DIET AND REPRODUCTION OF THE GOLIATH GROUPER, *EPINEPHELUS ITAJARA* (ACTINOPTERYGII: PERCIFORMES: SERRANIDAE), IN EASTERN BRAZIL

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**Background.** The goliath grouper, *Epinephelus itajara* (Lichtenstein, 1822), is the largest Atlantic grouper. It has been the first marine fish subjected to a fishing moratorium in Brazil (since 2002). The aim of this study was to investigate basic biological aspects, particularly the diet and reproduction of this endangered species. We believe that our results, together with information already available in the literature, may be a foundation for new management and conservation strategies for this grouper in Brazil.

**Materials and methods.** Specimens were obtained from an artisanal fish landings collaborative monitoring program in the cities of Caravelas and Alcobaça, Abrolhos Bank, eastern Brazil. Because of the fishing moratorium, we relied on sporadic incidental captures from fishermen or donation from the law enforcement agencies. Diet and reproduction of the goliath grouper was evaluated based on 34 specimens obtained between May 2005 and September 2010.

**Results.** Body size of 32 females ranged from 27.4 to 150.0 cm total length (TL) (mean 87.4 ± SD 34.8), while size of 2 males were 97.0 and 115.0 cm TL. Of these, 16 specimens (47%) were juveniles. Sex ratio was estimated as 1:16 (males to females). Two developing females were recorded, one obtained in February 2006 and another in January 2007. The length of first maturation (*L*₅₀) for females was estimated at 105.64 cm and the length where 100% of individuals are mature (*L*₁₀₀) at 126.0 cm TL. A total of 34 stomachs were analyzed, six of them were empty. Teleosts and decapods dominated the diet. The major food items of mangrove-associated fish were decapods *Callinectes* sp., while the coral reef-associated fish ingested mainly the boxfish *Acanthostracion* sp. The most important item for juveniles was *Callinectes* sp., while *Acanthostracion* sp. was the most important item for adults.

**Conclusion.** We suggest that the goliath grouper moratorium in Brazil should be maintained for a relatively long time frame—more than four decades.

**Keywords:** fish conservation; Abrolhos Bank; threatened fishes, fisheries management, ontogenetic variations, Epinephelinae

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INTRODUCTION

The goliath grouper, *Epinephelus itajara* (Lichtenstein, 1822), is the largest Atlantic grouper, exceeding 2 meters of total length (TL), and >400 kg of weight (Bullock et al. 1992). It inhabits tropical and subtropical coastal and estuarine areas on both sides of the Atlantic (Heemstra and Randall 1993) from Florida to southern Brazil, including the Gulf of Mexico, as well as along the west coast of Africa (Bullock et al. 1992, Heemstra and Randall 1993). The goliath grouper is a mangrove-dependent reef fish with the post-larval and juvenile phases thought to recruit to estuaries (Frias-Torres 2006, Frias-Torres and Luo 2009). In southern Brazil, however, juveniles (<100 cm) were recorded in open waters (Félix-Hackradt and Hackradt 2008). In the Northern Hemisphere, juveniles generally show high site fidelity, moving little within their home range and rapidly returning to their original habitat after handling (Eklund and Schull 2001, Frias-Torres et al. 2007). Goliath grouper is an opportunistic ambush predator and feeds at a relatively low trophic level (Koenig and Coleman 2009). The diet includes slow-moving fishes, invertebrates (particularly lobsters and crabs), and even sea turtles (Randall 1967, Bullock and Smith 1991).

