









## Filling gaps in the knowledge of grouper, especially Comb grouper (*Mycteroperca acutirostris*) (2013-2020) (Copacabana fishery, Rio de Janeiro, Brazil)<sup>1</sup>

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### ABSTRACT

There are gaps in our knowledge of important fish consumed by people in tropical countries. Small-scale fisheries are difficult to regulate, especially in countries with no species monitoring. At Copacabana (Rio de Janeiro), we observed 490 individuals of *badejo* (Comb grouper) (*Mycteroperca acutirostris*) in 2013-2014 and in 2018-2020. The average catch was 1.36 kg (n=466 individuals). A pattern of decreasing catches was observed for Comb grouper.

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Therefore, provided that the fishing gear and the number of fish have remained the same, the apparent decrease in Comb grouper needs to be further investigated. The results provide information about the major spawning season, around spring (September-December) with additional spawning during April. Genetic analyses in samples from 96 grouper obtained along the coast of Brazil were conducted. The Single Nucleotide Polymorphism (SNP) loci obtained for grouper species enabled us to determine the relative genetic proximity of *M. acutirostris* and *Mycteroperca bonaci*, information that can be useful for aquaculture and conservation. The objectives of this study were: a) to obtain data about the fishing activity (catch landings) of Comb groupers at Copacabana, Posto 6; b) to verify the reproductive period of Comb grouper, throughout the year, through the catches from the fishery; c) to develop a large numbers of SNP markers, which can be useful to studies about grouper populations (*Mycteroperca* and *Epinephelus*); and, d) to obtain data on the price of the Comb grouper. This study contributes data on the fishery and on the biology and ecology of groupers, and further contributes to conservation efforts.

**Keywords:** comb grouper, fisheries, genetics, *Mycteroperca*.

## **Preenchendo lacunas no conhecimento das garoupas, especialmente de *Mycteroperca acutirostris* (2013-2020) (Copacabana fishery, Rio de Janeiro, Brazil)**

### **RESUMO**

Existem lacunas em nosso conhecimento sobre a biologia de peixes importantes no consumo em países tropicais. A pesca em pequena escala é difícil de estudar e regulamentar, especialmente em países sem monitoramento sistemático de espécies. Em Copacabana (Rio de Janeiro) observamos 490 indivíduos de badejo (*Mycteroperca acutirostris*), nas pescarias, em 2013-2014 e 2018-2020. O kg médio dos badejos pescados foi de 1,36kg (n=466 indivíduos). Observou-se um padrão decrescente de capturas para o badejo. Portanto, considerando que a tecnologia pesqueira e o número de pescadores permaneceram os mesmos, a aparente diminuição do badejo necessita ser investigada mais detalhadamente. Os resultados desse estudo fornecem informações sobre a reprodução do badejo, com maior época de desova na primavera (setembro-dezembro) e desova adicional durante o mês de abril, no sudeste do Brasil. Análises genéticas sobre amostras de 96 badejos obtidos ao longo da costa do Brasil foram conduzidas. A informação genética obtida para as espécies permitiu-nos determinar a proximidade genética relativa de *M. acutirostris* e *Mycteroperca bonaci* e obter informações úteis para a aquicultura e conservação. Os objetivos deste estudo são: a) obter dados sobre a atividade pesqueira (desembarque de capturas) de badejo (Comb grouper, *Mycteroperca acutirostris*) em Copacabana, Posto 6; b) verificar o período reprodutivo dos badejos capturados nas pescarias, ao longo do ano; c) desenvolver um grande número de marcadores SNPs, que podem ser úteis para estudos de populações de garoupa (*Mycteroperca* e *Epinephelus*); d) obter dados sobre o preço do badejo. Existem muitas lacunas no conhecimento sobre *M. acutirostris*; este estudo pretende adicionar dados sobre biologia e ecologia do badejo e ajudar a subsidiar objetivos de conservação.

**Palavras-chave:** badejo, comunidades pesqueiras, genética, *Mycteroperca*.

### **1. INTRODUCTION**

Small-scale fisheries (SSFs) are ubiquitous along maritime coasts. Despite a significant lack of systematic data, such fisheries are a valuable food source for local populations and are

an important source of income (Begossi *et al.*, 2017; Béné, 2006). Small-scale fisheries account for approximately 50% of all fish captured for consumption worldwide; however, the lack of long-term monitoring in data-poor countries contributes to failures in fishery management (Kalidin *et al.*, 2020).

Estimation of grouper populations and their distributions in coastal aquatic ecosystems is complex, as exemplified in a study on Dusky grouper (Lopes *et al.*, 2019). Of course, even small-scale fisheries can impact aquatic fauna when considering late-maturing species and the reality that environmental problems are often not considered to be of primary importance by local authorities, as is the case in Brazil (Fearnside, 2019). On the other hand, it is known that SSFs can generally be sustainable even in developing countries (see examples of Caribbean and Latin American SSFs, such as those given by Salas *et al.* (2018)).

In this group are the “badejo”, as they are called in Brazil. Their dorsal fins have 11 spines and 15-18 rays. They are coastal fish with high commercial value, and are considered to be “noble fish”. Grouper are part of the Epinephelinae subfamily. Nevertheless, there is debate based on genetic data regarding whether the subfamily Epinephelinae should be treated as a family (Epinephelidae) rather than as a subfamily (Craig *et al.*, 2011).

*Mycteroperca acutirostris* has a dark brown color and a head with long striations, and it is characterized by 11 dorsal spines and 15-17 rays and 50-56 rakers in the first branchial arch, along with a rounded caudal fin. It is associated with rocky bottoms (adults), and juveniles are found in shallow waters and mangrove areas; its major threat is fishing pressure (Craig *et al.*, 2011; Figueiredo and Menezes, 1980). Our studies have shown that this species spawns in the spring in Brazil (Andreoli *et al.*, 2016; Silvano *et al.*, 2006).

