SHORT NOTES:

Filling gaps: fishing, genetics, and conservation of groupers, especially the comb grouper (badejo) (*Mycteroperca acutirostris*), in SE Brazil (2013-2020)

Short title

Fishing, genetics and conservation of groupers in Brazil

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Abstract

There are large gaps in our knowledge of the biology of important fish consumed by people in tropical countries, which makes conservation difficult. Small-scale fisheries are difficult to study and regulate, especially in countries with no systematic species monitoring. It is even more difficult to estimate the influence of these fisheries on vulnerable fish species and to diagnose possible damage to local fish populations. In this study, 490 individuals of **badejo,** or comb grouper (*Mycteropeca acutirostris),* were observed at the Posto 6 fishery in Copacabana, Rio de Janeiro, for the periods of 2013-2014 and 2018-2020. A pattern of decreasing catches was observed for comb grouper. 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 regarding the reproduction of comb grouper, with major spawning season around spring (September-December) and additional spawning during April in SE Brazil. Samples from 96 groupers along the coast of Brazil were obtained, and genetic analyses were conducted. The genetic information obtained for grouper species enabled us to determine the relative genetic proximity of *M. acutirostris* and *Mycteroperca bonaci* and to obtain information that can be useful for aquaculture and conservation.

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 [1,2]. Small-scale fisheries account for approximately 50% all fish captured for consumption worldwide; however, the lack of long-term monitoring in data-poor countries contributes to failures in fishery management [3].

Estimation of grouper populations and their distributions in coastal aquatic ecosystems is complex, as exemplified in a study on dusky grouper [4]. 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 [5]. 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. [6]). Therefore, the question is how are endangered, slowly growing species affected?

The genus *Mycteroperca* and the comb grouper (*Mycteroperca acutirostris*)

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, have high commercial value, and are considered to be “noble fish”. Groupers 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) and not a subfamily [7].

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 rackers 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 [7,8]. Our studies have shown that this species spawns in the spring in Brazil [9,10].

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 rackers in the first branchial arch. It is a reef species, but juveniles can be found in estuarine environments [7,8]. Another species included in the genetic study is *Mycteroperca insterstitialis*, called the yellowmouth grouper, which has small brown spots, 11 dorsal fins, 16-18 rays, a first branchial arch with 15-19 gill rackers, 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 [7].

The lack of data

When searching in FishBase [11] 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 high prices; there is no information regarding eggs, spawning processes, or periods; and food items are mentioned as "unidentified invertebrates" (Froese and Pauly [11]: February 17, 2021: 14:35). Nevertheless, it is considered to be a species of LC (least concern), and its population is estimated as stable (UICN [12]: last assessed November 20, 2016). For *Mycteroperca bonaci*, for example, we found slightly more biological information (Froese and Pauly[11]: February 18, 2021: 11:38): spawning in Brazil (one study including other fish species) in the period of June-December occurs for *M. bonaci* [13], and its diet was unidentified. *M. bonaci* is considered to be NT (near threatened, population decreasing) (IUCN [14]: February 18: 11:42). 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 [11]: February 18: 11:52). It is considered to be VU (vulnerable), and its population is decreasing (IUCN [14]: February 18: 11:55).

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 will be *M. acutirostris* because we have been following its catches for two periods since 2013 in Copacabana, Rio de Janeiro (State of Rio de Janeiro), with some scattered observations for Bertioga and Santos (State of São Paulo).

The objective of this study was to obtain data about the fishing activity and biology of the comb grouper. Given the substantial gaps in knowledge about an important food species and for the market, we believe that original and previously unpublished data regarding *M. acutirostris* are important for conserving this species.

Our earlier studies

This study follows a series of studies on small-scale fishing for groupers, especially *Epinephelus marginatus* (dusky grouper) and *Mycteroperca acutirostris* (comb grouper), that began in 2006 for both the dusky grouper [15] and comb grouper [10] at the Colônia de Pescadores do Posto 6 in Copacabana, Rio de Janeiro, Brazil.