The goliath grouper is extremely vulnerable to overfishing (principally spearfishing and long-lines), mainly due to critical life history traits such as slow growth, late gonadal maturity, long life span, strong site fidelity, and formation of seasonal spawning aggregations (Bullock et al. 1992, Sadovy and Eklund 1999, Morris et al. 2000, Eklund and Schull 2001). The International Union for Conservation of Nature (IUCN) lists it as a critically endangered species (Craig 2011). In Brazil, it has been fully protected by the federal law since September 2002 (Hostim-Silva et al. 2005) and the main demand to support new management measures would be to obtain information on the population status and species recovery. This moratorium was renewed twice in 2007 and 2012 for another three years*. In the South Atlantic there has almost been no studies on the basic life history traits of this fish. In Brazil, available information is fragmented and insufficient for a comprehensive evaluation of the population status. Available data comes from studies on the local ecological knowledge, from artisanal fishermen, spatio-temporal characteristics of aggregations (Ferreira and Maida 1995, Gerhardinger et al. 2006, 2009, Reuss-Strenzel and Assunção 2008), monitoring of population size (Félix-Hackradt and Hackradt 2008), genetic characterization of populations, and cryptic genetic divergence (Silva-Oliveira et al. 2008, 2013, Craig et al. 2009). No data, however, is available on its growth, maturity, fecundity, and diet.

Combined with commercial and recreational fisheries data, the biological information collected from fisheries can contribute to regular assessments of the status of fish stocks, helping the evaluation of fisheries management strategies (Pilling et al. 2008). Length and age at first maturation, maximum size, and reproductive patterns, for example, are essential for fishery management of larger groupers and snappers (Freitas et al. 2011b, 2014), as well as the information on predator/prey abundance and consumption rates of predators, which may be used to assess the magnitude of trophic fluxes (Heithaus et al. 2010).

Herein we investigate the diet and reproduction, of *Epinephelus itajara* specimens obtained from an artisanal fish landings collaborative monitoring program in the Abrolhos Bank, eastern Brazil. The region features the richest coralline complex in the South Atlantic known for its biodiversity and also for serious threats to its integrity and existence (Moura et al. 2013). The emphasis was placed on the assessment of ontogenetic and habitat shifts in diet, as such information may be relevant for future attempts to model trophic pathways and assess the possible ecosystem implications of *E. itajara* removal (Pauly et al. 1998, Farmer and Wilson 2011). We believe that our results, together with information already available in the literature, may be a foundation for new management and conservation strategies for the goliath grouper in Brazil.

MATERIAL AND METHODS

Study area. This study was carried out in the Abrolhos Bank, Bahia State, eastern Brazil, which encompasses a wide portion (42 000 km²) of the continental shelf, with depth rarely exceeding 30 m and a shelf edge at about 70 m depth (16º40′–19º40′S, 39º10′–37º20′W). The region comprises the largest and richest coral reefs in the South Atlantic, as well as an extensive mosaic of algal bottoms, mangrove forests, beaches, and vegetated sandbanks (Leão and Kikuchi 2005, Moura et al. 2013). Four marine protected areas (MPA) occur in the region, one of them with large mangroves—the Cassurubá Extractive Reserve— which in fact is a reserve only theoretically due to the lack of regulations (Francini-Filho and Moura 2008a, Francini-Filho et al. 2013). About 290 species of fishes are recorded in the region (Dutra et al. 2005, Previero et al. 2013). Most reef structures display a characteristic form of mushroom-shaped pinnacles, which attain 5 to 25 m in height and 20 to 300 m across their tops (Francini-Filho and Moura 2008b). Brazilian reefs are a conservation priority in the Atlantic Ocean due to high endemism levels (about 25% in fish and 30% in scleractinian corals) that are concentrated in only 5% of West Atlantic reefs (Francini-Filho et al. 2013). The rich biodiversity of the Abrolhos Bank is threatened by overfishing, pollution, dredging, sedimentation from coastal deforestation, and large-scale oil-gas exploration projects (Leão and Kikuchi 2005, Moura et al. 2013).