Other species are addressed here, specifically in the genetic study, including *Mycteroperca bonaci*, called the Black grouper, which has a dark brown color and regular, hexagonal spots; it has, among other traits, truncated caudal fins and 11-16 gill rakers in the first branchial arch. It is a reef species, but juveniles can be found in estuarine environments (Craig *et al.*, 2011; Figueiredo and Menezes, 1980). Another species included in the genetic study is *Mycteroperca interstitialis*, called the Yellowmouth grouper, which has small brown spots, 11 dorsal fins, 16-18 rays, a first branchial arch with 15-19 gill rakers, and an emarginate caudal fin. It is also a reef species. We considered other species for the genetic study, especially because they are found in the coast of Brazil: *M. acutirostris* is common in the SE, whereas *M. bonaci* is common in NE Brazil. *M. interstitialis* is found in both regions, among others.

These species are monandric hermaphrodites, and *M. bonaci* forms spawning aggregations. Fishing pressure is one of the main threats to these species; adults occupy reef habitats, and juveniles are found in estuarine and mangrove environments. Adults feed on fish (Craig *et al.*, 2011).

When searching in FishBase (Froese and Pauly, 2020) and the IUCN Red List (data from 2016 for these fish) for *Mycteroperca acutirostris* (Comb grouper), we found that the knowledge about this species had several gaps. There are gaps for *M. acutirostris*, which include important information, such as diet and reproduction. The available information is general (and is based on few studies) such as the following: It has high vulnerability and is expensive; there is no information regarding eggs, spawning processes, or periods; and food items are mentioned as “unidentified invertebrates” (Froese and Pauly, 2020). Nevertheless, it is considered to be a species of LC (least concern), and its population is estimated as stable (IUCN, 2020b). For *Mycteroperca bonaci*, for example, we found slightly more biological information (Froese and Pauly, 2020): spawning in Brazil (one study including other fish species) in the period of June-December occurs for *M. bonaci* (Trindade-Santos and de Meirelles Felizola, 2015), and its diet was unidentified. *M. bonaci* is considered to be NT (near threatened, population decreasing) (IUCN, 2020a). For *Mycteroperca interstitialis* (Yellowmouth grouper), we found some information regarding reproduction (a study in the United States) showing that its reproduction occurs in all months of the year and its diet consists of “nekton” (a study in Puerto Rico) (Froese

and Pauly, 2020). It is considered to be VU (vulnerable), and its population is decreasing (IUCN, 2020a). For *Mycteroperca interstitialis* (Yellowmouth grouper), we found some information regarding reproduction (a study in the United States) showing that its reproduction occurs in all months of the year and its diet consists of “nekton” (a study in Puerto Rico) (Froese and Pauly, 2020). It is considered to be VU (vulnerable), and its population is decreasing (IUCN, 2020a).

*Epinephelus marginatus* is found in the Atlantic and in the western Indian Ocean. It has a decreasing population trend and is considered by the IUCN to be “EN” (endangered); *Mycteroperca acutirostris* is found in the western Atlantic and is considered to be heavily fished, but it is viewed by the IUCN to be of “LC” (least concern) (Craig *et al.*, 2011; Froese and Pauly, 2020).

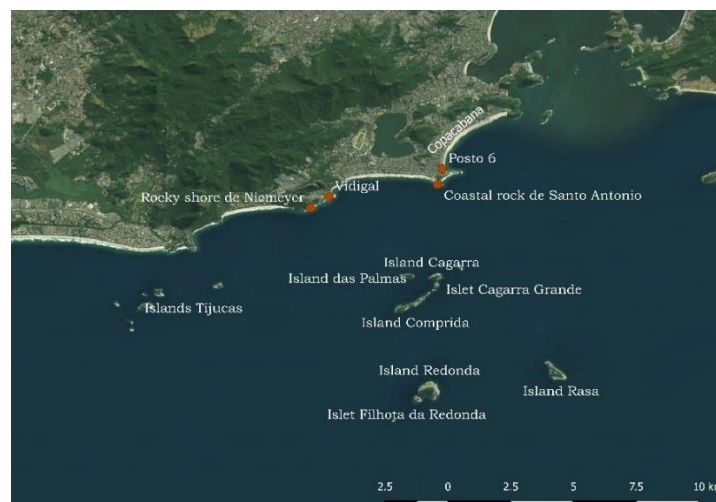
Given the lack of data on Comb grouper (*M. acutirostris*), our objectives in this study were: a) to obtain data about the fishing activity of the Comb Grouper (*M. acutirostris*)(spots used, landings); b) to obtain data on its reproductive period (s) throughout the year; c) to develop a large numbers of SNPs markers, which can be useful to studies of grouper populations (*Mycteroperca* and *Epinephelus*); and, d) to obtain data on the price of the Comb grouper (price fluctuation can probably be used indirectly evaluate resource availability). It is important to mention here that even though we included some genetic data for other species of *Mycteroperca* in this study, which allows us to make comparisons, our main focus is the study of *M. acutirostris* in the Copacabana fishery of Posto 6, since we have been following its catches for two periods since 2013.

For the genetic study, our objective was to use genotyping by sequencing to rapidly develop a large number of SNPs markers. These markers can be useful in future studies to improve genetic inferences within each species of *Mycteroperca* and *Epinephelus* here related to (among others) migration and population structure in a cost-effective manner (Bernos *et al.* 2020). We also examine whether divergence among these grouper genera and species are consistent with the early division proposed by Ma and Craig (2018).

