

Collaborative approach in the study of the reproductive biology of the dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Perciformes: Serranidae)

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ABSTRACT. Several aspects of the reproductive biology of the dusky grouper *Epinephelus marginatus* were investigated using a collaborative methodology. 193 specimens of dusky groupers were obtained in collaboration with fish market personnel in the city of São Francisco do Sul, Santa Catarina State, Southern Brazil. Fish market staff were trained and encouraged to collect biological material required for reproductive studies, giving access to many specimens which otherwise would have been unavailable. *E. marginatus* was found to reproduce in early summer, spawning between November and December. Females reached sexual maturity at approximately 460 mm total length. Sampled males ranged from 900-1000 mm and females from 300-960 mm in total length. No transitional (i.e. sex changing) males were observed. The regression equation for the relationship between total length (TL) (mm) and total weight (TW) (g) was $TW = 4.4 \times 10^{-5} TL^{2.8}$. Suggestions to improve the performance of future collaborative sampling projects are discussed.

Key words: collaborative research, reproductive biology, dusky groupers, *Epinephelus marginatus*, marine conservation, southwest atlantic.

RESUMO. Abordagem colaborativa no estudo da biologia reprodutiva da garoupa verdadeira *Epinephelus marginatus* (Lowe, 1834) (Perciformes: Serranidae). Diversos aspectos da biologia reprodutiva da garoupa verdadeira *Epinephelus marginatus* foram abordados através de uma metodologia colaborativa. Ao todo, 193 exemplares da garoupa verdadeira foram obtidas em peixarias colaboradoras no município de São Francisco do Sul, Estado de Santa Catarina, Brasil. Os funcionários das peixarias foram treinados e incentivados a realizar a coleta de todo material biológico necessário para estudos reprodutivos, possibilitando o acesso a um grande número de vísceras que teriam de outra maneira sido dispensadas. *Epinephelus marginatus* esteve em atividade reprodutiva no início do verão, com desova provável entre novembro e dezembro. Fêmeas atingiram a maturidade sexual com aproximadamente 460 mm de comprimento total. Machos variaram de 900mm a 1000 mm e fêmeas de 300 mm a 960 mm. Não foram observados machos em transição sexual. A equação de regressão da relação entre o comprimento total (CT) (mm) e peso total (PT) (g) foi dada ($PT = 4.4 \times 10^{-5} CT^{2.8}$). Sugestões são dadas ainda para o aumento da performance de futuras abordagens colaborativas de pesquisa.

Palavras-chave: pesquisa colaborativa, biologia reprodutiva, garoupa verdadeira, *Epinephelus marginatus*, conservação marinha, sudoeste atlântico.

Introduction

Understanding how ecosystems work is essential to designing effective management procedures.

However, the current research effort directed to marine and coastal environments has not been enough to provide the amount of information needed (Johannes, 1998). Such deficiencies are ongoing in

Brazil mainly due to the scarcity of marine research institutions, the lack of suitably trained professionals, and inadequate funding for basic biology (e.g. natural history) and fishery systems research (e.g. data on artisanal and industrial fishery landings).

The dusky grouper (Figure 1) is an anfi-atlantic species that presents a complex reproductive strategy, including protogynous hermaphroditism, reproductive aggregations and complex social interactions (Culioli and Quignard, 1999; Pelaprat, 1999; Marino *et al.*, 2001). These characteristics, together with other critical characteristics shared by many *Epinephelinae* species (e.g. slow growth, late age at maturity) (Coleman *et al.*, 1999) has brought the attention of many researchers and conservation entities throughout the Atlantic and Mediterranean areas of its distribution (Chauvet, 1981; La Mesa *et al.*, 2002; Bertoncini *et al.*, 2003). In Brazil, this species was recently included in the “over-exploited or threatened with over exploitation” list of marine resources (Ministério do Meio Ambiente, Instrução Normativa n. 5, 2004, May 21th). Recognizing the broad deficiency of biological information regarding those species included in this list, the document compromises Ibama (The Brazilian Environmental Agency) to motivate research and implement management strategies before 2010.