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*In December 2014, the Brazilian government published a new endangered species list according IUCN criteria. Goliath grouper was categorized as critically endangered, because has not shown signs of population recovery.*
Sampling and analytical procedures. Specimens of *Epinephelus itajara* were obtained from an artisanal fish landing monitoring program of commercially important reef fishes (see Freitas et al. 2011b) that focused on fish caught using hook and line, longline, spearfishing, and gillnets in the main fish markets in the cities of Caravelas and Alcobaça (Fig. 1). All the mangrove individuals were collected in the CER. Sampling was performed in collaboration with local fishermen opportunistically between June 2005 and September 2010. Because of the moratorium on fishing of the species, biological studies rely on donation by fishermen after sporadic incidental captures and/or donation by the law enforcement agencies after confiscations, (permits # 038/2004 DIFAP-IBAMA, 042/2006 DIFAP-IBAMA and SISBIO 15080-5).

In the laboratory, the total length (TL) of individuals was measured to the nearest 1 mm and the total weight (TW) was determined to the nearest 10 g. Stomach and gonads were removed and immediately fixed in 10% formalin solution for 24 h and subsequently transferred and stored in 70% ethanol. Stomach contents were examined in laboratory using a stereomicroscope. Prey individuals were identified to the highest possible taxonomic level according to specialized literature (Matsuura 2001) and consultation with experts. Parameters such as percentage Frequency of occurrence (F) (i.e., percentage of stomachs where the food item was present), gravimetric abundance a food item (W_A) (i.e., percentage of the weight of a food item in relation to the weight of total food), as well as the numerical abundance of a food item (N) (i.e., percentage of the number of food item i in relation to the total number of all food items) were determined (Hyslop 1980, Bowen 1996). These variables were used to calculate the Index of Relative Importance (IRI) (see Pinkas et al. 1971), according to the equation:

$$RI = (N + W_A) \times F$$

The IRI establishes the order of importance of food items in the diet. IRI values were standardized to percentages following Cortés (1999).

Variations on diet according to the factors habitat (coral reefs or mangroves) and ontogeny (juveniles: <100 cm TL, or adults: >100 cm TL) were examined using multivariate analyses considering all individuals with no empty stomachs (n = 28). Size classes were established according to our results on *L_50* for *Epinephelus itajara* in Abrolhos Bank (see results). A two-way permutational multivariate analysis of variance (PERMANOVA) examined the effects of the factors habitat (fixed, two levels) and ontogeny (fixed, two levels) on the standardized and transformed (Log x + 1) weight contribution of items. The Bray–Curtis dissimilarity was used in all tests, using unrestricted permutation of raw data with 999 random permutations. Similarity of percent contribution (SIMPER) analysis was employed to examine the prey categories most responsible for the between-factors separation (Clarke and Gorley 2001). Non-metric multidimensional scaling (NMDS) was employed to visually assess dispersion of individuals. Vectors superimposed to the NMDS diagram were created by using the Spearman’s correlation on the proportionate contributions of food items. The final solution represents the set of relations between samples and food items in a two-dimensional, with determination of the stress level of the representation (Clarke and Gorley 2001). All analyses were performed using the PRIMER-E 6.0 software (Plymouth Marine Laboratory, Plymouth, England).

 Gonad weight (GW) was determined to the nearest 0.01 g. Sex and maturity were macroscopically determined from gonad size, consistency, colour, vascularization, presence of lateral sperm sinuses, ovarian cavity, ovarian lamellae, and identifiable oocytes (Colin et al. 2003). After macroscopic classification, gonads were dehydrated with increasing alcohol concentration series, cleared in xylene, and then embedded in histological paraffin. Histological sections of 4–6 μm were stained in Harris haematoxylin and eosin. Five developmental phases were considered following Brown-Peterson et al. (2011): immature (IM), developing (DV), spawning capable (SC), regressing (RG), and regenerating (RT). The IM phase corresponds to a fish that has never spawned, characterized histologically in females by the presence of oogonia and primary growth oocytes through the perinuclear stage, as well as little space among oocytes in the lamellae and ovarian wall generally thin (Brown-Peterson et al. 2011). In DV females, the ovary is beginning to develop, but not ready to spawn. The SC fish are developmentally and physiologically able to spawn; the RG represents the cessation of spawning and the RT phase corresponds to a sexually mature, but reproductively inactive individual.