Studies of other Epinephelinae: Epinephelus marginatus, dusky grouper

Epinephelus marginatus is found in the Atlantic and in the western Indian Ocean with 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) [7,11].

Here, we chronologically describe the research results for garoupa, dusky grouper. The most recent study [16] showed relative catches and price stability. The studies that were begun by Begossi and Silvano [15] 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. [17], which included fishing locations (fishing spots), weight and length (TL), macroscopic analysis of gonads by trained fishers (800 individuals from 2013 to 2015); Begossi et al. [18]: 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 work by Begossi and Salivonchyk [16]: 1,896 groupers from 2013-2018, with catches and prices being relatively stable in Copacabana (the https://www.biorxiv.org/content/10.1101/759357v1).

Methods

The fishery

138 The “Colônia de Pescadores do Posto 6” includes a small-scale fishing community on
139 Copacabana Beach that was established in 1923. Fishing is conducted from small motorboats by
140 using set gillnets, hooks and lines and by spearfishing [18,19]. Recently, spearfishing by diving
141 has become important, especially among young fishers. Fishing effort has been similar over the
142 last 11 years: there are approximately 20-25 active fishers, including approximately 10 divers
143 who spearfish close to islands.

144 Fieldwork at Copacabana was undertaken from September 2013 to February 2020 at the
145 landing point or fish market of Posto 6, Copacabana. One of the authors (AB) performed visits
146 on approximately 5 days/month and compiled information on weights, prices, and locations,
147 among other data. Two fishers were trained by following a protocol that is explained in detail in
148 Begossi [20] and was used in Begossi et al. [17,18,21]. These methods included local
149 knowledge, tracking fishery landings (systematic visits 3-5 days/month), including fishing
150 locations, weight and length measurements (TL), and reproduction (gonad macroscopic
151 analysis), which were the same as in the earlier studies [detailed in 18,20]. Garoupa, or dusky
152 grouper (*E. marginatus*), was observed in 2013-2018, and comb grouper (*M. acutirostris*), was
153 observed in two periods, namely, 2013-2014 and 2018-2020.

154

155 **Genetics of groupers**

156 **Sampling of fins for genetic analyses.** A total of 96 samples were obtained along the
157 coast of Brazil (see details in [18]) from specimens belonging to two genera (*Epinephelus* and

158 *Mycteroperca*) and five grouper species: *E. marginatus* (N = 28), *E. morio* (N = 19), *M.*
159 *acutirostris* (N = 16*), M. bonaci* (N = 27), and *M. interstitialis* (N = 6). All individuals were
160 caught by commercial fishers and identified based on their morphological characteristics as
161 described in Begossi et al. [18].

162 **Molecular techniques.** Total genomic DNA was extracted from approximately 20 mg of
163 tissue using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, GE). DNA concentrations were
164 estimated using a Qubit v4.0 fluorometer (Thermo Fisher Scientific, Waltham, USA). Thermo
165 Fisher Scientific) and were normalized to 20 ng/µl. Genomic libraries were constructed
166 according to the Genotype-by-Sequencing double digestion protocol described by Poland et al.
167 [22], using the restriction enzymes NsiI (NEB, Ipswich, USA) and MseI (NEB). The resulting
168 libraries were pooled at 96-plex and sequenced on the Illumina NextSeq 500 sequencing
169 platform (Illumina, Inc, USA) at the Hemocentro of Ribeirão Preto facilities (Brazil), in mid-
170 output mode and set to produce 150 bp single-end reads. The quality of the obtained raw reads
171 was assessed using FastQC software
172 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) at the Hemocentro of Ribeirão
173 Preto facilities (Brazil).