Figure 1. The dusky grouper *Epinephelus marginatus* (approximately 250 mm of total length). Photo by Áthila Bertoncini Andrade (Instituto Vidamar).

In Brazil, several aspects of dusky grouper biology have been addressed, such as feeding preferences (Machado *et al.*, 1997), used of intertidal pools (Bertoncini *et al.*, 1999), juvenile habitat use (Machado *et al.*, 2003) and reproductive biology (Bertoncini *et al.*, 2003). However, further refinement of this information is needed, especially concerning spawning period, sex inversion and changes in size at maturity over time. Such information is important for the species management (e.g. seasonal fish closures,

minimum and maximum sizes of capture) and conservation (e.g. seasonally or fully restricted marine protected areas).

Consistent results on the reproductive biology of *Serranidae* species are ideally assessed through extended sampling programs covering several annual cycles, and consequently involving a high number of analyzed individuals. The sampling period required for publication of adequate results ranged from three up to thirteen years in serranid reproductive studies conducted by Hood and Schlieder (1992), Bullock *et al.* (1992; 1996), Bullock and Murphy (1994). These studies considered almost solely specimens from artisanal fisheries sampled by a specialized research team. However, logistical and financial factors are likely to be limiting in attempting to adequately study some *Serranids* species.

This work reports on the implementation results of a collaborative research program, which couples scientists and community effort with the purpose of researching a poorly known and regionally important marine fisheries resource, the dusky grouper *Epinephelus marginatus* (Lowe, 1834). Collaborative approaches has been successfully developed for marine fish conservation (Rosa *et al.*, 2006), fostering dialogue among relevant stakeholders while helping the surveillance of species biology and other conservation issues. Herein, we propose an alternative sampling approach to that normally executed by a specialized research team. Three fish markets and their staff were involved in the collection of biological material (fish viscera), which were used in laboratory analysis for the evaluation of some aspects of the reproduction of the dusky grouper. We show that collaborative research can contribute in counterbalancing logistic limitations and in providing biological data needed for management.

Material and methods

One factor constraining research on some coastal serranids (and other species occurring in artisanal fisheries) is the access to sufficient biological material (e.g. fish viscera/guts). As a function of the collaborative methodology used here, fish markets and their staff are able to participate actively in the research process on the dusky grouper. This partnership allows the access to fish viscera that would otherwise be discarded by the fish markets.

Three fish markets located in the city of São Francisco do Sul, Santa Catarina State, were incorporated in the project. The coastal area adjacent to these fish markets constitutes one of the largest estuarine systems of Southern Brazil, and provides a

diverse range of habitats such as mangroves, salt marshes and rocky shores (Figure 2).

Fish markets were visited approximately every 10-15 days from August 2002 to April 2004. Telephone contacts were frequently undertaken in order to verify sample occurrence. The following topics were repeatedly emphasized through informal conversations with fish market staff and owners during visits: 1) general and specific objectives of the research; 2) discussions on grouper biology; 3) information on the status of the present research, including preliminary results; 4) discussions on how this research could potentially benefit and subsidize management practices; 5) practical and logistical difficulties of this kind of research and the importance of collaborative approaches; 6) potential benefits to fish markets resulting from their participation; and 7) reinforcement of sampling procedures.

Sampling materials were made available to fish markets including pencils, rubbers, plastic bags, labeling papers and measuring tapes. The following biological material and data were solicited: individual grouper viscera together with a label comprising individual total fish weight (TW) in grams, total fish length (TL) in centimeters, catch date and locality. Staff were trained in the necessary procedures to collect and freeze the samples. Female fish for which either TL or TW data was not available had their sizes/weights estimated using the regression equation between TL/TW.

Gonads were sexed and weighed (GW) using an analytical balance (0.01 g precision) and stomachs were preserved for future research. Gonads were then fixed

in 10% formaldehyde for 24 hours and transferred to 70% alcohol (Beçak and Paulete, 1976). Finally, gonads were embedded in paraffin, sectioned at 4 to 7 μm and stained with Harris' hematoxylin and eosin following Horobin and Bancroft (1998).

