube arrays that had consumed crabs that were 18.4 ± 1.1 mm and 1.7 ± 0.3 g.

Weight of Ingested Prey: Predictive regressions relating partial dimensions of non-digested prey (e.g., fish vertebral column length) to their weight were significant (Table 2.3) and therefore used to back-calculate the original weight of prey ingested by gag.

On a weight basis, fish dominated the diet of gag from 4-cube arrays (85% by weight), of which 63% was from baitfish species, primarily tomtate and Spanish sardine (Table 2.1). The diet of gag from 16-cube arrays was also comprised of a majority of fish prey (63% by weight), with baitfish comprising 60% (Table 2.2). Spanish sardine and tomtate were also the predominant prey species by weight for gag on 16-cube arrays. Gag from 16-cube arrays, relative to gag from 4-cube arrays, also had a large component of crab in their diet (26% versus 8% by weight, respectively).

Calorific Conversion: Caloric density of prey species varied between 0.980 to 2.045 kcal/g wet weight (Table 2.5). Caloric density of pelagic baitfishes overall was 1.114 kcal/g wet weight The relatively low energy density estimated for a variety of baitfishes was most likely a result of most of the baitfish being juveniles. Pigfish (Orthopristis chrysoptera) and slippery dick (Halichoeres bivittatus) had the greatest caloric density estimates of all fishes sampled. Only two prey species exhibited a significantly positive relationship in increasing caloric density with increasing prey fish size (sandperch Diplectrum formosum and Scaled sardine). Average caloric density was therefore used rather than a size-specific caloric density for all prey species, including sandperch and Scaled sardine, because the size range that the fish were sampled over was narrow (Table 2.5). Obtaining additional prey samples throughout each species potential size range will be a priority in 2002-2003.

On a gross energy basis, prey consumed by gag on 4-cube arrays was primarily various fish species (88% by gross energy), primarily baitfishes (59%), such as tomtate, Spanish sardine, scaled sardine, and round scad (Table 2.1). Crustaceans, such as portunid crabs, were relatively unimportant energetically to 4-cube gag (7% by energy), as was squid (4% by gross energy). Fish, and especially baitfish, were also important on a gross energy basis to gag on 16-cube arrays (67% fish, or specifically 63% baitfish by energy). However, portunid crabs were considerably more important on a gross energy basis to 16-cube gag (24% by energy) relative to 4-cube gag (7% by energy).

Diel Feeding Pattern

Gag had a greater percentage of empty stomachs during mid-day (~1000-1400 h) and just before night (~1700-1800 h) than during early morning (~0700-0800 h), mid-afternoon (~1500 h), or late evening (~2000-2100 h) (Figure 2.1). Concurrently, the stomach fullness index (SFI) based on all prey was lowest around 0700-0800 h and after 1800 h at night (Figure 2.1), with values below 1%. Although more variable, the SFI was at its peak during 1600-1700 h at 3.5-4% body weight. The SFI based only on prey that were fish (Prey Fish SFI) followed a similar trend as the SFI for all prey types because the majority of prey consumed were fish (Figure 2.2). The Prey Fish SFI showed a peak at 1600-1700 h, with otherwise low values (<0.5% body weight) throughout the day sampled (0700-2100 h). The % Digested Prey Fish showed minima between ~0700-1000 h, 1600 h, and 2100 h (Figure 2.2), with an increasing index (i.e., increasing amount of digestion) from 1000-1400 h and from 1700-2000 h. The overlap between these two indices between 1600-1700 h indicated that the large amount of fish prey consumed by gag during this time was relatively undigested, which together indicated recent feeding.

The Index of Recent Feeding (IRF) for baitfish present only in the summer months (Spanish sardine, scaled sardine, and round scad) indicated that baitfish were consumed early in the morning (before 0700 h) and in the late afternoon or early evening (after ~1600 h) (Figure 2.3), with a decreasing IRF from ~0700 h to 1400 h. This modality was indicative of a crepuscular feeding periodicity and was consistent with the overall % Digested Prey Fish Index. For other prey species (tomtate and crabs), however, their occurrence as recent prey in the stomachs did not appear to be bimodal. Although variable, the trend for tomtate and crabs appeared to be towards gag consuming them primarily during the daytime (Figure 2.3).

Food Evacuation Rate

Although food evacuation rates need to be determined under controlled laboratory feeding experiments, there was an indication from the % Digested Prey Fish (Figure 2.2), as well as the IRF for summer baitfish (Figure 2.3), that the evacuation rate of fish from gag stomachs was relatively rapid. This observation is based on the increasing digestion of prey fish from 0700h to 1400h (Figure 2.2), as well as a corresponding decreasing trend in the IRF for baitfish from 0700 h to 1400 h (Figure 2.3). At 0700 h, over 70% of gag sampled with stomach contents present had recently consumed baitfish. Presumably, this percentage would increase even more prior to 0700 h. However, by 1400 h only 10% of gag sampled had recent baitfish in their stomachs with 90% having relatively digested baitfish.

As an estimation of an evacuation rate for gag, the linear extrapolation of the relationship between the % Prey Fish Remaining in gag stomachs as a function of time after feeding (post-prandial time) indicated that gag evacuate their stomachs completely in ~16 hr (Figure 2.4). However, Swenson and Smith (1973), using a linear digestion model to estimate food consumption in walleye (*Stizostedion vitreum vitreum*), recommend estimating the evacuation time based on an "effection phase" or phase of rapid digestion. This evacuation time corresponds to ~90% digestion of the stomach contents. The final 10% of the stomach contents are passed during a "residual phase", which has been observed to vary based on a number of factors, including the type of hard parts associated with the food (Kionka and Windell 1972). On

the basis of using the effection phase of digestion then, gag evacuation time is ~15 h (Figure 2.4).

Average Daily Consumption (% Body Weight)

Average daily consumption on a per body weight basis was not different between sublegal- and legal-size gag on either 4-cube arrays (Wilcoxon: P=1.00) or 16-cube arrays (Wilcoxon: P>0.86) (Figure 2.5). In addition, consumption by gag was not different between sublegal-sized gag on 4-cube versus 16-cube arrays (Wilcoxon: P>0.47) or between legal-size gag on 4-cube versus 16-cube arrays (Wilcoxon: P>0.60). Legal-sized gag on 4-cube arrays had more variation in their interquartile range than all other groups (Figure 2.5). Overall, average daily consumption by gag on 4-cube arrays was observed to be greater than for gag on 16-cube arrays (1.8% versus 1.2%, respectively), but the difference was not statistically significant (Wilcoxon: P>0.31) (Figure 2.6).

Average Daily Gross Energy Consumption (cal/g body weight)

The trends in average daily gross energy consumption by gag on 4-cube and 16-cube arrays was similar to average daily consumption by weight because most prey species had caloric densities close to 1 kcal/g wet weight (Table 2.5). Baitfishes were primarily juveniles and were therefore relatively low in caloric density compared to the potential caloric density of adult baitfish, which could typically be 2-3 times greater. Gross energy consumption of sublegal- and legal-sized gag from 4-cube arrays were therefore not different (Wilcoxon: P> 1.00), and neither was consumption of the two sizes of gag from 16-cube arrays (Wilcoxon: P>0.60) (Figure 2.7). Gross energy consumption was also not different between sublegal-sized gag on 4-cube versus 16-cube arrays (Wilcoxon: P>0.47) or legal-sized gag on 4-cube versus 16-cube arrays (Wilcoxon: P>0.60) (Figure 2.7). As with average daily consumption on a per body weight basis, legal-sized gag from 4-cube arrays had a broader interquartile range than any other group. Overall, the gross energy consumption of gag on 4-cube arrays tended to be higher than energy consumption of gag from 16-cube arrays (22 versus 11 cal/g body weight, respectively), but the difference was not significant (Wilcoxon: P>0.31) (Figure 2.8).

Average daily consumption by weight and gross energy consumption estimates in the future could be improved by: 1) modeling evacuation rates of gag under experimental feeding trials to determine if evacuation is linear or exponential, rather than assuming that it is a linear; and 2) estimating caloric density of some additional prey species through bomb calorimetry rather than approximations or literature values for species from different areas.