## 2. MATERIAL AND METHODS

### 2.1. The fishery at Posto 6, Copacabana

The main fishing locations of the studied individuals of Dusky grouper and Comb grouper caught in 2013-2014 and 2018-2020 were the area in 2-7 km south from Copacabana Beach, where a lot of archipelagos and islands are located: Cagarra Islands, Tijucas Islands, Redonda Islands, Rasa Island and others (Figure 1).



**Figure 1.** Fishing Locations Used for Dusky grouper and Comb grouper (2013-2018).

The “Colônia de Pescadores do Posto 6” includes a small-scale fishing community on Copacabana Beach that was established in 1923. Fishing is conducted from small motorboats by using set gillnets, hooks and lines and by spearfishing (Begossi *et al.*, 2013; 2019). Recently, spearfishing by diving has become important, especially among young fishers. Fishing effort has been similar over the last 11 years: there are approximately 20-25 active fishers, including approximately 10 divers who spearfish close to islands.

Fieldwork at Copacabana was undertaken from September 2013 to February 2020 at the landing point or fish market of Posto 6, Copacabana. One of the authors (AB) visited approximately 5 days/month and compiled information on weight, price, and location, among other data. Two fishers were trained to follow a protocol that is explained in detail in Begossi (2008) and was used in Begossi *et al.* (2012; 2016; 2019). These methods included local knowledge, tracking fishery landings (systematic visits 3-5 days/month), including fishing locations, weight and length measurements (TL), and reproduction (gonad macroscopic analysis), which were the same as in the earlier studies [detailed in (Begossi and Silvano, 2008; Begossi *et al.*, 2019)]. Macroscopic gonad analysis included the observation (naked eye) of the presence or absence of mature gonads. Garoupa, or Dusky grouper (*E. marginatus*), was observed in 2013-2018, and Comb grouper (*M. acutirostris*) was observed in two periods, namely, 2013-2014 and 2018-2020.

This study follows a series of studies on small-scale fishing for grouper, especially *Epinephelus marginatus* (Dusky grouper) and *Mycteroperca acutirostris* (Comb grouper), that began in 2006 for both the Dusky grouper (Begossi and Silvano, 2008) and Comb grouper (Andreoli *et al.*, 2016) at the Colônia de Pescadores do Posto 6 in Copacabana, Rio de Janeiro, Brazil.

Here, we chronologically describe the research results for garoupa, Dusky grouper. The most recent study (Begossi and Salivonchik, 2019) showed relative catches and price stability. The studies that were begun by Begossi and Silvano (2008) included results about the local knowledge of fishers regarding diet and habitat, fishing locations, weight and length (TL), and stomach contents, among other features (sample of 40 individuals from 2006-2007). This study was followed by that of Begossi *et al.* (2016), which included fishing locations (Figure 1), weight and length (TL), macroscopic analysis of gonads by trained fishers (800 individuals from 2013 to 2015); Begossi *et al.* (2019): locations, weight and length (TL), macroscopic analysis of gonads by trained fishers (sample of 222 individuals, from 2016-2017), and prices from October, 2016 to November, 2017; and the study by Begossi and Salivonchik (2019): 1,896 grouper observed from 2013-2018, showing catches and prices as relatively stable in Copacabana (the <https://www.biorxiv.org/content/10.1101/759357v1>). Genetic analyses were performed earlier for Dusky grouper (Priolli *et al.*, 2014; 2016).

Given the substantial gaps in knowledge about an important food species and for the market, we believe that these original and previously unpublished data regarding *M. acutirostris* are important for conserving this species.

## 2.2. Genetics of grouper

### 2.2.1. Sampling of fins for genetic analyses

A total of 96 samples were obtained along the coast of Brazil (see details in (Begossi *et al.*, 2019)) from specimens belonging to two genera (*Epinephelus* and *Mycteroperca*) and five grouper species: *E. marginatus* (N = 28), *E. morio* (N = 19), *M. acutirostris* (N = 16), *M. bonaci* (N = 27), and *M. interstitialis* (N = 6). All individuals were caught by commercial fishers and identified based on their morphological characteristics as described in Begossi *et al.* (2019).

### 2.2.2. Molecular techniques

Total genomic DNA was extracted from approximately 20 mg of tissue using a DNeasy

Blood and Tissue Kit (Qiagen, Hilden, GE). DNA concentrations were estimated using a Qubit v4.0 fluorometer (Thermo Fisher Scientific, Waltham, USA). Thermo Fisher Scientific) and were normalized to 20 ng/ $\mu$ L. Genomic libraries were constructed according to the Genotype-by-Sequencing double digestion protocol described by Poland *et al.* (2012), using the restriction enzymes *NsiI* (NEB, Ipswich, USA) and *MseI* (NEB). The resulting libraries were pooled at 96-plex and sequenced on the Illumina NextSeq 500 platform (Illumina, Inc, USA) at the Hemocentro of Ribeirão Preto facilities (Brazil), in mid-output mode and set to produce 150 bp single-end reads. The quality of the obtained raw reads was assessed using FastQC software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) at the Hemocentro of Ribeirão Preto facilities (Brazil).

For each genus, samples were demultiplexed, and the raw read sequences were filtered with the module “process\_radtags” in the Stacks program (Version 1.42) (Catchen *et al.*, 2013). SNP calling retained only SNP per sequenced tag, with a minimum sequencing depth of 5X, minor allele frequency  $\geq 0.05$  and occurring in at least 90% of individuals within each species. The SNP identification was performed considering each genus separately, and also for the five species simultaneously. Population genomic analyses were performed by applying additional filtering parameters to obtain the maximum-quality SNPs: 1) individual samples with >55% missing data were excluded, and 2) SNPs with missing data in 25% of the samples or a minor allele frequency (MAF) <0.05 were removed.