To estimate the length at which 50% of the females were sexually mature (L_{m50}), 139 specimens were considered. Gonads were classified into four maturity stages through microscopic evaluation of histological sections (maturing, mature, recovering and resting) (see Bertoncini *et al.*, 2003 for histological descriptions of maturity stages of southern Brazilian dusky groupers). Macroscopic evaluation of gonads (following Marino *et al.*, 2001) was employed as a means of corroborating the histological analysis. The approximate length at first maturity (L_{m50}) was obtained through the distribution of relative frequencies (%) of juveniles (immature gonads) and adults (other development stages of gonads) by TL classes. Each analysis considered only those individuals for which applicable data was present.

Monthly variation in mean values of the Gonosomatic Index (GSI) (Vazzoler, 1996) was used in the determination of the reproductive period of *E. marginatus* females' as follows:

$$GSI^1 = GW/(TW-GW) \times 100 \text{ (individual value)}$$

$$GSI^2 = \sum GSI^1 / n \text{ (monthly mean value)}$$

where,

GW = gonad weight; TW = total weight; n = monthly sample size.

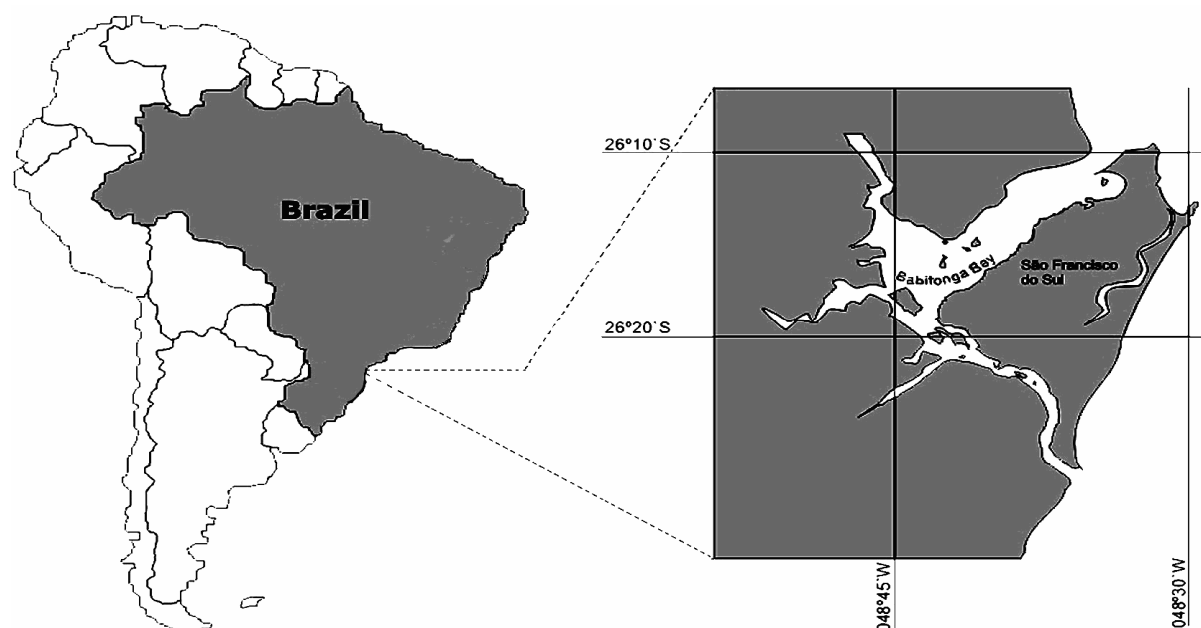


Figure 2. Babitonga bay and São Francisco do Sul Island, Southern Brazil.