Summary:

Objective 2: Prey consumed by gag from 4-cube and 16-cube arrays was predominantly pelagic baitfishes. Gag on 4-cube arrays had consumed a greater diversity of prey species, whereas the diet of gag on 16-cube arrays was less diverse but contained relatively more portunid crabs than gag on 4-cube arrays. Although trends in average daily food consumption and average daily gross energy consumption indicated that gag from 4-cube arrays had consumed greater quantities of prey than gag from 16-cube arrays, the differences were not significant either between reef array sizes or between sublegal-sized and legal-sized gag.

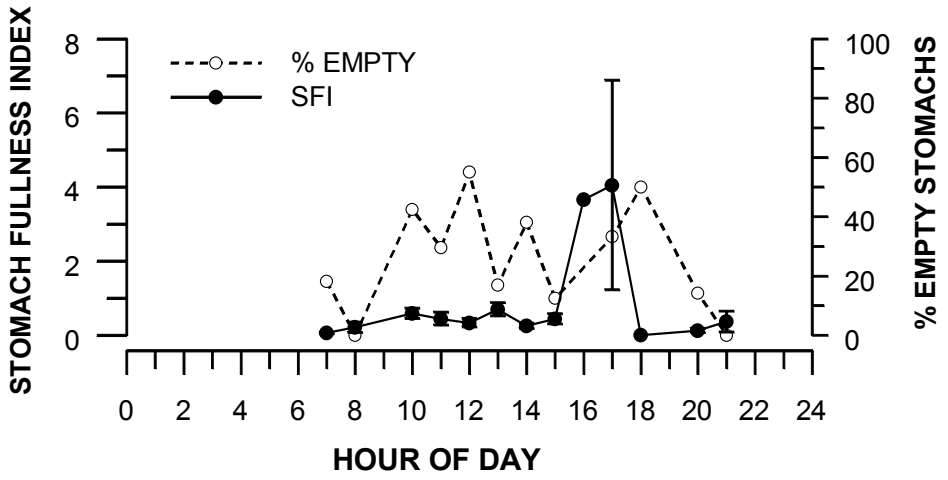

Figure 2.1. Stomach fullness index and % empty stomachs as a function of time of collection for gag.

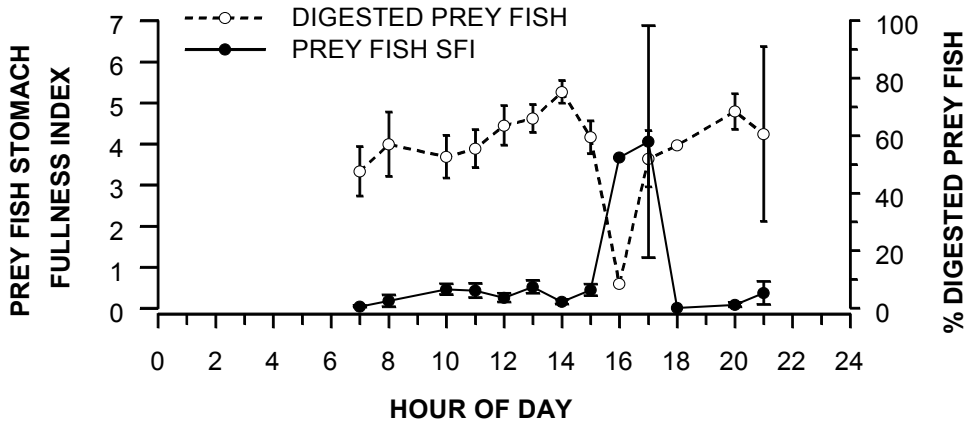

Figure 2.2. Prey fish stomach fullness index and % digested prey fish as a function of time of collection for gag.

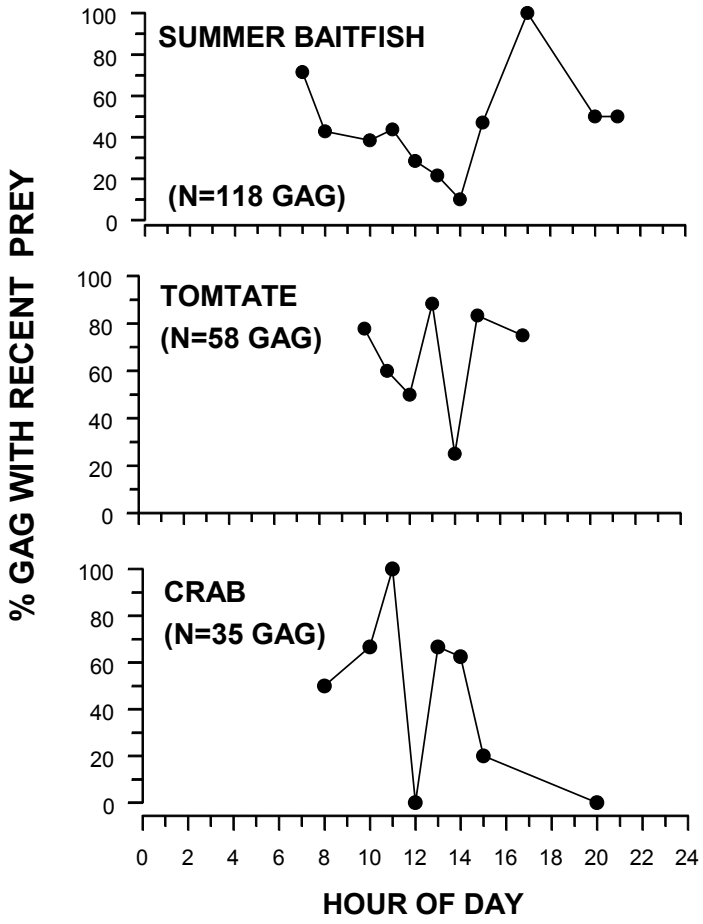

Figure 2.3. Percent occurrence of recent prey in the stomach contents of gag as a function of time of collection.

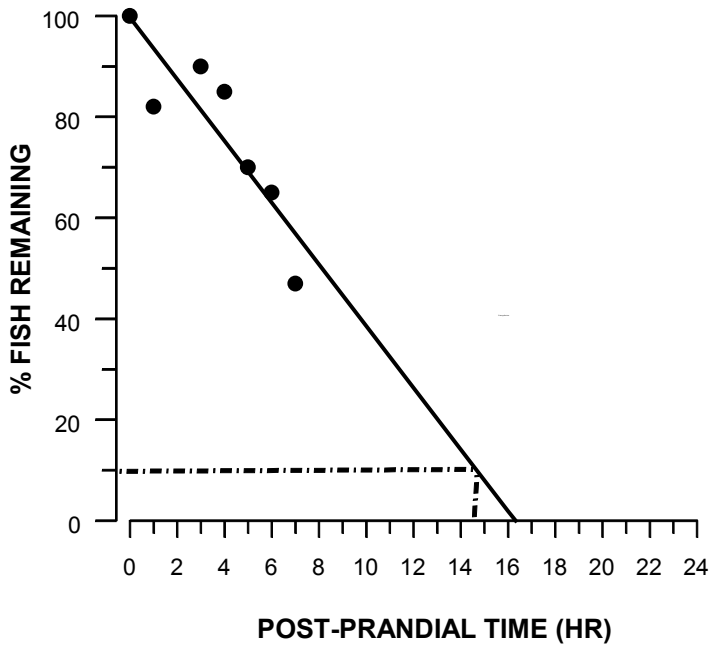

Figure 2.4. Percent fish remaining in gag stomachs as a function of standardized post-prandial time. Dashed line denotes demarcation between effection and residual phases of digestion.