### 2.2.3. Genetic analyses

The filtered data were imported as a genind object into R and were analyzed mainly by using several packages for population genetics. An outlier locus approach was taken using the R packages PCAdapt Version 3.0.4 (Luu *et al.*, 2017) and fsthet (Flanagan and Jones, 2017) and the software package SelEstim (Vitalis *et al.*, 2014), and only the loci that were identified as outliers by at least two of the three methods were considered as candidates for selection. The number of groups considered in fsthet and SelEstim analyses were three for *Mycteroperca* two for *Epinephelus* (corresponding to the number of species within each genus). The outputs from these analyses were used to create a neutral loci dataset and an adaptive loci dataset for further analysis. Neighbor-joining trees were generated for both the neutral and outlier datasets using Nei’s genetic distance method. Analyses of population structure were performed using discriminant analysis of principal components (DAPC) with the ‘Adegenet’ package (Jombart, 2008).

## 3. RESULTS AND DISCUSSION

We observed a total of 490 individuals, among which weights were obtained for 466 individuals and showed a total catch of 630.39 kg. The average catch was 1.36 kg. While 410,55 kg was obtained in 2014, we observed a relative decrease in Comb grouper catches, with 2019 being the year in which the species became rare in Copacabana fishing catches, and this trend continued in 2020. The number of individuals and the weights of Comb grouper found and studied at the landing point and fish market at Posto 6, Copacabana significantly varied by year and month (Tables 1 and 2).

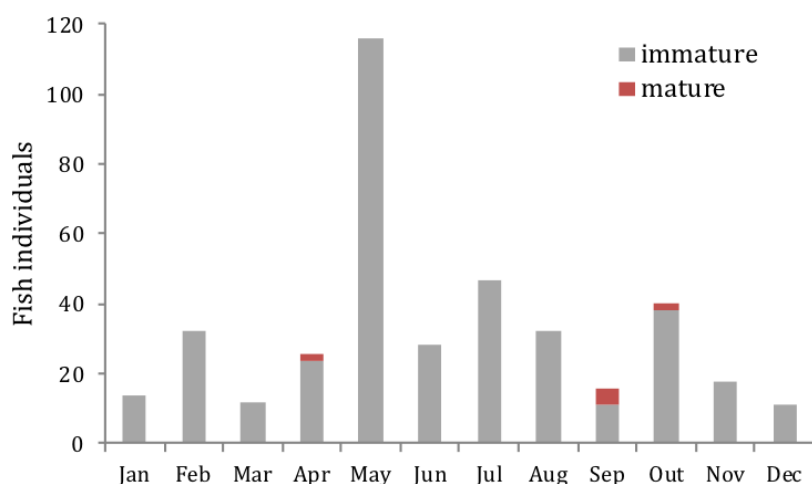
Mature gonads for Comb grouper were observed in April, September and October: these spring months coincide with mature gonads being observed in Dusky grouper (Begossi and Lopes, 2020; Begossi and Salivonchyk, 2019; Begossi *et al.*, 2019) (Figure 2). The months mentioned by fishers from Copacabana (13 fishers) as the months in which Comb grouper was “*ovado*” (with mature gonads) were September (5 fishers) and November-December (8 fishers).

**Table 1.** Comb grouper (badejo) observed at Copacabana fishery from 2013 to 2021. The data in parentheses indicate the number of individuals for which the weight was determined, when it was not possible to weigh the fish caught. Total number of individuals is 490 and weight (kg) was obtained for 464 individuals (\*COVID period) (Table 2). In the COVID period of the study (May, 2020 to March 2021), occasional visits were made, and two trained fishers contributed through *whatsapp*, when possible. In this period of 27 days of observations (19 visits and 8 *whatsapp*s), 19 *M. acutirostris* were observed.

Month	2013	2014	2018	2019	2020
Jan		14			23 (3)
Feb		32	1		10 (5)
Mar		12			2
Apr		26	3 (2)		*
May		116			*
Jun		28			*
Jul		46	13		*
Aug		20	29		*
Sep	6		17		*
Oct	34		21		*
Nov	18			2	*
Dec	3	1		17	*
Total	61	291	84 (83)	19	35 (10)

**Table 2.** Weight (kg) of the badejo (Comb grouper) caught at Copacabana fishery (2013-2020). Total weight is 630.39 (n=464 individuals) and average catch is 1.36kg. For the COVID period (March 2020-March 2021), 27 days, the weight amounted to 42.4 kg (n=19), average 2.23kg per individual.

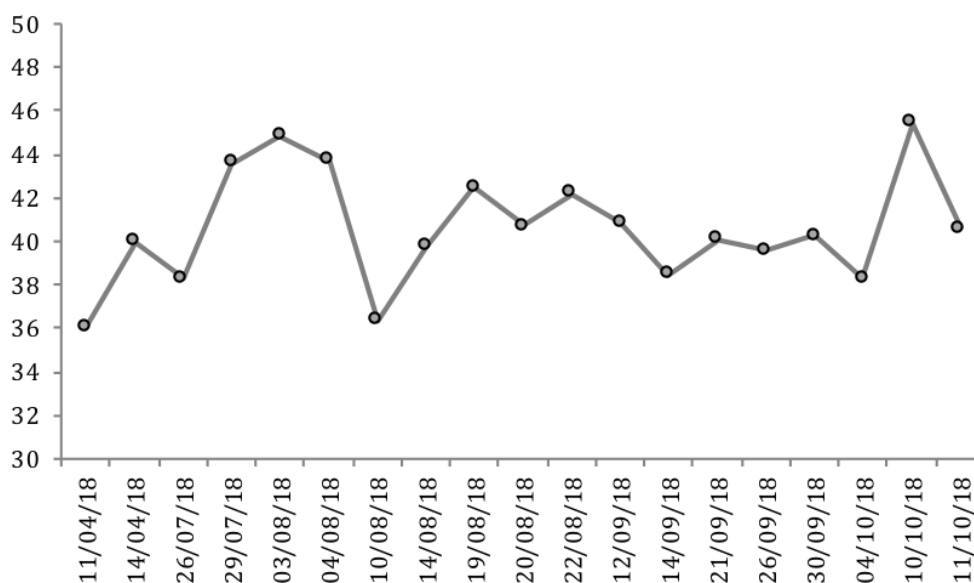
Mon	2013	2014	2017	2018	2019	2020
Jan		13.01				6.90
Feb		58.75	8.78	1.30		4.20
Mar		10.51				2.50
Apr		40.25		1,05		
May		178.05				
Jun		23.16				
Jul		67.60		24.77		
Aug		17.20		55.89		
Sep	5.17			34.70		
Oct	35.98			22.68		
Nov	13.39				3	
Dec	11.30	2.00			33.41	
Total	65.84	410.55	8.78	140.40	36.41	13.60