Sex ratio was estimated using Bayesian method using the total numbers of females (f) and the number of females plus males (n). We used the conjugate beta-binomial model to estimate the proportion of females with uninformative beta (1,1) as a prior distribution and beta (f + 1, n – f + 1) as posterior distribution (Gelman et al. 2004). The minimum size at maturity (*L_m*50) was recorded for females. The TL at which 50% of females were mature (*L_50*) (King 2007) was estimated using Bayesian methods (Kinas and Andrade 2010). The following logistic regression model was used:

$$PMF = 1 \times (1 + 1 \exp (a + \beta \times L))^{-1}$$

where PMF is the proportion of mature females in length.
class L, a is a parameter, and $L_{50} = -a \times \beta^{-1}$. For the $L_{50}$ analysis, developing, spawning capable, actively spawning, regressing, and regenerating individuals were considered as sexually mature (adults). The model was implemented using the Automatic Differentiation Model Builder (ADMB) software (Fournier et al. 2012) and uninformative priors. Samples from the posterior distributions of the parameters were obtained using the Markov Chain Monte Carlo algorithm (Gelman et al. 2004) with a 10 million chain and samples saved once every 10 000 interactions.

RESULTS
General biological parameters and reproduction. A total of 34 specimens (32 females and 2 males) of Epinephelus itajara were analyzed (Fig. 2), and from these 16 (47%) specimens were juveniles. Body size of females (94.1% of sampled animals) ranged from 27.4 to 150 cm TL (mean 87.4 ± SD 34.8), while the size values of males (5.9%) were 97.0 and 115.0 cm TL. The length–weight relation was estimated at TW = 0.0004TL^3.8127 ($r^2 = 0.7004, n = 34$). Sex ratio was 1:16 males to females, with the median of the posterior distribution of the proportion of females equal to 0.924 (90% credibility interval = 0.831–0.976) (Fig. 3). Analyses of gonadal histological sections confirmed the predominance of immature females. Only two developing females were recorded (Fig. 4), one obtained on 2 February 2006 and another on 28 January 2007. The beginning of the reproductive period is indicated by the presence of the early developing sub-phase, in which only the primary growth and cortical alveolar are present. Thus, the spawning season of Epinephelus itajara in the Abrolhos Bank may occur during austral summer (January to March). Nine RT females were registered between April and October. The two males were classed as RT and caught in January and April 2007. We did not record any histological evidence of hermaphrodite individuals. The $L_{50}$ for females was estimated at 105.5 cm (mean of the posterior distribution, 90% credibility interval = 992.57–1116.72, Figs. 5 and 6) and $L_{100}$ at 126.0 cm TL.

Diet. A total of 34 stomachs were analyzed, six of them were empty (17.4%). Among the 12 items consumed by Epinephelus itajara, the most important were fishes (IRI = 46.31%), fish remains (IRI = 28.14%), the boxfish, Acanthostrocion sp., (IRI = 12.32%), and decapods (IRI = 52.77%), with Callinectes sp. corresponding to IRI = 42.59%. Teleosts and decapods dominated the diet based on all dietary metrics. Among identifiable prey items, the blue crab, Callinectes ornatus ($F = 25.0\%; n = 1$), and the blackpoint sculling crab, Cronius ruber ($F = 7.14\%; n = 5$), were the most important prey based on number and frequency of occurrence (Table 1). The major food items of mangrove-associated fish were Callinectes sp. (IRI = 87.97%) and fish remains (IRI = 10.93%), while coral reef-associated fish ingested mainly Acanthostrocion sp. (IRI = 37.92%), fish remains (IRI = 29.10%), and Brachyura (IRI = 5.91%). The analysis performed for different size classes showed that Callinectes sp. (IRI = 66.51%) and fish remains (IRI = 27.40%) were the most important items for juveniles. Most important items for adults were Acanthostrocion sp. (IRI = 70.79%), followed by yellowtail snapper, Ocyurus chrysurus (Bloch, 1791) (IRI = 8.57%) and Haemulon spp. (IRI = 6.58%).