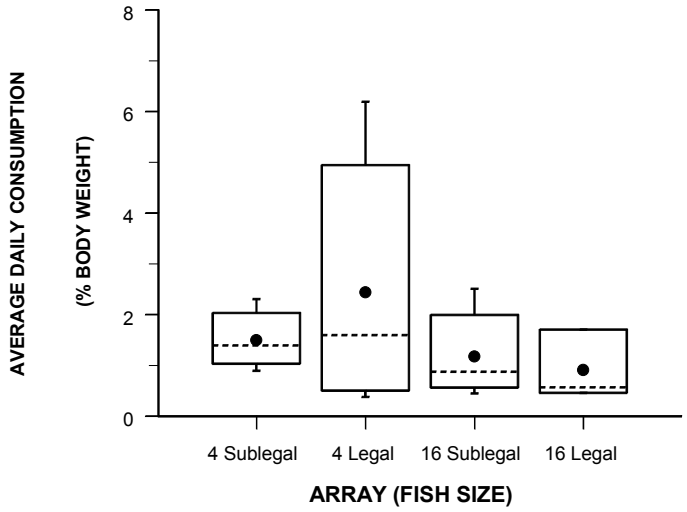

Figure 2.5. Average daily food consumption as % body weight for sublegal- and legal-sized gag from 4-cube and 16-cube arrays in the SRRS. Data are summarized in box plots as a range (vertical bars), 25% and 75% quartiles (box), median (dashed line), and mean (solid circle). N=4 arrays for all except 16-cube legal-sized fish where N=3.

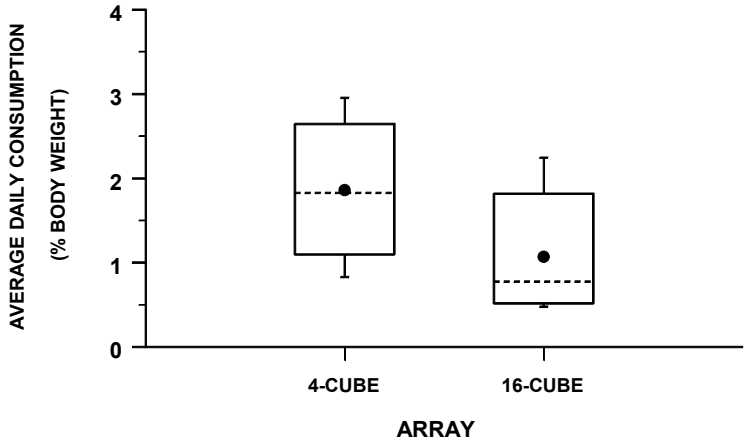

Figure 2.6. Average daily food consumption as % body weight for gag from 4-cube and 16-cube arrays in the SRRS. Data are summarized in box plots as a range (vertical bars), 25% and 75% quartiles (box), median (dashed line), and mean (solid circle).

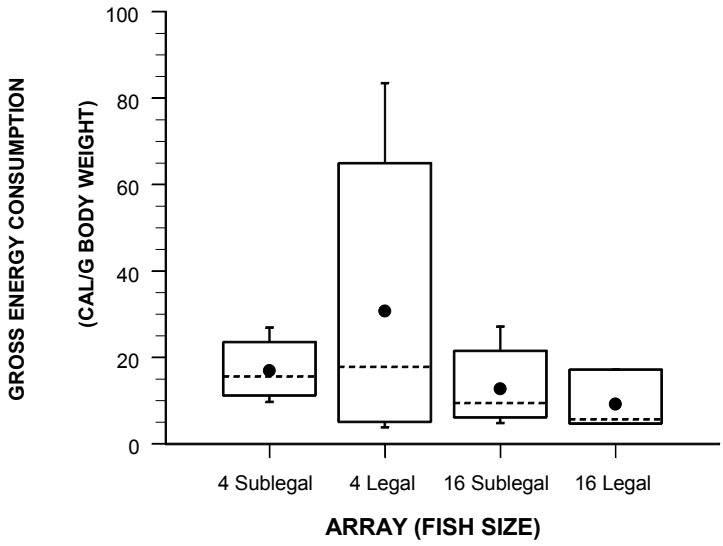

Figure 2.7. Average daily gross energy consumption as calories per gram body weight for sublegal- and legal-sized gag from 4-cube and 16-cube arrays in the SRRS. Data are summarized in box plots as a range (vertical bars), 25% and 75% quartiles (box), median (dashed line), and mean (solid circle). N=4 arrays for all except 16-cube legal-sized fish where N=3.

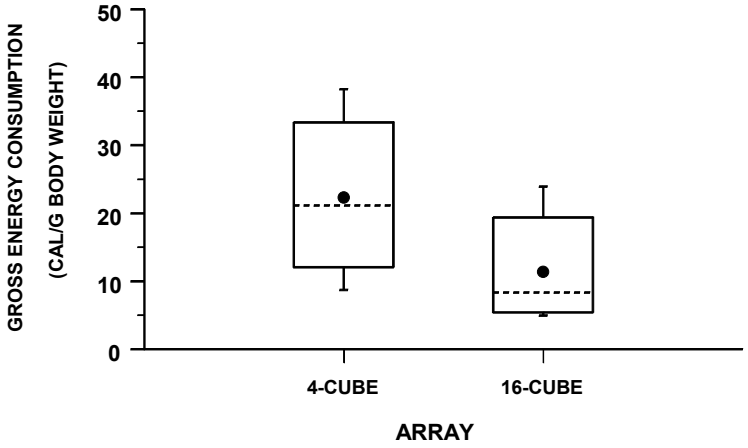

Figure 2.8. Average daily gross energy consumption as calories per gram body weight for gag from 4-cube and 16-cube arrays in the SRRS. Data are summarized in box plots as a range (vertical bars), 25% and 75% quartiles (box), median (dashed line), and mean (solid circle).

Table 2.1. Frequency of occurrence, numerical abundance, mass, and gross energy of prey recovered from stomachs of gag grouper sampled from 4-cube reef arrays of the SRRS, Florida.

Species (Common Name)	Occurrence (%)		Numerical Abundance (%)		Mass (grams) (%)		Gross Energy (kcal/g) (%)	
FISHES								
Centropristis striata (Gulf Black Sea Bass)	1	(1.0)	1	(0.2)	45.7	(2.8)	47.3	(2.5)
Decapterus punctatus (Round Scad)	25	(25.3)	106	(17.8)	165.1	(10.3)	188.8	(9.9)
Diplectrum formosum (Sandperch)	2	(2.0)	3	(0.5)	1.5	(0.1)	1.5	(0.1)
Haemulon aurolineatum (Tomtate)	37	(37.4)	141	(23.6)	359.1	(22.4)	370.6	(19.5)
Halichoeres bivittatus (Slippery Dick)	1	(1.0)	1	(0.2)	36.4	(2.3)	50.1	(2.6)
Harengula jaguana (Scaled Sardine)	28	(28.3)	100	(16.8)	166.0	(10.4)	198.6	(10.4)
Lagodon rhomboides (Pinfish)	5	(5.1)	5	(0.8)	16.1	(1.0)	26.3	(1.4)
Orthopristis chrysoptera (Pigfish)	2	(2.0)	2	(0.3)	144.6	(9.0)	295.8	(15.6)
Sardinella aurita (Spanish Sardine)	9	(9.1)	61	(10.2)	246.3	(15.4)	273.7	(14.4)
Serranus subligarius (Belted Sandfish)	1	(1.0)	1	(0.2)	12.5	(0.8)	14.4	(0.8)
Baitfish (Mixed spp.)	27	(27.3)	63	(10.6)	69.8	(4.4)	77.8	(4.1)
Fish (Unknown spp.)	1	(1.0)	1	(0.2)	107.0	(6.7)	123.6	(6.5)
Larval fish (Unknown spp.)	1	(1.0)	21	(3.5)	0.1	(0.0)	0.1	(0.0)
Subtotal	87	(87.9)	506	(84.8)	1370.3	(85.5)	1668.7	(87.7)
CRUSTACEANS								
Portunus floridanus	21	(21.2)	80	(13.4)	136.2	(8.5)	136.2	(7.2)
Crab sp.	1	(1.0)	1	(0.2)	6.5	(0.4)	6.5	(0.3)
Shrimp sp.	5	(5.1)	7	(1.2)	2.3	(0.1)	2.3	(0.1)
Subtotal	25	(25.3)	88	(14.7)	145.0	(9.0)	145.0	(7.6)
MISCELLANEOUS								
Loligo pleii (Pleii's Striped Squid)	2	(2.0)	2	(0.3)	80.1	(5.0)	80.1	(4.2)
Polychaete worm (Unknown species)	1	(1.0)	1	(0.2)	8.1	(0.5)	8.1	(0.4)
Subtotal	3	(3.0)	3	(0.5)	88.2	(5.5)	88.2	(4.6)
TOTALS	99	(100.0)	597	(100.0)	1603.5	(100.0)	1902.0	(100.0)

Table 2.2. Frequency of occurrence, numerical abundance, mass, and gross energy of prey recovered from stomachs of gag grouper sampled from 16-cube reef arrays of the SRRS, Florida.