Results and discussion

Data quality

A total of 193 specimens of *E. marginatus* were sampled by fish market staff. Individuals were captured in the Central/Northern coast of Santa Catarina state using one of the following fishing gears: spear fishing or rods and artificial baits around islands, shipwrecks and rocky outcrops and; long-line or line and hook over rocky bottoms.

Sampling success improved progressively with time due to increased commitment of the market collaborators to the research. Among the fish market staff, identifying those who were in fact interested in establishing a link with the research team was essential. The market owners' permission was fundamental for the development of the collaboration. However, people in charge of cleaning and gutting the fish were the ones that really made the collaborative research work possible.

A relatively high level of data integrity was observed. From the total number of fish sampled by fish market staff, only 15% of the gonads were either absent or fragmented. Fragmented portions of gonads, although not functional for GSI determination, were used in histological procedures.

We did, however, encountered problems in trying to assess the hepatosomatic index. It was not possible to calculate it due to the advanced degenerative state of most livers, and thus changes from their original mass by the time they were weighed. This organ generally suffers injuries during the handling and cleaning procedures, and apparently undergoes fast degeneration, especially in the case of those livers which had been perforated by metal spears.

The value for TW was only infrequently missing from labels (1%), probably because this is usually a routine measurement at these fish markets. TL data was lacking from 5.6% of the sample labels, but this was not considered a significant rate of omission. Capture location and date values, when absent from labels, were usually recovered by asking fish market staff during field visits. The occasional lack of this sort of information was not considered a major problem because market visits were frequent and thus not many fish accumulated at the markets between visits. Data on fishing gear was difficult to assess because fish market owners were not always interested in this sort of information at the time the fish was bought.

We suggest that most of the problems presented here can be minimized by training collaborators and increasing their immersion in the project's objectives. They must thus be made aware of their effective importance to the success of the project. We felt that this collaboration is likely to be achieved

only by maintaining a frequent market visiting timetable. Thus, a healthy relationship is constructed and an effective collaboration is built between researchers and market staff. In this sense, developing the conversation on the topics 1 to 7 (presented previously in this paper), becomes fundamental. The relationship between scientists and staff at the fish markets encouraged a productive dialogue and information exchange on the biology and conservation issues on dusky grouper, hopefully helping to raise their awareness on these matters.

Further comments on data quality and suggestions for improving the performance of future collaborative sampling projects are given in Table 1.

Considerations on the sampled dusky groupers

The number of specimens sampled varied monthly, probably due to decreased fishing effort as a reflection of meteorological adversities, or changes in the vulnerability of the dusky grouper population to fishing activities (Table 1). Problems identified during the collaborative sampling methodology, sources of problems, and suggestions for future sampling improvements.

For instance, migration to deeper areas is known for south Brazilian dusky groupers (Machado et al., 2003), and may influence its vulnerability to coastal artisanal fisheries such as those practiced in the study area. The fishery system within Babitonga Bay is also multi-specific (Pinheiro and Cremer, 2004). Fishermen change their target species seasonally according to the dynamics of the most valuable resources.

Dusky groupers sold by fish markets came from a diverse range of fishing gears with different selectivity levels. Further information on fishing gear was not registered, preventing any assessment of the size frequency distribution of the *E. marginatus* population in the studied area. We should also be aware that the size frequency distribution of sampled groupers (Figure 3) may not be a faithful representation of all fishes being landed.

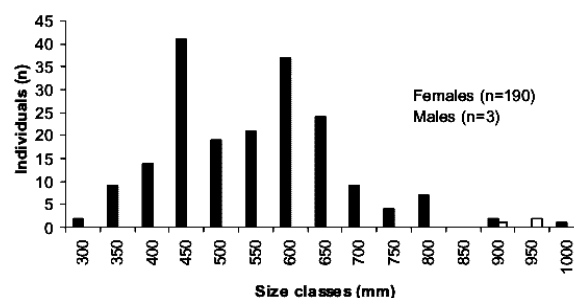


Figure 3. Length-frequency distribution of sampled dusky groupers *Epinephelus marginatus*. Females are shown by black and males by white bars.