174 For each genus, samples were demultiplexed, and the raw read sequences were filtered
175 with the module "process_radtags" in the Stacks program (version 1.42) [23]. SNP calling
176 retained only SNP per sequenced tag, with a minimum sequencing depth of 5X, frequency of the
177 least common allele ≥ 0.05 and occurring in at least 90% of individuals within each species. The

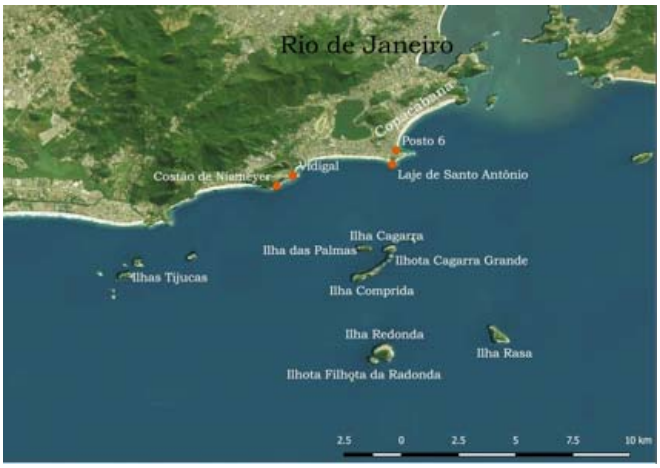
178 SNP identification was performed considering each genus separately, and also for the five
179 species simultaneously. Population genomic analyses were performed by applying additional
180 filtering parameters to obtain the maximum-quality SNPs: 1) individual samples with >55%
181 missing data were excluded, and 2) SNPs with missing data in 25% of the samples or a minor
182 allele frequency (MAF) <0.05 were removed.
183
184 **Population genetic analyses.** The filtered data were imported as a genind object into R
185 and were analyzed mainly by using several packages for population genetics. An outlier locus
186 approach was taken using the R packages PCAdapt version 3.0.4 [24] and fsthet [25] and the
187 software package SelEstim [26], and only the data that were identified as outliers by at least two
188 of the three methods were considered as candidates for selection. The number of groups
189 considered in fsthet and SelEstim analyses were three for *Mycteroperca* two for *Epinephelus*
190 (corresponding to the number of species of each collection sampled). The outputs from these
191 analyses were used to create a neutral locus dataset and an adaptive locus dataset for further
192 analysis. Neighbor-joining trees were generated for both the neutral and outlier datasets using
193 Nei's genetic distance method. Analyses of population structure were performed using
194 discriminant analysis of principal components (DAPC) with the 'Adegenet' package [27].
195
196 ## Results
197

198 **The fishery at Posto 6, Copacabana**

199 The main fishing locations of the studied individuals of dusky grouper and comb grouper
200 caught in 2013-2014 and 2018-2020 were the area in 2-7 km south from Copacabana beach,
201 where a lot of archipelagos and islands are located: Cagarras Islands, Tijucas Islands, Redonda
202 Islands, Rasa Island and others (Fig. 1).

203

204 **Fig 1. Fishing Locations Used for dusky grouper and comb grouper (2013-2018).**



205

206 The number of individuals and the weights of comb grouper found and studied at the
207 landing point and fish market in Posto 6, Copacabana significantly varied by year and month of
208 the year (Tables 1 and 2).

209 We observed a total of 490 individuals, among which weights were obtained for 466
210 individuals and showed a total catch of 630.39 kg. The average catch was 1.36 kg. While 410,55
211 kg was obtained in 2014, we observed a relative decrease in comb grouper catches, with 2019

212 being the year in which the species became rare in Copacabana fishing catches, and this trend

213 continued in 2020.

214 **Table 1**. Comb groupers observed at Copacabana fishery from 2013 to 2021. The data in
215 parentheses indicate the number of individuals for which the weight was determined, when it
216 was not possible to weight the fish caught. Total number of individuals is 490 and weight
217 obtained for 464 individuals (*_**covid**_ period). In the _**covid**_ period of the study (May, 2020 to
218 March 2021), occasional visits were made, and two trained fishers contributed through
219 *whatsapp*, when possible. In this period of 27 days of observations (19 visits and 8 *whatsapp*
220 messages), 19 *M. acutirostris* were observed.
221

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		*
Out	34		21		*
Nov	18			2	*
Dec	3	1		17	*
Total	61	291	84 (83)	19	35 (10)