Species (Common Name)	Occurrence (%)		Numerical Abundance (%)		Mass (grams) (%)		Gross Energy (kcal/g) (%)	
FISHES								
Diplectrum formusum (Sand Perch)	1	(2.0)	1	(0.5)	15.0	(2.9)	14.7	(2.6)
Decapterus punctatus (Round Scad)	10	(20.0)	32	(15.6)	26.8	(5.1)	30.7	(5.5)
Haemulon aurolineatum (Tomtate)	21	(42.0)	38	(18.5)	86.4	(16.5)	89.2	(16.0)
Harengula jaguana (Scaled Sardine)	6	(12.0)	17	(8.3)	27.5	(5.2)	32.9	(5.9)
Lagodon rhomboides (Pinfish)	1	(2.0)	1	(0.5)	2.0	(0.4)	3.4	(0.6)
Sardinella aurita (Spanish Sardine)	8	(16.0)	48	(23.4)	123.0	(23.5)	136.6	(24.6)
Baitfish (Mixed spp.)	16	(32.0)	35	(17.1)	47.3	(9.0)	52.7	(9.5)
Subtotal	47	(94.0)	172	(83.9)	328.0	(62.6)	360.1	(64.7)
CRUSTACEANS								
Portunus floridanus	13	(26.0)	28	(13.7)	133.5	(25.5)	133.5	(24.0)
Subtotal	13	(26.0)	28	(13.7)	133.5	(25.5)	133.5	(24.0)
MOLLUSKS								
Loligo pleii (Pleii's Striped Squid)	5	(10.0)	5	(2.4)	62.5	(11.9)	62.5	(11.2)
Subtotal	5	(10.0)	5	(2.4)	62.5	(11.9)	62.5	(11.2)
TOTALS	50	(100.0)	205	(100.0)	524.0	(100.0)	556.1	(100.0)

Table 2.3. Regressions for predicting length and/or mass of common prey species consumed by gag collected from arrays of the SRRS. All regressions were significant at $P<0.0001$. CL = Carapace Length; CW = Carapace Width; MTL = Maximum Total Length; OL = Otolith Length; TWT = Total Weight; and VCL = Vertebral Column Length.

Prey Species	n	Regression	r^2	Size Range (cm)
Baitfish[a]	105	$MTL = 1.5039VCL + 3.7057$	0.99	3-21
	108	$TWT = 0.000034083VCL^{2.9856}$	0.98	
	106	$TWT = 0.0000084348MTL^{3.0038}$	0.99	
Centropristis striata	85	$MTL = 1.7107VCL - 7.0284$	0.99	9-37
(Gulf Black Sea Bass)	84	$MTL = 32.2478OL - 61.8509$	0.94	
	89	$TWT = 0.00008809VCL^{2.9390}$	0.99	
	99	$TWT = 0.00003380MTL^{2.8401}$	0.99	
Decapterus punctatus	48	$MTL = 1.4880VCL + 4.7370$	0.98	4-20
(Round Scad)	44	$MTL = 48.4267OL - 34.8600$	0.91	
	50	$TWT = 0.00003406VCL^{2.9831}$	0.99	
	49	$TWT = 0.00001173MTL^{2.9389}$	0.99	
Diplectrum formosum	72	$MTL = 1.7077VCL + 0.6764$	0.96	7-24
(Sandperch)	35	$MTL = 29.9919OL - 19.0629$	0.97	
	73	$TWT = 0.00004927VCL^{3.0152}$	0.98	
	94	$TWT = 0.00001185MTL^{2.9734}$	0.99	
Haemulon aurolineatum	69	$MTL = 1.5028VCL + 6.1524$	0.98	4-23
(Tomtate)	68	$MTL = 24.6074OL - 22.4461$	0.98	
	72	$TWT = 0.00009389VCL^{2.8682}$	0.98	
	85	$TWT = 0.000009535MTL^{3.0679}$	0.99	
Harengula pensacolae	30	$MTL = 1.6613VCL - 2.7537$	0.98	3-12
(Scaled Sardine)	17	$MTL = 39.3502OL + 15.8728$	0.95	
	31	$TWT = 0.000003877VCL^{3.5918}$	0.99	
	30	$TWT = 0.000001608MTL^{3.4073}$	0.99	
Lagodon rhomboides	4	$MTL = 1.6698VCL - 0.7970$	0.99	15-21
(Pinfish)	4	$MTL = 43.5380OL - 89.7249$	0.94	
	4	$TWT = 0.0003059VCL^{2.6720}$	0.89	
	4	$TWT = 0.00008467MTL^{2.6578}$	0.89	

Table 2.3 (Cont'd). Regressions for predicting length and/or mass of common prey species consumed by gag collected from arrays of the SRRS. All regressions were significant at $P<0.0001$.

Prey Species	n	Regression	r^2	Size Range (cm)
Portunus floridanus	15	$CW = 10.9032CL + 1.1233CL$	0.76	1-5
(Portunid Crab)	15	$TWT = 0.0001593CL^{2.9066}$	0.95	
Sardinella aurita	22	$MTL = 1.41229VCL + 15.1439$	0.98	6-21
(Spanish Sardine)	21	$MTL = 54.6007OL + 9.4307$	0.74	
	22	$TWT = 0.0001901VCL^{2.6045}$	0.97	
	22	$TWT = 0.00001564MTL^{2.8677}$	0.98	

a Average of round scad, Spanish sardine, and scaled sardine. Regression of MTL versus OL could not be pooled for these baitfish because of significant differences.

Table 2.4. Size of prey consumed by gag collected on arrays of the SRRS.

Prey Species	n	Maximum Total Length (mm) Mean	±SE	Weight (g) Mean	±SE
FISHES					
Centropristis striata (Gulf Black Sea Bass)	1	143.5		45.69	
Decapterus punctatus (Round Scad)	138	46.1	1.7	1.39	0.26
Diplectrum formosum (Sandperch)	4	56.3	19.7	4.13	3.62
Haemulon aurolineatum (Tomtate)	179	55.0	1.1	2.49	0.13
Halichoeres bivittatus (Slippery Dick)	1	141.0		36.35	
Harengula jaguana (Scaled Sardine)	117	47.1	1.7	1.65	0.44
Lagodon rhomboides (Pinfish)	6	48.5	5.9	3.02	0.96
Orthopristis chrysoptera (Pigfish)	2	193.0		72.31	38.08
Sardinella aurita (Spanish Sardine)	109	73.5	1.1	3.39	0.16
Serranus subligarius (Belted Sandfish)	1	85.0		12.5	
Baitfish (Mixed species)	98	48.4	1.2	1.19	0.10
Fish (Unknown species)	1	203		107	
Larval fish (Unknown species)[a]	21	11		0.0059	
CRUSTACEANS					
Portunus floridanus (Portunid Crab)	108	22.2	1.1	2.50	0.30
Crab sp.	1	25.0		6.52	
Shrimp sp.	7	14.5	2.4	0.32	0.10
MOLLUSKS					
Loligo pleii (Pleii's Striped Squid)	7	105.0	36.1	20.38	9.61
MISCELLANEOUS					
Polychaete worm (Unknown species)	1	210		8.15	

[a] All larval fish were between 10-12 mm in length; weight was determined by weighing all larval fish together and dividing by 21 individuals.

Table 2.5. Caloric density of potential prey species consumed by gag collected on arrays of the SRRS. All prey species were collected in the general vicinity or inshore of the SRRS.