The PERMANOVA analysis revealed differences in diet between juveniles and adults (Pseudo-$F = 2.44$ and $P = 0.009$) and also considering the interaction between the factors size and habitat (Pseudo-$F = 2.03$ and $P = 0.034$) (Table 2). However, the diet between habitat categories was marginally significant (Pseudo-$F = 1.85$ and $P = 0.052$). Pairwise tests found that the diet of juveniles vary significantly between habitats (Table 3). In spite of the dietary

| No. of individuals | Total length [cm] |
|--------------------|------------------|
| 1                  | 20–29.9          |
| 2                  | 30–39.9          |
| 3                  | 40–49.9          |
| 4                  | 50–59.9          |
| 5                  | 60–69.9          |
| 6                  | 70–79.9          |
| 7                  | 80–89.9          |
| 8                  | 90–99.9          |
| 9                  | 100–109.9        |
| 10                 | 110–119.9        |
| 11                 | 120–129.9        |
| 12                 | 130–139.9        |
| 13                 | 140–149.9        |

Fig. 2. Size and sex composition of Epinephelus itajara individuals in individual size groups, sampled between May 2005 and September 2010 in the Abrolhos Bank, eastern Brazil

| Male | Female |
|------|--------|
| n = 2| n = 32 |

Fig. 3. Posterior distribution of the proportion of females of Epinephelus itajara from the Abrolhos Bank, eastern Brazil (south-western Atlantic population) estimated through the beta-binomial model; For comparison, the estimates of Bullock et al. (1992) obtained in the eastern Gulf of Mexico are shown
overlap among individuals of *Epinephelus itajara*, NMDS analysis allowed the visualization of slightly distinct groups considering the factors studied (Fig. 7).

The SIMPER analysis performed considering juveniles and adults showed that fish remains and *Acanthostracion* sp. were the most important items contributing to the between-groups dissimilarities. The percentage contribution of *Callinectes* sp. (76.33%) and fish remains (22.54%) were the most important items for juveniles, while *Acanthostracion* sp. (100%) was the most important items for adults. Considering habitats, SIMPER analysis showed that most of the dissimilarity between groups was explained by the percentage contribution of fish remains, *Callinectes* sp. and *Cronius ruber* in the diet. In mangrove samples, *Callinectes* sp. contributed with 83.36% and fish remains with 16.34%, while in coral reef grouper, *Acanthostracion* sp. represented 51.01% of food, followed by fish remains (42.41%).

**DISCUSSION**

This is the first study providing basic biological information about the largest Epinephelinae species in the Atlantic Ocean. The size at first maturation of the goliath grouper in Abrolhos Bank estimated here (105.5 cm TL) was lower than that estimated by Bullock et al. (1992) in the Gulf of Mexico (males: 110 to 115 cm and females: 120–135 cm TL). This suggests that the population of the Abrolhos is not recovering yet from the great fishing pressure that motivated the establishment of the fishing moratorium in 2002. According to Bullock et al. (1992) and McClanahan (2009) *Epinephelus itajara* is highly vulnerable to overfishing due mainly to its large size and late maturity. The size reduction at first maturity due to overfishing was reported for several other commercially important marine species (Carlson and Baremore 2003, Hutchings 2005, McBride et al. 2013). Alternative explanations for the differences between the present results and that from Bullock et al. (1992) are the relatively low sample size in the presently reported study, as well as difference between years of data collection (1980s vs. 2000s), natural variability in temperature, food availability, and predation rates. Clearly, more studies focusing on the reproduction of goliath grouper from different geographical locations are necessary to adequately address these questions.