Table 1. Problems identified during the collaborative sampling methodology, sources of problems, and suggestions for future sampling improvements.

DATA	PROBLEMS	SOURCE OF PROBLEMS	PROPOSED SOLUTIONS
1. Gonads	1. Gonads not present or cut in pieces. Low histological quality.	1. Small sized gonads located close to the fish's anus, where the knife is generally firstly introduced during fish gut clearance. Degenerative processes commence once the fish is dead.	1. Attention during the cleaning process should be motivated. Encourage freezing of fish on its arrival and/or after fish gut clearance and sampling.
2. Liver	2. Accelerated decomposition. Organs mechanically fragmented.	2. Liver seems to have a fast decomposition, especially if perforated by metal spears. Livers are damaged by spears at capture (by spear fishing) or by knife during fish gut clearance.	2. Encourage freezing of fish on its arrival and/or after fish gut clearance and sampling. Attention during the cleaning process should be encouraged.
3. Stomach	3. Stomachs perforated by spears or knives. Food items in advanced stage of decomposition	3. The person who cleans the fish frequently cuts the organ in pieces. Long periods of exposure of fresh fish to consumers increase digestive levels	3. Attention during the cleaning process should be encouraged. Encourage freezing of fish on its arrival and/or after fish gut clearance and sampling.
4. Total Length	4. Information lacking or not precisely written on labels.	4. Measuring total length requires an extra effort during fish gut clearance. During busy periods and days this data is frequently ignored.	4. Training those in charge of fish gut clearance. Keep sampling measurement tapes available. Emphasize importance of this information. Maintain a regular visit timetable.
5. Total Weight	5. Information lacking or not precisely written on labels.	5. Sometimes there is a tendency to do gross rounding of weight values.	5. Emphasize importance of information.
6. Locality	6. Information on fish capture locality absent.	6. The exact locations of fishing grounds are not asked of local fishermen, and may not be revealed by them. The region of capture is, however, given most of the time.	6. Emphasize importance of information to market owner. Motivate the communication of this message among staff and fishermen or intermediate sellers.
7. Date of Capture	7. Imprecision on the date of capture.	7. Fish sometimes arrives at markets in large quantities and frozen. Sometimes not all fishes belong to the same fishing day, nor the same site.	7. Emphasize importance of information to market owner. Maintain a regular visit timetable.
8. Labels	8. Labels lacking or incorrect.	8. Attention is not given to labeling procedures when: no interest in the research is shown; sampling materials are lacking (pencils, measurement tapes and labeling paper); numerous consumers are at the markets on busy days.	8. Training staff on the labeling process. Collaboration of research specialists on busy days. Provide and replace sampling materials whenever they get lost.

Not all individuals arriving at the fish markets were sampled for this research due to the following situations: occasionally collaborators did not consider small fish relevant to the research; large fish were often sold to consumers with guts; sometimes consumers preferred to buy whole ungutted fish, as the gut clearance is charged.

Comments on sex ratio and sexual inversion

The overall sex-ratio of our sample was 46 females for each male. However, this value should be considered with prudence for two main reasons: the impossibility of measuring fishing selectivity effects on population estimates of sex-ratio and; collaborator's sampling effects on sex-ratio (e.g. not all fishes at fish markets were sampled).

Long-term data on sex ratio is important when addressing the conservation status of protogynous hermaphroditic fish stocks. Males of such species (which are mainly larger fish) play a key role in reproduction, and declining proportions of them over time should increase conservation concerns (Coleman *et al.*, 2000).

Males occupied the largest size-classes and were not present in the smallest size-classes (Figure 3). This finding is consistent with patterns observed for the dusky grouper, which has been diagnosed as a

protogynous hermaphroditic species (Marino *et al.*, 2001; Bertoncini *et al.*, 2003).