**Figure 2.** For the period 2013-2020, 392 individual Comb grouper were examined for gonads.

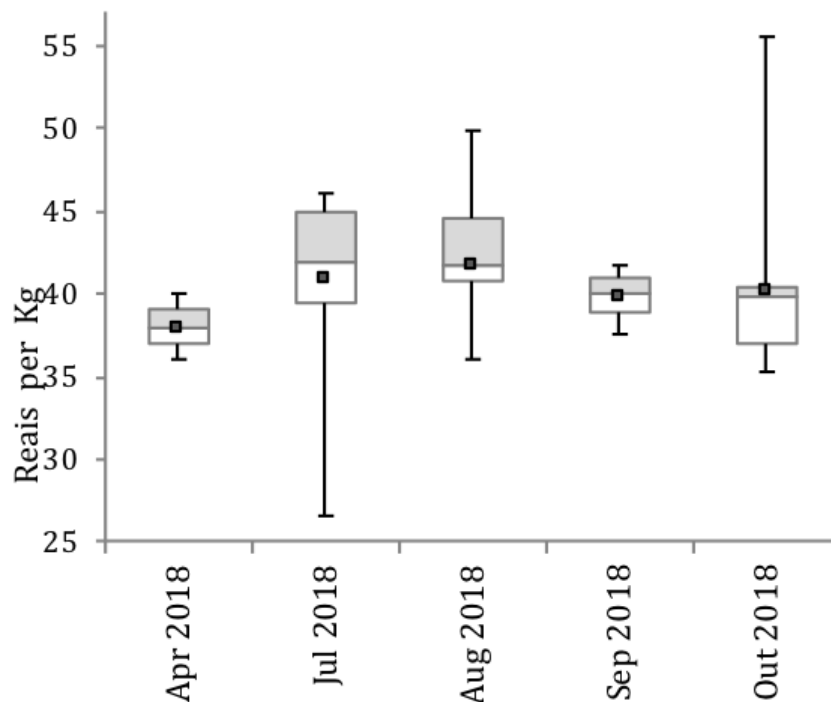
Mature gonads were observed in only 9 cases: 5 in September and 2 in April and October.

Prices were examined for April and July October of 2018. The average price of Comb grouper was 40.8 Reais/kg. The prices ranged from 26.5 (26/07/2018) to 55.5 Reais/kg (10/10/2018). The average daily prices varied from 36 (11/04/2018) to 45.4 Reais/kg (10/10/2018) (the exchange rate on July 6, 2018, was 3.93 Reais; Figure 2). The average monthly prices were slightly higher in July and August than in other months. They fluctuated most strongly in July and October (Figure 3a and b). The average exchange rate for 2018 was US\$1.00=R\$3.65)(<https://www.exchangerates.org.uk/USD-BRL-spot-exchange-rates-history-2018.html>). Even though prices can be an indirect way to evaluate resource availability, in the observed months of 2018 we did not observe significant fluctuations that showed differences in the availability of the resource. Fish prices reflect fish unpredictability and its supply tends to be inelastic (Sathiadhas and Kumar, 1994). Actually, in 5 months of 2018, prices did have apparent fluctuations, but a long time series is needed to better evaluate the relationship between prices and supply of the Comb grouper in Copacabana, Rio. Price fluctuations were not noticed for Dusky grouper, in the same fishery (Begossi and Salivonchyk, 2019).



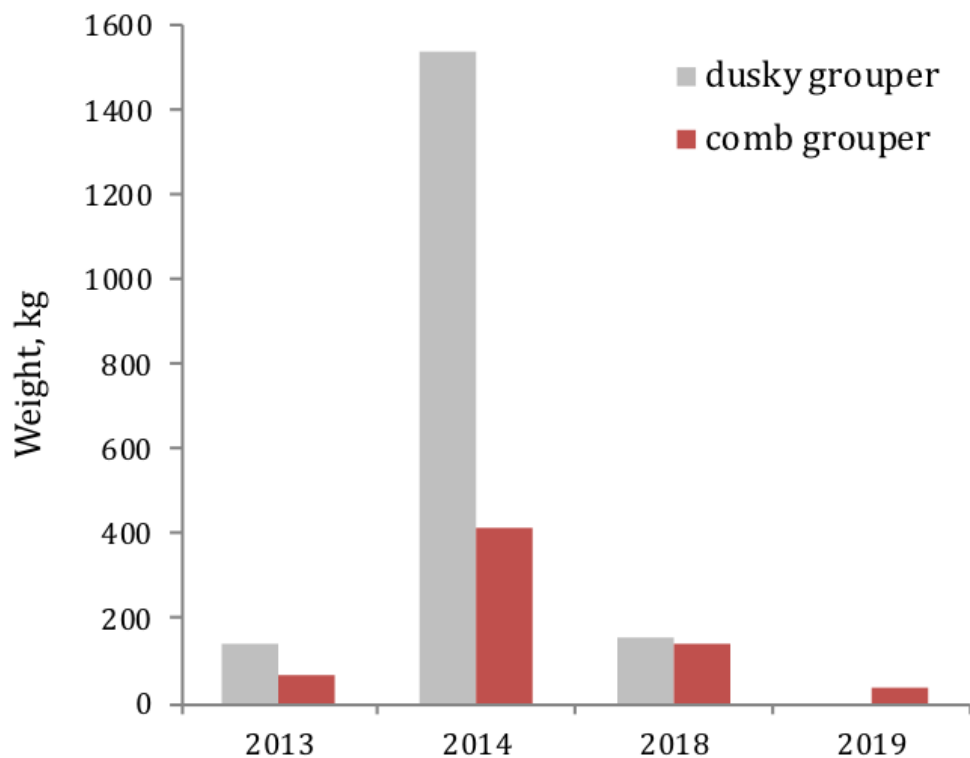
**Figure 3a.** Daily Average Price (in Brazilian Reais) per 1 kg of Comb Grouper in Copacabana (2018, US\$1.00=R\$3.65).