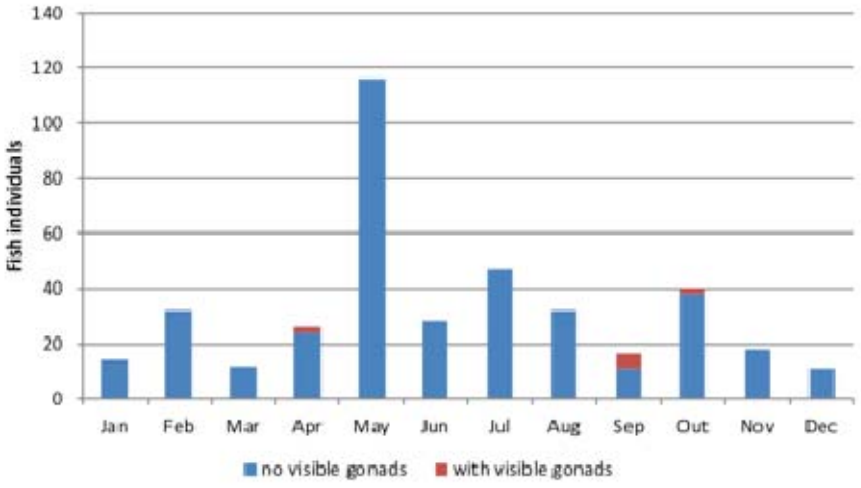
222
223
224 **Table 2**. Weight (kg) of comb grouper caught at
225 Copacabana fishery (2013-2020). Total weight is 630.39 (n=464 individuals)
226 and average catch is 1.36kg. For the _**covid**_ period (March 2020-March 2021),
227 27 days, the weight amounted 42,4 Kg (n=19), average 2,23Kg per individual.
228

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		
Out	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

Mature gonads for comb grouper, were observed in April, September and October: these spring months coincide with mature gonads being observed in dusky grouper [16,18,28] (Fig 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).

Fig 2. For the Period 2013-2020, 392 Individuals of Comb Grouper were Examined for Gonads. Visible Gonads were Observed in Only 9 Cases were: 5 in September and 2 in April and October.

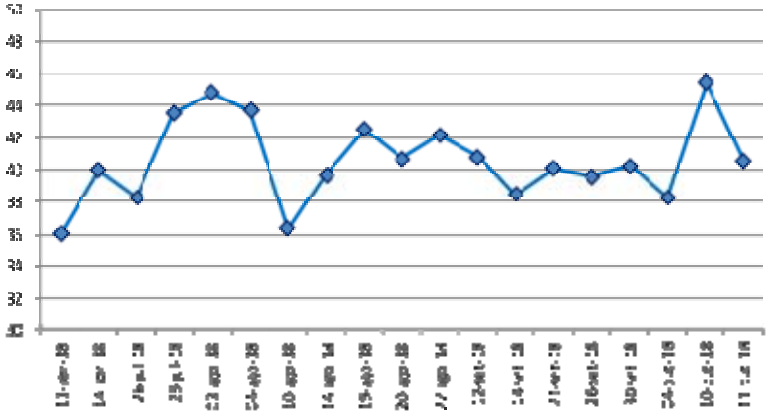


Prices were examined for April and July October of 2018. The average price of comb grouper, was 40.8 Reais/kg. The prices ranged from 26.5 (26/07/2018) to 55.5 reais/kg (10/10/2018). The average daily prices varied from 36 (11/04/2018) to 45.4 reais/kg

241 (10/10/2018) (the exchange rate on July 6, 2018, was 3.93 reais; Fig 2). The average monthly
242 prices were slightly higher in July and August than in other months. They fluctuated most
243 strongly in July and October (Figs 3a and b). The average exchange rate for 2018 was
244 US$1.00=R$3.65) (https://www.exchangerates.org.uk/USD-BRL-spot-exchange-rates-history-
245 2018.html).