Prey Species	Caloric Density (kcal/g wet weight)			Size Range (cm)
	Mean	±SE	n	
FISHES				
Caranx crysos (Blue Runner)	1.1661	0.0254	9	15-19
Centropristis striata (Gulf Black Sea Bass)	1.0358	0.0722	3	18-24
Chloroscombrus chrysurus (Atlantic Bumper)	1.307		1	19
Decapterus punctatus (Round Scad)	1.1434	0.0198	20	9-18
Diplectrum formosum (Sandperch)	0.9799	0.0222	17	7-21
Diplodus holbrooki (Spottail Pinfish)	1.3910	0.1012	8	15-20
Haemulon aurolineatum (Tomtate)	1.0321	0.0199	19	7-16
Halichoeres bivittatus (Slippery Dick)	1.3773	0.0254	10	12-17
Harengula jaguana (Scaled Sardine)	1.0321	0.0405	11	8-12
Lagodon rhomboides (Pinfish)	1.6403	0.0120	2	16-17
Opisthonema oglinum (Atlantic Thread Herring)	0.6513		1	19
Orthopristis chrysoptera (Pigfish)	2.0451	0.2877	2	18-20
Sardinella aurita (Spanish Sardine)	1.1111	0.0264	8	16-20
Serranus subligarius (Belted Sandfish)	1.1553[a]			
Synodus foetens (Inshore Lizardfish)	1.0248	0.0383	5	14-20
Synodus intermedius (Sand Diver)	1.0714	0.0314	9	13-32
Baitfish (Mixed species)	1.1140[b]	0.0148	57	
Fish (Unknown species)	1.1553[a]	0.0220	124	
Larval fish (Unknown species)	1.1140[b]			
CRUSTACEANS				
Portunus floridanus (Portunid Crab)	1.0000[c]			
Crab sp.	1.0000[c]			
Shrimp sp.	1.0000[c]			
MOLLUSKS				
Loligo pleii (Pleii's Striped Squid)	1.0000[c]			
MISCELLANEOUS				
Polychaete worm (Unknown species)	1.0000[c]			

[a] Average caloric density of all fish species except baitfish.
[b] Caloric density of a mixture of round scad, Spanish sardine, scaled sardine, and tomtate.
[c] Estimated caloric density. Requires completion of direct estimation from bomb calorimetry.

Objective 3. To confirm prior experimental results showing differences in gag growth and condition between patch reefs of contrasting size, and to estimate those differences concurrent with Objectives 1 and 2 in order to perform Objective 4.

Methods

In reference to the Suwannee Regional reef System (SRRS), we use the terms "reef", "reef array", and "array" interchangeably, and the terms "patch reef" and "patch" interchangeably. Two factors, patch size and fishing pressure (i.e. published location), were combined to create four treatments for this survey; 4-cube published (4-P), 4-cube unpublished (4-U), 16-cube published (16-P), and 16-cube unpublished (16-U).

Gag exhibit strong site fidelity and have an established home range. Prior research has shown that the home range of gag may include as many as three patch reefs on the 225 m patches used in this study. For this reason we chose to use the array as our analytical unit.

Gag Abundance

Data collection: Monthly visual fish counts were conducted on the Suwannee Regional Reef sites beginning in January 2001 thru September 2001. Fish counts documented gag abundance and size distribution for each of the 8 reefs involved in the study (Reef #'s 0, 4, 7, 11, 13, 16, 20, 21). A diver on SCUBA conducted counts and estimated total length based on 10 cm intervals aided by a meter stick t-bar. One diver was used per count to eliminate disturbance due to the presence of two or more divers. Census times were standardized to 10 minutes for gag counts.

Data analysis: For the purpose of this report the August 2001 census was used for comparative analyses. This was done for two reasons: (1) The condition data were collected and considered as a summer "block" and (2) It was consistent with summer counts conducted in previous years. Additionally, within an array, only patches from which fish were collected for condition data were used (i.e. 3 patches per array, or half of an array). This was done because some patch reefs on two of the arrays, 16 and 20, were being manipulated for a shelter experiment and, therefore, some of the manipulated patches were considered inappropriate for this project. The data were analyzed by number of gag <50 cm in length, number of gag >50 cm in length, and total number of gag per array. The 50 cm distinction is the break in our size classes where gag become legal to harvest. Analysis of variance was performed on the data to determine the effects of patch size. Reef array 20 had substantially fewer gag than the other 16-cube arrays and was considered an outlier and removed from abundance analysis (considered biased by intense fishing pressure). Analysis of variance was also performed on the percent legal gag to determine the effects of patch size and publication of reef locations.

Growth and Condition

Data collection: Diver deployed fish traps were used to live capture gag off of the reefs. Fish were corralled into the traps and sent to the surface for measurement and lavaging. Fish were speared on patches where trapping was unsuccessful due to low densities of gag. Soon after

capture, fish were measured for maximum total length, fork length, and girth and then released or sacrificed for stomach content, muscle tissue, and otolith analysis (see objective 2).

Data analysis: Initially, relative weight was to be used as an indicator of fish condition. Relative weight was to be calculated by collecting a field weight via a laptop computer and digital scale and then standardizing the field weight for wave action/sea height. However, this method did not allow for sufficiently precise accounting of the wave-induced variation in measurement. Relative weights were analyzed only for those fish that were sacrificed and weighed in the lab. Analysis of variance was performed on the relative weights using the grand means obtained from each array and looking at the effect due to patch size and publishing with n=4.

Alternatively, girth measurements were taken as an indicator of fish condition. A girth-length regression was run for each treatment (4-U, 4-P, 16-U, 16-P) and for pooled 4-cube and 16-cube reefs; the regressions were then compared to determine if they were significantly different.

Growth was estimated from otolith measurements. Otoliths were taken from all sacrificed gag and processed for ageing. Otoliths were first cross-sectioned using a Buehler variable speed sectioning saw, with sections permanently mounted on glass slides. Ages of gag were estimated based on counting annuli apparent in the otolith sections. Otolith sections were then measured using an Image1 computer analysis system to determine the growth increment, which was the difference between the radius of the otolith and the distance to the ultimate annulus. The data were analyzed by size class and by age using an ANOVA, with the two patch sizes (4 and 16 cube) and two fishing pressures (published and unpublished).

Muscle energy was determined from sacrificed gag as described in Objective 2. As a proxy to physiological condition of the gag, somatic muscle samples were collected from all sacrificed gag. Based on previous sampling protocol, a 25-50 g sample of white muscle was collected from the anterior portion of the epaxial musculature. This sample was ground for homogenization, freeze dried to determine % moisture, and re-ground to suitable particle size for analysis. A 1-g freeze-dried sample of muscle tissue was then combusted in a Parr adiabatic bomb calorimeter to determine total energy content. The data were analyzed by size class and by age ???using an ANOVA, with the two patch sizes (4 and 16 cube) and two fishing pressures (published and unpublished) as treatments.

Results

Gag Abundance

Mean overall abundance of gag was 2.32 times greater on 16-cube reefs than on 4-cube reefs (Fig. 3.1a); this difference was significant ($p=0.0051$). Mean abundance of gag >50 cm was 3.21 times greater on 16-cube reefs (Fig. 3.1b); this too was significant ($p=0.0169$). Mean abundance of gag <50 cm was 1.97 times greater on 16-cube reefs (Fig. 3.1c); this was not statistically significant ($p=0.0567$). There was no significant differences in the overall abundance or in the abundance of gag <50 cm TL or >50 cm between published and unpublished reefs.

The mean percentage of legal gag present was higher on 16-cube reefs (Fig 3.2a). Published reefs had a lower mean percentage of legal gag present than unpublished reefs (Fig. 3.2b). However, these differences were not significant (p=0.8014 and p=0.6218, respectively).

Relative Weight

The mean relative weights for gag from 4-cube and 16-cube reefs were not statistically different (p=0.1931, Fig. 3.3a) in a test with low statistical power (1-ß = 0.0736). The values from 4-cube reefs tended to be higher than from16-cube reefs. Similarly, relative weights for gag from published reefs tended to be higher than from unpublished reefs (Fig. 3.3b), and these differences were not significant (p=0.2470). There was no significant difference in relative weight of gag <50 cm from 4-cube and 16-cube reefs (p=0.7563). However, gag >50 cm, which were not numerous (see Fig. 3.4), had significantly higher relative weights on 4-cube reefs (p=0.0050).