**Figure 3b.** Monthly Average Prices (in Brazilian Reais) per 1 kg of Comb grouper, in Copacabana (2018, US\$1.00=R\$3.65).

When comparing the yield (weight) of both species over the years, we observed higher catches for Dusky grouper, compared to Comb grouper, along with a decrease in Comb grouper in 2018 and 2019 (Figure 4).



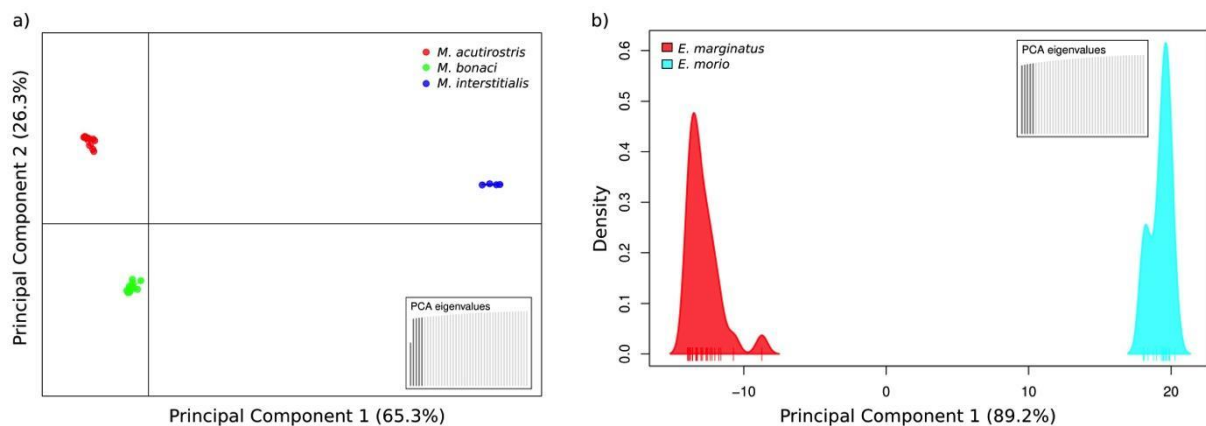
**Figure 4.** Annual Catches by Weight of Dusky grouper (A) and Comb grouper (B). The results from samples taken from September 2013 to February 2018 for and from September 2013-December 2014 and January 2018- December 2019 for Comb grouper at the Copacabana fishing post.

### 3.1. Genetic identification of groupers in the coast of Brazil

Sequencing of GBS libraries produced 150,458,819 raw reads for the 96 grouper, and 139,429,799 reads were retained following “process\_radtags” filtering. After applying the Stacks pipeline to each genus and additional filtering, 1,313 SNP loci were identified in 43 individuals of *Mycteroperca* (*M. acutirostris* = 16, *M. bonaci* = 23 and *M. interstitialis* = 4). For *Epinephelus*, 3,528 SNP loci were identified in 45 individuals (*E. marginatus* = 27 and *E. morio* = 18) (Figures A1 and A2, Appendix).

Outlier analyses identified 38 total consensus outlier loci in the three *Mycteroperca* species. Population structure analyses were conducted using the remaining 1,275 SNPs that were deemed to be neutrally evolving after outlier analyses. Overall,  $F_{ST}$  was 0.95 and highly significant ( $p < .001$ ), while the species pairwise values varied from 0.935 to 0.967.

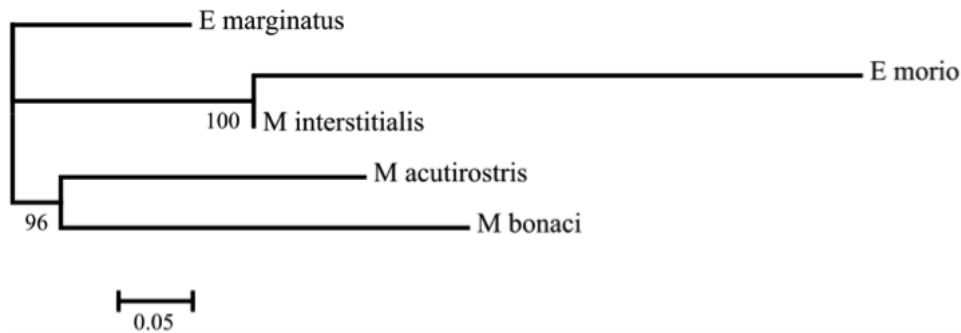
The high genetic divergence suggested by the pairwise  $F_{ST}$  estimates was also observed in the DAPCs. For *Mycteroperca*, the analysis based on SNPs with neutral behavior retained two principal components, which explained almost 92% of the total variation and clearly showed that samples of *M. acutirostris*, *M. bonaci* and *M. interstitialis* were highly distinct from each other (Figure 5a). For *Epinephelus*, the analyses based on SNPs with neutral behaviour retained one principal component, which explained more than 89% of the total variation and showed that samples of *E. marginatus* and *E. morio* were highly distinct (Figure 5b).