In the Northern Hemisphere *Epinephelus itajara* spawns between July and September (Colin 1994, Bullock et al. 1992, Sadovy and Eklund 1999, Koenig and Coleman 2009, Mann et al. 2009). Results from this study (based solely on females) suggest that in the Abrolhos Bank the spawning season of *E. itajara* occurs in austral summer (January to March). Similar conclusions were
obtained to the South Atlantic in previous studies based on local ecological knowledge (Gerhardinger et al. 2006, Reuss-Strenzel and Assunção 2008) and indirect evidences (see Colin et al. 2003) such as colour patterns (Félix-Hackradt and Hackradt 2008). Although Smith (1971) assumed protogynous hermaphroditism for the goliath grouper, no conclusive evidences for this reproductive strategy were obtained here (see similar results in Bullock et al. 1992). Besides the data determined by us, the only information on sex ratios available for E. itajara was provided by Bullock et al. (1992), who estimated the sex ratio as 1.75 : 1 (females to males) in the Gulf of Mexico. For other Epinephelinae species in general the female : male proportion is around 4 : 1 (Claro et al. 1990, Brulé et al. 2003). The presently determined sex ratio is highly skewed towards females, suggesting that large males are overfished in the Abrolhos Bank. A caution is necessary, however, when comparing sex ratio estimates from different studies, because of the possible influence of gear selectivity, differences between sexes in time spent at aggregations sites, and/or migrations patterns (Sadovy and Eklund 1999).

As expected, this study supports the idea that Epinephelus itajara is a carnivorous fish (Bullock and Smith 1991, Koenig and Coleman 2009). Decapod crustaceans and fish were the most important food items observed. Similar results were obtained in previous studies. A broad array of preys is known to be consumed by E. itajara, including: Atlantic spadefish, Chaetodipterus faber (Broussonet, 1782); scrawled cowfish, Acanthostracion quadricornis (Linnaeus, 1758); striped burrfish, Chilomycterus schoepfi (Walbaum, 1792); gastropods; or decapod crustaceans (e.g., lobsters, Panulirus argus and Scyllarides sp., as well as the crabs Calappa flamma and Callinectes sp.) (see Bullock and Smith 1991, Koenig and Coleman 2009). In Brazil, Gerhardinger et al. (2006) also confirmed this pattern through surveys of fishermen ecological knowledge, which indicate that most commonly food items of the goliath grouper are lobsters, spadefish Chaetodipterus faber, octopuses, and catfish (Family Ariidae). Altogether, information on the diet of E. itajara available

Fig. 5. Estimated logistic regression of proportion of mature females of Epinephelus itajara (maximum of the posterior distribution) in relation to total length, from the Abrolhos Bank, eastern Brazil (May 2005 to September 2007); Grey lines indicate length at which 50% (\(L_{50}\)) of individuals were mature (mean of the \(L_{50}\) posterior distribution), and dashed lines indicate the 90% of credibility interval

Fig. 6. Posterior distribution of the length when 50% of the females of Epinephelus itajara from the Abrolhos Bank, eastern Brazil (south-western Atlantic population) are mature (\(\alpha = 18.43, \beta = -0.017\) and \(L_{50} = 105.5\)); For comparison, the range of possible values for \(L_{50}\) from Bullock et al. (1992) are also shown

Fig. 7. Ordination plot based in the non-metric multidimensional scaling analysis (nMDS) for diets of adults (A) and juveniles (J) of Epinephelus itajara from the Abrolhos Bank, eastern Brazil using the index of relative importance (IRI) of food categories [Log(\(x + 1\)]. Symbols represent the factor habitat: mangrove (▼) and coral reefs (●); Vectors superimposed on the nMDS diagram were created by using the Spearman’s correlation on the proportionate contributions of the food categories that most influenced the ordination
to date indicates predominance of large sized and slow moving preys (Bullock and Smith 1991). A relevant number of cowfish Acanthostracion sp. were recorded here, all from coral reef habitats. Because only dermal plates were detected, it is suggested that these structures take longer periods to be digested and are thus more frequent inside E. itajara stomachs.