Girth

Girth-length regression analysis performed on the gag collected from the four treatments (4-U, 4-P, 16-U, 16-P) show no significant differences between 4-cube published and unpublished reefs or between 16-cube published and unpublished reefs.

Overall girth-length regression analysis comparing 4-cube and 16-cube gag showed no significant difference between the slopes (p=0.075), although the 4-cube gag tended to have a steeper slope, with the intersection of the two lines at about 410 mm (Fig. 3.4). There was no significant difference in girth per length in gag <50 cm from 4-cube and 16-cube reefs (p=0.3309). Gag >50 cm had significantly greater girth per length on 4-cube reefs (p=0.0181).

Marginal Growth

At this time the otolith data allowed analysis of only the 30-39 cm size class; there were no significant differences in marginal growth based upon patch size or fishing pressure (p=0.4193 and p=0.1155, respectively). The available data was also analyzed by age and size class for age 1, 30-39cm, age 2, 30-39cm, and age 2, 40-49cm; none of which had significant differences in marginal growth based upon patch size or fishing pressure (ranged from p=0.1595 to p=0.9080).

Muscle Energy

At this time only a small portion of the tissue samples have been processed. The available data allowed analysis only of size classes 30-39cm, 40-49cm, and 50-59cm; none of which had significant differences in muscle energy based upon patch size or fishing pressure. The data sorted by age and size class allowed analysis of age 2, 30-39cm and age 3, 30-39cm; neither of which had significant differences in muscle energy based upon patch size or fishing pressure. These results may be modified as more data become available.

Summary

Results from 1997 on the Suwannee Regional Reef System showed significantly higher abundances of gag on 16-cube reefs than on 4-cube reefs as well as higher abundances of gag on

published than on unpublished reefs. It was also observed that reefs exposed to fishing pressure had a significantly lower percentage of legal-sized gag present than those not exposed.

While our data from 2001 support the differences between 16-cube and 4-cube reefs, we found no differences in gag abundances between published and unpublished reefs. This was true of the total abundances as well as the abundances of legal-sized gag. Additionally, abundances of gag on unpublished reefs were drastically reduced from 1997 and were close to the abundances of published reefs. We now believe that the majority of Suwannee Regional Reefs are subject to fishing pressure, and that there is a high likelihood that 16-cube reefs have been compromised to a greater degree than 4-cube reefs. Total numbers of gag were significantly higher on 16-cube patches than on 4-cube patches; this was consistent with results from 1997. An overall reduction from 850 to 685 gag was observed from 1997-2001, this translates to a 20% reduction in gag abundance (Fig 3.5a). The bulk of this reduction was from 16-cube unpublished reefs (Fig. 3.5b). The overall abundance of gag on all 16-cube patches in 2001 was 38% lower than in 1997; concurrently the overall abundance of gag on all 4-cube patches increased by 29% (Fig. 3.5c). The total numbers of gag and the magnitude of differences between reef treatments were both lower in 2001 than in 1997.

The working hypothesis on the Suwannee Regional Reef System is that juvenile-to-adult gag select reef habitat primarily on the basis of shelter and secondarily on the basis of food, and that shelter limits local densities, which in turn regulates growth and condition. Previously, results showed that 4-cube reefs had a higher average growth rate and produced fish with higher relative weights than 16-cube reefs.

The condition factors of relative weight and girth both tended toward gag from 4-cube reefs being in better condition, however, these were significant only for gag greater than 50 cm. The recent results are consistent with, but much less striking than, results from 1997. In 1997 there was a marginal interaction effect between fishing pressure and patch reef size on relative weight, this was not apparent in 2001. Mean relative weights in 2001 were lower than in 1997 for all reef types (Fig. 3.6a). Mean relative weight on 4-cube reefs decreased from 111.75 in 1997 to 97.53 in 2001, a 13% reduction. Mean relative weight on 16-cube reefs decreased from 108.10 in 1997 to 95.65 in 2001, a 12% reduction (Fig.3.6b). The statistical power associated with analysis of relative weights in 1997, though not high, was four and a half times greater than what it was in 2001 ($1-\beta = 0.3360$ vs. $1-\beta = 0.0736$).

The processing of samples for muscle energy was added during this project, and not originally proposed. Differences in muscle energy and marginal growth may be evident when age-specific analyses of these data are done.

Fig 3.1a. Mean total abundance of gag.

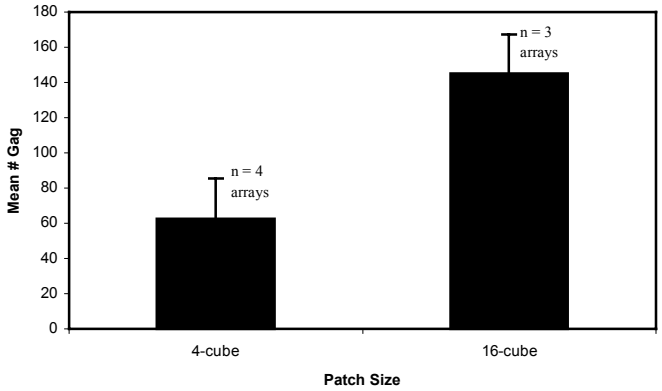

Fig 3.1b. Mean abundance of gag >50cm.

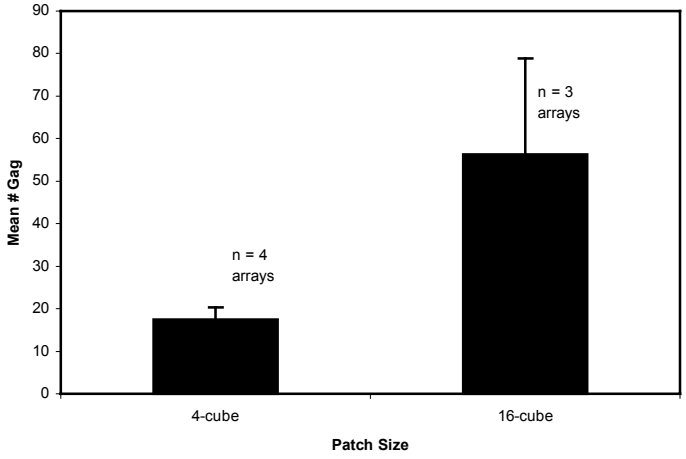

Fig 3.1c. Mean abundance of gag <50cm.

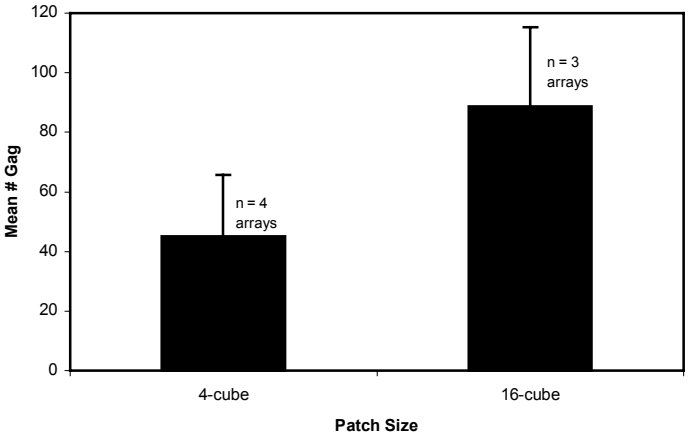

Fig 3.1*x*. Mean abundance of Gag on 4 and 16 cube reefs. These values represent half of an array.

Fig 3.2a. Percent legal gag: 4-cube vs. 16-cube.