**Figure 5.** DAPC results for the genus *Mycteroperca* and *Epinephelus*. In a), samples of *M. acutirostris*, *M. bonaci*, and *M. interstitialis* are represented by colored dots, and their dispersal along the first two components is shown. In b), samples of *E. marginatus* and *E. morio* are represented by colored horizontal ticks along the first principal component and their density is represented in the vertical axis.

The same clustering patterns were observed for the neighbor-joining trees using both the neutral (A) and outlier (B) datasets for *Mycteroperca* (Figure A1) and *Epinephelus* (Figure A2). Outliers loci showed the species more divergent than neutral loci in both trees confirming the power of these for assigning individuals.

The genetic distances between the five grouper species (Figure 6) were determined from a set of neutral SNP loci and revealed that *M. acutirostris* is more closely related to *M. interstitialis* (0.343) than to *M. bonaci* (0.48). *Epinephelus morio* was the species that was most distant from the others, even for the species from the same genus, *E. marginatus* (0.73). Mitochondrial molecular markers and ribosomal genes showed *E. morio* in a branch different from *M. acutirostris*, *M. bonaci* and *M. interstitialis*; while *E. marginatus* was reclassified by the authors as *Mycteroperca marginatus* because was in the same monophyletic branch (Ma and Craig, 2018). In Figure 6, neighbor-joining tree based on Nei’s genetic distances was produced using the set of 817 SNP loci identified with Stacks pipeline considering all the five grouper species simultaneously.



**Figure 6.** Neighbor-joining trees based on Nei's genetic distances. Branch nodes are denoted as the percentage of bootstrap support that was generated with 1,000 replicates.

#### 4. CONCLUDING REMARKS

Exploratory research is very important for species that require management, especially species that are important for human consumption and for the livelihoods of poor communities that depend on small-scale fisheries for consumption and cash. This study provides important information for the conservation of this species and provides information on the reproduction period and on genetics that may be useful for managing this species. In addition, it provides alerts for a species that is becoming scarce and for a species with poor data and that is still considered of LC (least concern).

In this study, we found different catch patterns for each of the grouper species, namely, stability of catches and prices for Dusky grouper, but a decreasing pattern in the catches of Comb grouper, for the Copacabana small-scale fishery. We added genetics to better understand the species and to gain insights into their conservation and biology. With this study, we hope to fill some knowledge gaps in Brazil and contribute to management efforts.

By comparing catches of Dusky grouper and Comb grouper, contrasting patterns are found in the catches of the studied groupers: while we found relative stability for the catches of Dusky grouper, we found very irregular and decreasing catches in terms of number and weight for Comb groupers. Even considering that Comb grouper is of LC (least concern, IUCN Red List), there is an information gap concerning its diet and reproduction (Froese and Pauly, 2020). Thus, an optimistic prognosis is offered from the studies of Dusky grouper and Copacabana (Begossi and Salivonchyk, 2019), but there is a pessimistic prognosis for Comb grouper due to its decreasing catches when the fishing effort is maintained at a constant level, as was observed for the Copacabana fishery. Even though prices can help reveal the decrease or increase in the abundance of noble fish species for the market, such as *M. acutirostris*, the results did not add much for that objective. Long-term analysis of price fluctuation could add to this kind of estimation. Therefore, the results obtained from the landing points of Copacabana show decreases in catches: fishers report the fish must be obtained farther, at more distant areas and that it has been scarcer in the catches.

The results about the genetic diversity of the five species of grouper showed that *E. morio* is more distant than the other four species and that neutral and outlier SNPs are powerful to identify and to assign individuals.

How could these data aid in the conservation of species? Such information can help directly and indirectly.

Directly, information regarding the reproduction period is helpful for managing fish resources, as fishing closures for Comb grouper in the spring could help maintain stocks. Information on diet, in addition to its importance in maintaining available prey, could provide insights for possible aquaculture, which exists in SE Brazil for Dusky grouper (Glamuzina,

2020; Kerber, 2020).

Indirectly, grouper genetics data can provide basic information for aquaculture; it engenders information that could aid in conservation for assigning individuals in future studies. Here, genetic information could also be helpful for taxonomic analysis of the subfamily *Epinephelus*. Grouper systematics has been reevaluated, restricting the genus *Epinephelus* and expanding *Mycteroperca* when several species of *Epinephelus* were included in *Mycteroperca*, including *E. marginatus* (Craig *et al.*, 2011; Ma and Craig, 2018).

Summing up, the fishing locations found for the catch of Comb groupers by fishers of Posto 6 are relatively close to the coast of Copacabana, including rocky shores, such as islands of Cagarras, Redonda, among others (Figure 1). Average catch was 1.36 (n=446 individual). Mature gonads were found especially in the spring (September, October), but also in April (Autumn). Prices did not show conspicuous fluctuations in the observed months of 2018, indicating minor fluctuations in a resource that was already scarce in 2018 (the year in which prices were collected). GBS libraries revealed 1,313 SNP loci to *Mycteroperca* and 3,528 SNP loci to *Epinephelus* which can be useful in future studies to improve genetic inferences in each species.