Ontogenetic shifts in diet of epinephelins and lutjanids may occur when juvenile and adult fish occupy different habitats. For example, this pattern was recorded for dusky grouper, Epinephelus marginatus (Lowe, 1834) (see Machado et al. 2008); mutton snapper, Lutjanus analis (Cuvier, 1828) (see Freitas et al. 2011a); and grey snapper, Lutjanus griseus (Linnaeus, 1758) (see Randall 1967). The goliath grouper is considered a mangrove-dependent species (Frias-Torres 2006), with clear ontogenetic shifts in habitat use. In general, juveniles are found in fringing mangrove shorelines, while adults occur in coral reefs, isolated patch reefs, reef/rock ledges, and artificial structures (Bohnsack and Ault 1996, Sadovy and Eklund 1999, Félix-Hackradt and Hackradt 2008). In our study, crustaceans predominated in the diet of juveniles associated with mangroves and fishes predominated in diet of adults associated with coral reefs. This variation may be due to crustaceans (the primary food item), are low in abundance in coral reef habitat and/or inaccessible to adults because of the extreme rugosity of coral reefs (Koenig and Coleman 2009, Koenig et al. 2011).

Despite of the fact that high trophic level species like adult Epinephelus itajara may cause indirect effects in the community (Heithaus et al. 2008, 2010, Ferretti et al. 2010) and influence a large range of ecological processes (Babcock et al. 1999, Pinney et al. 2000, Willis and Anderson 2003), the goliath grouper in the Abrolhos Bank feed primarily on prey from relatively low trophic levels (detritivorous and herbivorous), many of them with no direct economic value.

In Florida (USA), population recovery of the goliath grouper did not affect the abundance of commercially important snappers and lobsters (Frias-Torres 2012). Conversely, Graham et al. (2003) observed that the reduction in groupers abundance by fishing pressure in Australia caused an increase on their small fish preys.

Alterations in large groupers populations, such as Epinephelus itajara, can have significant commercial and ecological impacts. For example, the diet composition of 28 individuals of Epinephelus itajara, captured between May 2005 and September 2010 from Abrolhos Bank, is shown in Table 1.

### Table 1

| Prey item                     | Total | Mangrove | Coral reef | Juvenile | Adult |
|------------------------------|-------|----------|------------|----------|-------|
|                              | n     | F        | IRI        | n        | F     | IRI    |
| TOTAL FISH                   | 23    | 64.29    | 46.31      | 46.31    | 5     | 30.77  |
| Fish remains                 | 10    | 12.14    | 28.14      | 28.14    | 5     | 30.77  |
| Acanthostracion sp.          | 6     | 14.29    | 12.32      | 12.32    | 0     | 0.00   |
| Synodus sp.                  | 1     | 3.57     | 0.00       | 0.00     | 1     | 6.25   |
| Diodon sp.                   | 2     | 3.57     | 0.00       | 0.00     | 2     | 6.25   |
| Gymnolobus spp.              | 1     | 3.57     | 1.30       | 1.30     | 0     | 0.00   |
| Haemulon spp.                | 4     | 3.57     | 1.37       | 1.37     | 0     | 0.00   |
| Ocyurus chrysurus            | 1     | 3.57     | 2.00       | 2.00     | 0     | 0.00   |
| TOTAL DECAPODS               | 24    | 71.43    | 52.77      | 52.77    | 14    | 61.54  |
| Panulirus argus              | 1     | 3.57     | 0.43       | 0.43     | 0     | 0.00   |
| C. ruber                     | 5     | 7.14     | 3.51       | 3.51     | 2     | 0.00   |
| C. ornatus                   | 1     | 25.00    | 3.41       | 3.41     | 1     | 7.69   |
| Brachyura                    | 4     | 10.71    | 2.82       | 2.82     | 0     | 0.00   |
| CORAL FRAGMENTS              | 2     | 7.14     | 0.92       | 0.92     | 0     | 0.00   |
| TOTAL                        | 51    | 142.80   | 100        | 100      | 19    | 92.23  |

n = number, F = frequency of occurrence of the food items consumed [%], IRI = index of relative importance of individual food items [%].