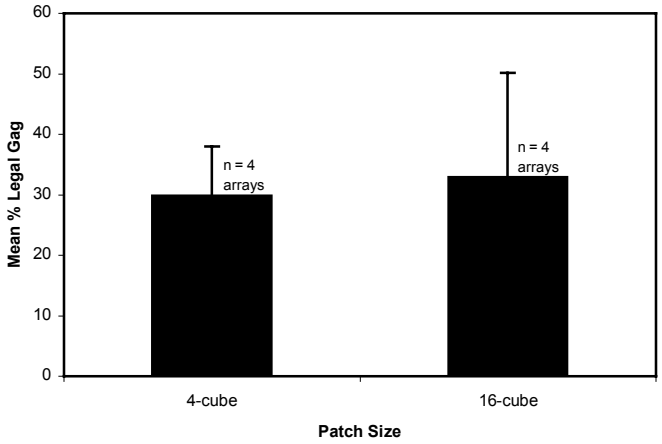

Fig 3.2b. Percent legal gag: published vs. unpublished.

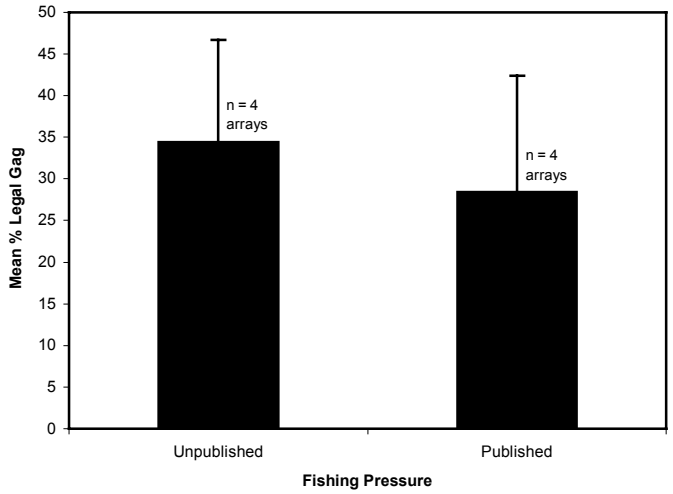

Fig 3.2*x*. Mean percentage of total abundance of gag that are of legally harvestable size. 3.2a shows differences in reef patch size, 3.2b shows differences in reef fishing pressure.

Fig 3.3a. Mean relative weight of gag: 4-cube vs. 16-cube.

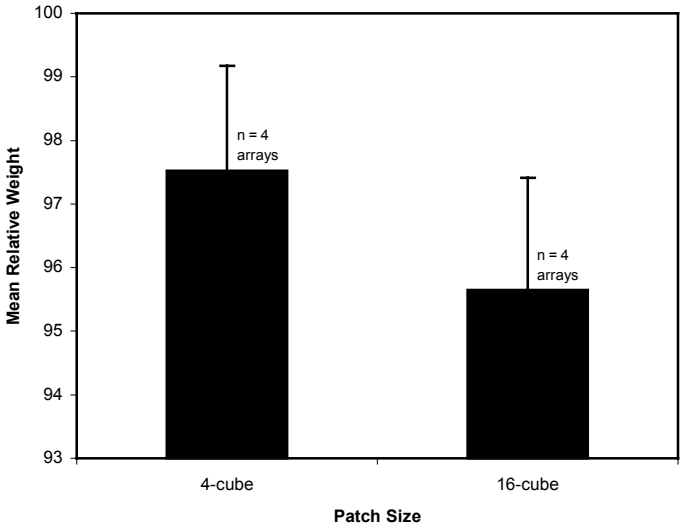

Fig 3.3b. Mean weight of gag: published vs. unpublished.

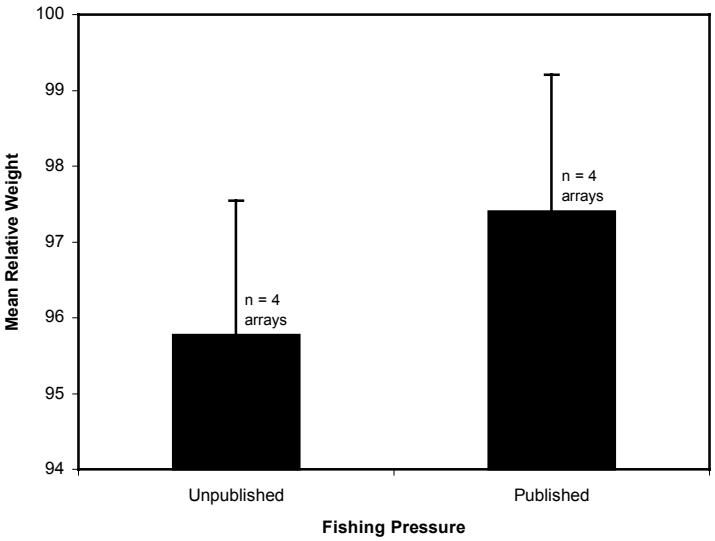

Fig 3.3*x*. Mean relative weight of gag. 3.3a shows differences in reef patch size, 3.2b shows differences in reef fishing pressure.

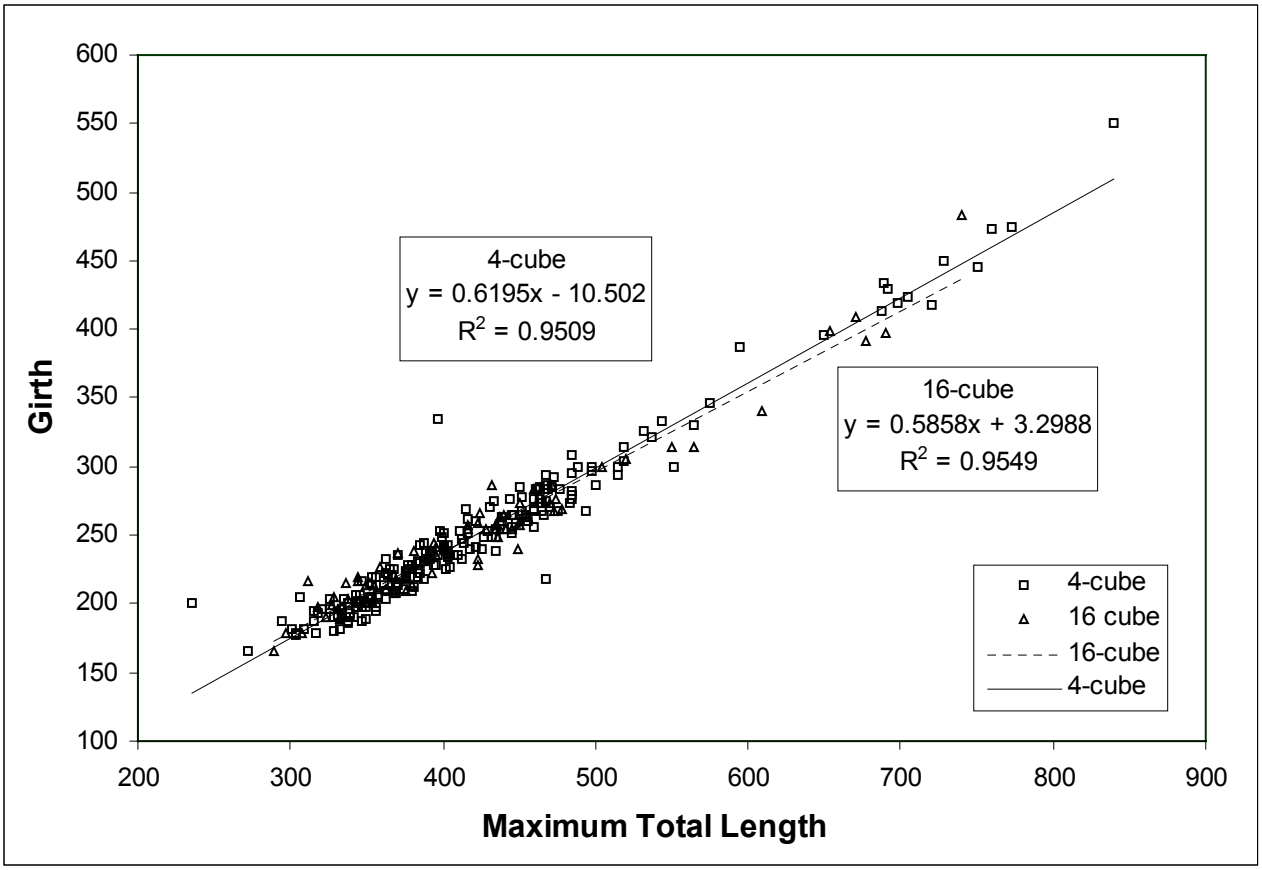

Fig 3.4. Girth-length regression for 4-cube and 16-cube reefs.