Spawning aggregation behavior is common among Serranidae species. During the reproductive event, a smaller number of males fertilize larger number of females (Zabala *et al.*, 1997). In the hypotheses presented by Shapiro *et al.* (1993), decreasing encounters between males and females and increasing interactions among females promotes sexual inversion. Koenig and Coleman (1999) suggested that the process of sexual inversion is triggered during spawning aggregations in populations of gag grouper *Mycteroperca microlepis*. Once such spawning aggregations take place, social interactions are intensified among individuals in the population. Dusky grouper spawning aggregations were observed in the study area (LCG, pers. obs.). Investigating these aggregations is a fundamental issue for local dusky grouper conservation. Fishermen's ecological knowledge seems to be regionally rich on this subject, and can potentially play an important role in this regard.

Male dusky groupers ranged from 900 to 1000 mm in TL, and females from 300 to 960 mm. Bertoncini *et al.* (2003) also noticed a sex overlap at the 800 mm size class. We can infer that sexual inversion does not happen at a fixed size or nor do all individuals undergo

sexual inversion. In the Northeastern Atlantic, sexual inversion occurs in individuals between 14 to 17 years old, at sizes ranging from 800 to 900 mm (Chauvet, 1981; Barreiros, 1995; Bouchereau et al., 1999). In the Mediterranean Sea, sex change occurred in fish 690–930 mm total length (Marino et al., 2001).

Large sized specimens, which are usually difficult to capture by research teams during regular field surveys, were made available to this research through the collaborative methodology. In Brazil, the assessment of such large size classes still represents a critical constraint on the study of the whole ontogenetic range of the dusky grouper's life history.

Our results do not allow further conclusions in relation to the sexual inversion strategy and a precise evaluation of the operational population sex-ratio. However, we strongly recommend the improvement of this collaborative methodology to a point where reliable data on these subjects can be assessed.

Length-weight relationship

The regression equation of the relationship between TL (mm) and TW (g) ($TW = aTL^b$) is: $TW = 4.4 \times 10^{-5} TL^{2.8}$, $R^2=0.97$ (Figure 4). The value of $b=2.8$ was similar to that found by Kara and Derbal (1995), Chauvet (1981, 1988), Bou-Ain (1984) and Bertoncini et al. (2003), with these values generally being close to 3.0 (Figure 4).

Bouchereau et al. (1999), studying dusky grouper growth at the Lavezzi Islands marine reserve, obtained the exponent 2.59. Such a value might have been influenced by the low number of individuals involved in their study ($n=22$) and by the absence of size classes below 200 mm TL. The value herein obtained did not consider individuals smaller than 300 mm, mainly because of their absence in the fish market sample. Data on juveniles can be found in the relationship presented by Bertoncini et al. (2003), which also considered tide pool specimens.

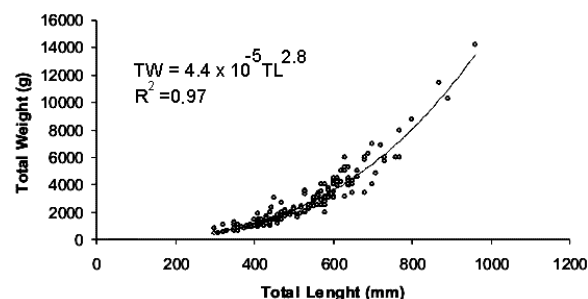


Figure 4. Relationship between total length (TL) and total weight (TW) for female dusky groupers *Epinephelus marginatus* from Babitonga Bay. Data collected by fish market staff during the collaborative research project ($n=173$, from which both TW and TL data were available).

Size at maturity

Adjusting the logistic model on the frequency distribution of mature individuals we obtained an approximate value of 460 mm for L_{m50} (Figure 5). This value is quite similar to the 470 mm obtained by Bertoncini et al. (2003) in Santa Catarina state.

Off the coast of Tunisia, 50% of the dusky groupers were sexually mature at 400 to 500 mm TL, and at about five years of age (Chauvet, 1988). Marino et al. (2001) estimated a value of 438 mm for a Mediterranean Sea population. No transitional males were observed during the present study.