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## 6. COMPLIANCE WITH ETHICAL STANDARDS

This research was approved and signed by B. R. Martins dos Santos, Comitê de Ética, Universidade Santa Cecília, number 1.747.889 on September 27, 2016 (Plataforma Brasil). It is approved under number 53824 at the SISBIO and is registered under number AB53669 at the SISGEN, MMA (Ministério do Meio Ambiente, Brasil). No animal ethics committee was relevant for this study, since all specimens were collected dead from fishery markets, and specimens were caught by small-scale fishers through artisanal methods. Anesthesia, euthanasia or animal sacrifices are not part of the methods used in this study.

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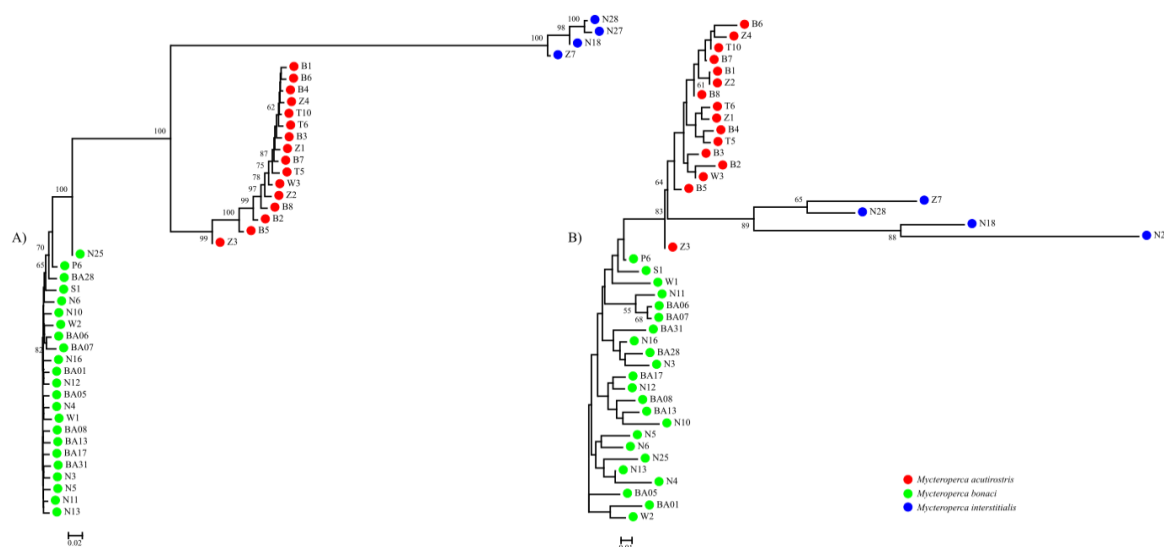
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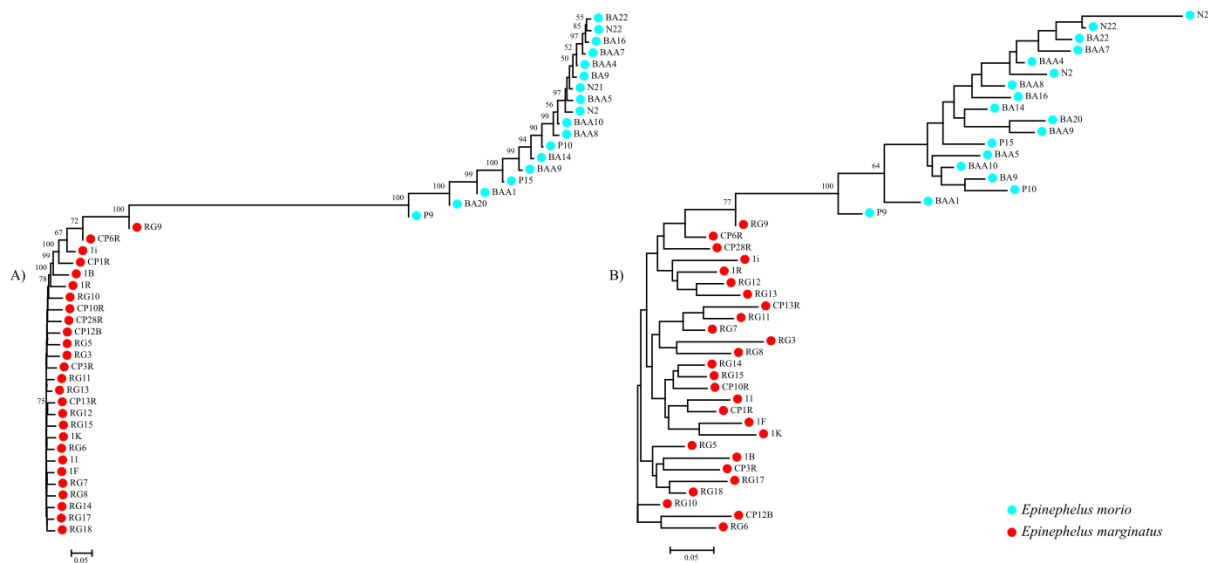
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Appendix:



**Figure A1.** Neighbor-joining Trees Based on Nei's Genetic Distances using the Following Sets of Loci: (A) a panel of 1,275 putatively neutral SNPs and (B) a panel of 38 putatively adaptive SNPs. Branch nodes are denoted as the percentage of bootstrap support that was generated with 1,000 replicates. Collection codes correspond to *M. acutiostis* (red dots), *M. bonaci* (green) and *M. interstitialis* (blue).



**Figure A2.** Neighbor-joining Trees Based on Nei's Genetic Distances Using the Following Sets of Loci: (A) a panel of 3,490 putatively neutral SNPs and (B) a panel of 38 putatively adaptive SNPs. Branch nodes are denoted as the percentage of bootstrap support that was generated with 1,000 replicates. Collection codes correspond to *E. marginatus* (red dots) and *E. morio* (blue).