Fig 3.5a.

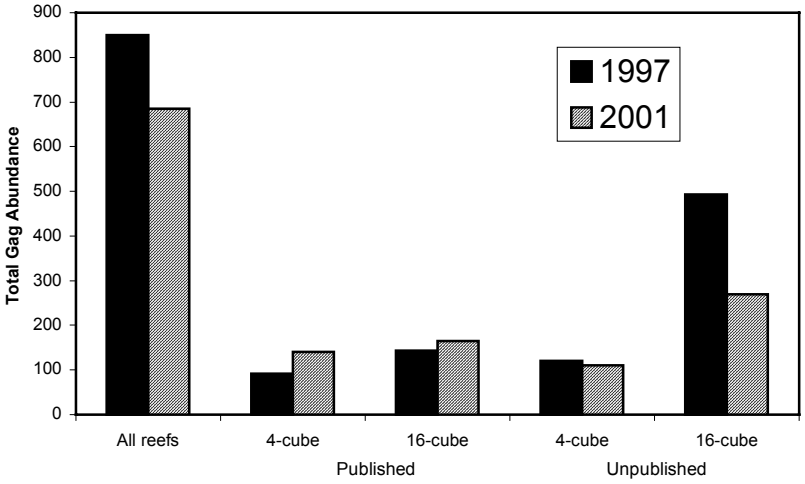

Fig 3.5b. Percentage of gag present in 2001 from 1997.

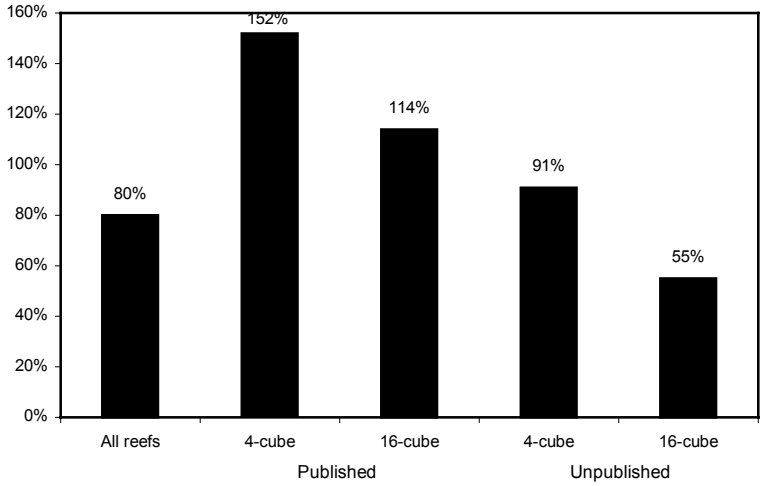

Fig 3.5c. Percentage of gag present in 2001 from 1997.

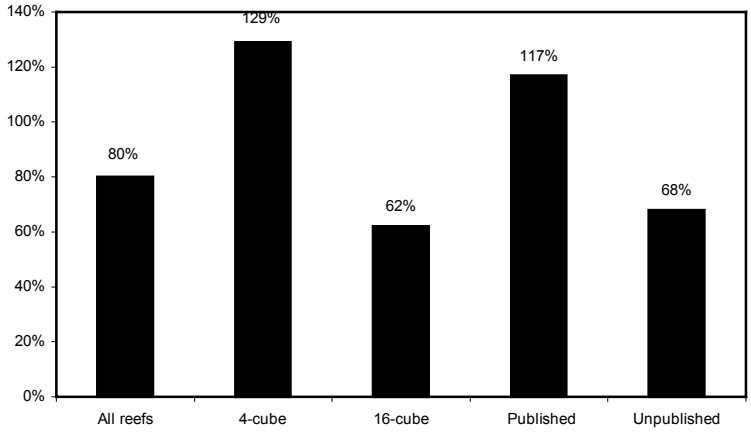

Fig 3.5*x*. Comparisons of gag abundance between 1997 and 2001.

Fig 3.6a.

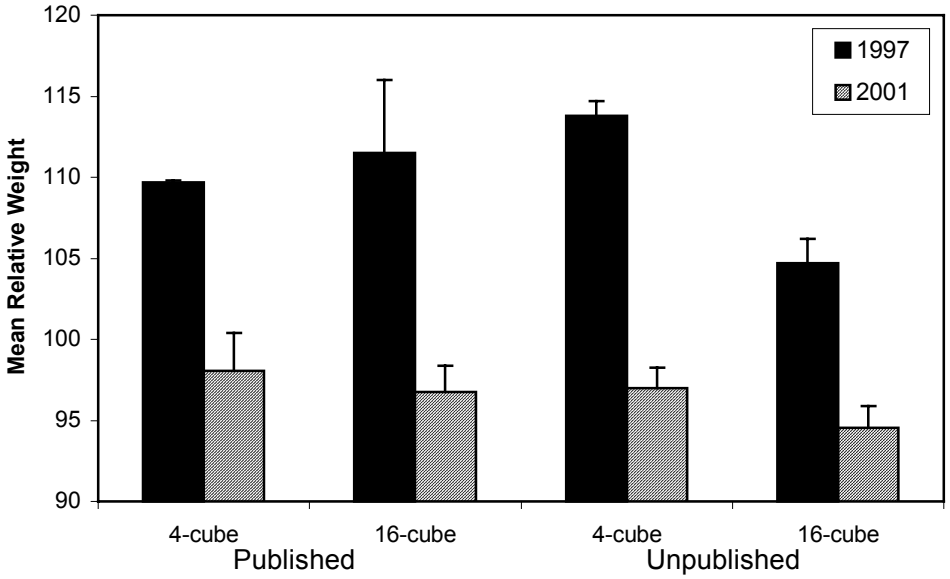

Fig 3.6b.

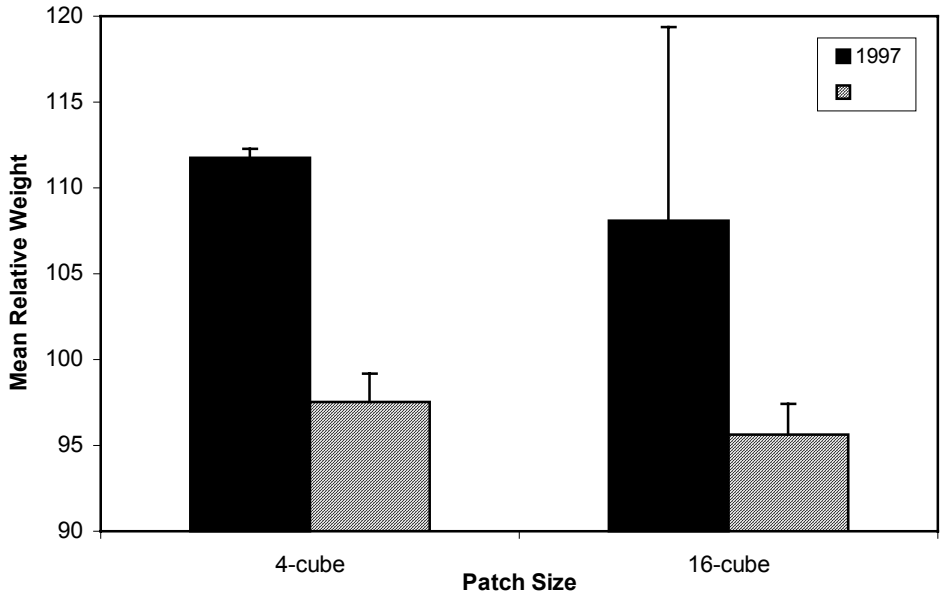

Fig 3.6*x*. Comparisons of gag abundance between 1997 and 2001.

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