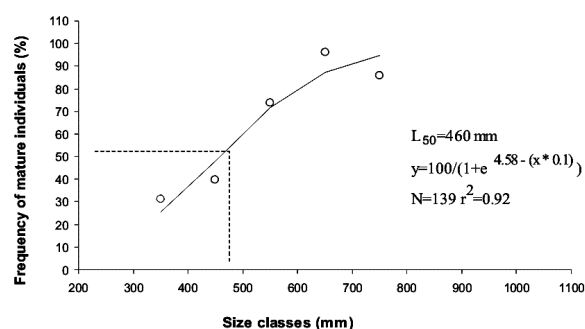


Figure 5. Maturation curve of dusky grouper *Epinephelus marginatus* females ($N=139$). L_{m50} represents the estimated length value at which 50% of the sampled individuals reached sexual maturity.

Reproductive period

A close look at the monthly variability of female GSI (Figure 6) shows higher GSI values dispersed from November 2002 to March 2004. Dusky groupers reproduced during early summer, apparently spawning from November to December. Bertoncini et al. (2003) have also verified increasing reproductive activity of *E. marginatus* during summer months.

The high amplitudes of standard deviation are related to the presence of few female specimens at advanced maturity stages, i.e. showing high individual GSI values. We advance the following hypotheses regarding the low occurrence of adult females found with mature gonads: 1) short maturation cycle (i.e. vitellogenic process prior to spawning); and 2) some females may not undergo the vitellogenic process. In the later regard, several large females analyzed during summer showed resting gonads. In aquaculture experiments, many females failed in completing the maturation process, and thus did not complete the vitellogenesis process resulting in mature oocyte development (Marino and Mandich, 2000). This can be due to adverse behavioral and/or environmental conditions, with a

consequent failure in hormonal production and thus inhibition of reproduction in captivity (Marino and Mandich, 2000); 3) Unknown gear selectivity of reproductively active females may also have played a part.

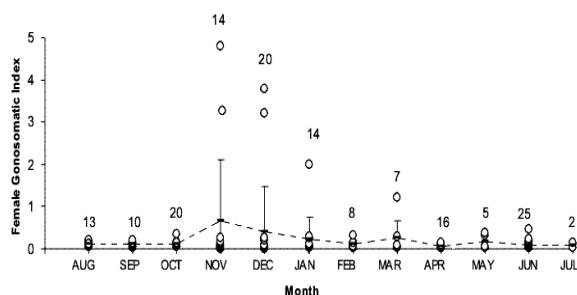


Figure 6. Numbers of female dusky groupers *Epinephelus marginatus* sampled by month (empty circles and respective monthly upper value) and mean values for Gonosomatic Index (dotted line), with upper limit of standard deviation (full line) for 154 analyzed specimens.

Conclusion

In spite of the fragmented data and the impossibility of incorporating abiotic parameters in our analysis, the collaborative methodology approach was a feasible supplement to targeted research on this species, which would demand a considerable logistic effort and costs.

Few research efforts have been directed towards the reproductive biology of Brazilian serranids. This is partly due to the difficulty in assessing a large number of individuals over a reasonable time period.

The use of marketed fishes is a useful conservationist approach, especially for species showing critical natural history characteristics such as groupers. However, it is clear that periodic and intense training should be a central component of such collaborative schemes in order to refine and support a further robust data-base.

Considering the need for information related to reproductive aspects of *E. marginatus*, we encourage the establishment of a medium/long term data collection campaign. Thus, not only a broad range of reproductive aspects can be approached, but also growth, feeding habits and population genetics can be elucidated through the collection of scales, otoliths, tissues and the analysis of stomach contents. Furthermore, the relationship between researchers, fish market staff and consumers throughout the study allowed a rich information exchange and discussion of the conservation needs and biological features of the dusky grouper. Although this was not a primary intended outcome of our research, it hopefully helped to raise people's

awareness on these matters (including ourselves). We suggest that this important outcome may in future be explored as an intended product of such a collaborative approach, broadening the effects of such academic research aimed at marine conservation objectives.

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