### Table 2

| Factor                     | df | SS   | MS   | Pseudo-F | P(perm) | UP |
|----------------------------|----|------|------|----------|---------|----|
| Size                       | 1  | 8842.2 | 8842.2 | 2.44 | 0.009s | 999 |
| Habitat                    | 1  | 6695.5 | 6695.5 | 1.85 | 0.052  | 999 |
| Size × habitat             | 1  | 7341.9 | 7341.9 | 2.03 | 0.034s | 999 |
| Residuals                  | 24 | 86788 | 3616.2 |     |        |    |
| Total                      | 27 | 1.159E5 |      |     |        |    |

s = significant results, df = degrees of freedom, SS = sum of squares, MS = mean squares, P(perm) = permutation P-value, Pseudo-F = PERMANOVA’s statistics, UP = unique values obtained under permutation.
ecological consequences (Brulé et al. 2005). Goliath grouper is dependent on the lower trophic level, and its presence, diet, and reproductive aspects could be used as indicators of the ecosystem health. The occurrence of E. itajara in the region, that comprises the largest and richest reefs in the South American (Leão and Kikuchi 2005, Moura et al. 2013), may indicate that this system still resists anthropogenic pressures, such as overfishing, pollution, and dredging (Leão and Kikuchi 2005, Moura et al. 2013).

Data presented here may help to support future conservation efforts for this threatened group, particularly supporting the moratorium that started in Brazil in 2002. Despite the moratorium, unfortunately, the goliath grouper is still being captured and illegally commercialized along Brazilian coast (Félix-Hackradt and Hackradt 2008, Reuss-Strenzel and Assunção 2008, Silva-Oliveira et al. 2008, Alarcon et al. 2009, Soares et al. 2011, Bender et al. 2013, Giglio and Freitas 2013), including mentioning in the official Brazilian fishery report, with more than 300 t of annual illegal catches (Giglio et al. 2014). Effective protection in Florida (USA), led to the population initial recovery after 20 years, with increases in juvenile abundance (Frias-Torres 2006) and return of adults to certain reef locations and spawning aggregation sites (Koenig and Coleman 2009). According to Bannerot et al. (1987) and Russ (1991) fisheries theory developed for gonochoristic populations does not apply to hermaphrodite fishes. Based on the large size of gonadal maturity observed in this study (>100 cm TL), consequently late gonadal maturity (up to 7 years), and high longevity (around 40 years) (Bullock et al. 1992), it is suggested here that the goliath grouper moratorium in Brazil should be maintained for a relatively long time frame (more than four decades). Additional measures, such as protection of key habitats such as mangroves and aggregation sites, as well as greater enforcement to avoid poaching, are urgently needed. In the absence and difficulty to collect biological data, we suggested the use of the local ecological knowledge, in conjunction with information from fisheries biology, providing essential information on biological aspects, such as spawning aggregation areas and seasonality, patterns of abundance, and feeding.

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### Table 3

| GRF | Size × habitat | Test | P-value |
|-----|----------------|------|---------|
|     | Juvenile (17) | Adult (11) | t-test |
|     | M (11) vs. C (6) | M (2) vs. C (9) | P(perm) |
| GRF | 1.853 | 1.104 | 0.033 |
| UP  | 964 | 17 | 0.426 |

**Notes:**
- GRF = grouping factor, M = mangrove, C = coral reef, t = two-sample t-test, P(perm) = permutation P-value, UP = unique values obtained under permutation; Number in brackets shows the number of samples, 5 indicates significant results.

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