246 **Fig 3a. Daily Average Prices (in Brazilian Reais) per 1 kg of Comb Grouper, in**
247 **Copacabana (2018, US$1.00=R$3.65).**

248



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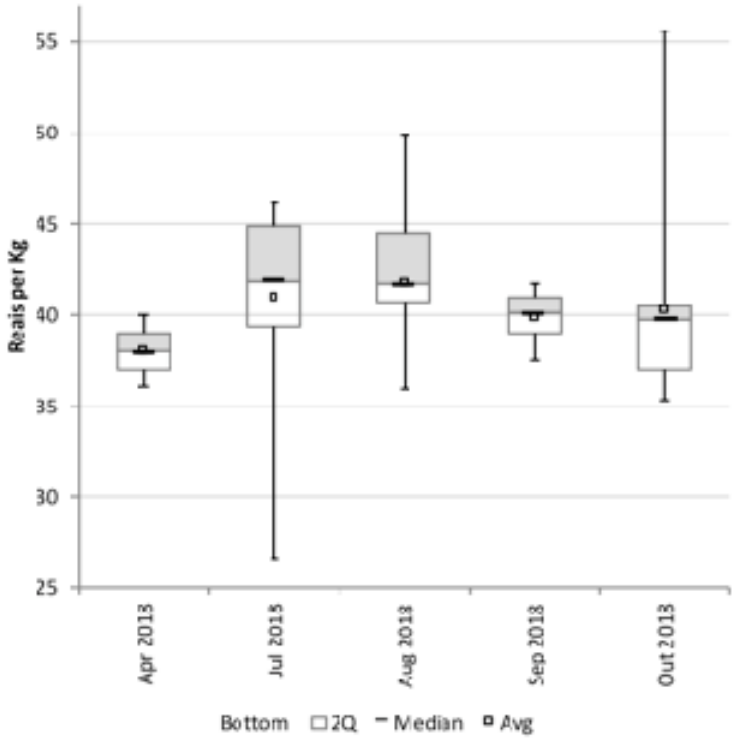
250

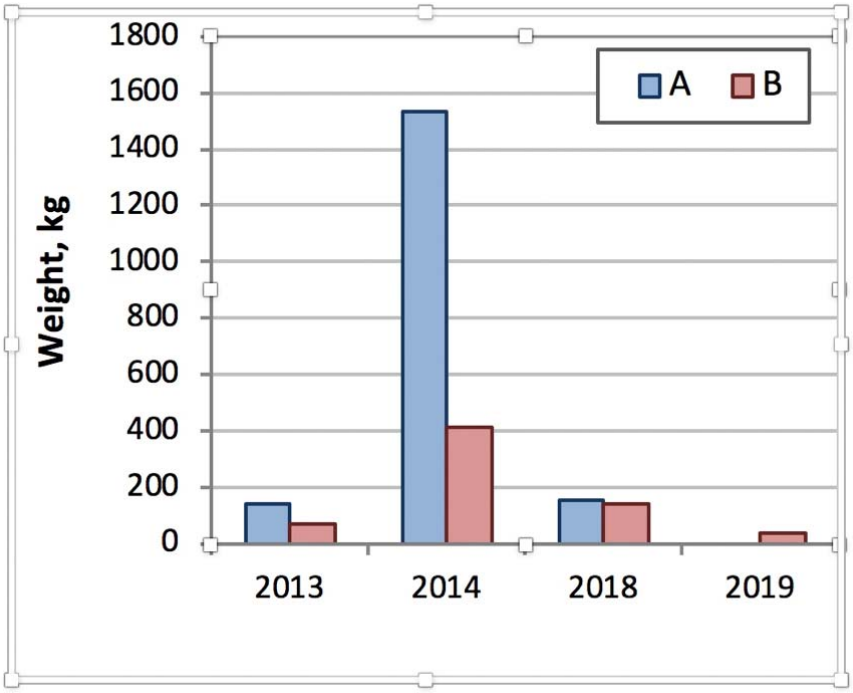
251 **Fig 3b. Monthly Average Prices (in Brazilian Reais) per 1 kg of Comb Grouper, in**
252 **Copacabana (2018, US$1.00=R$3.65).**



253

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

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



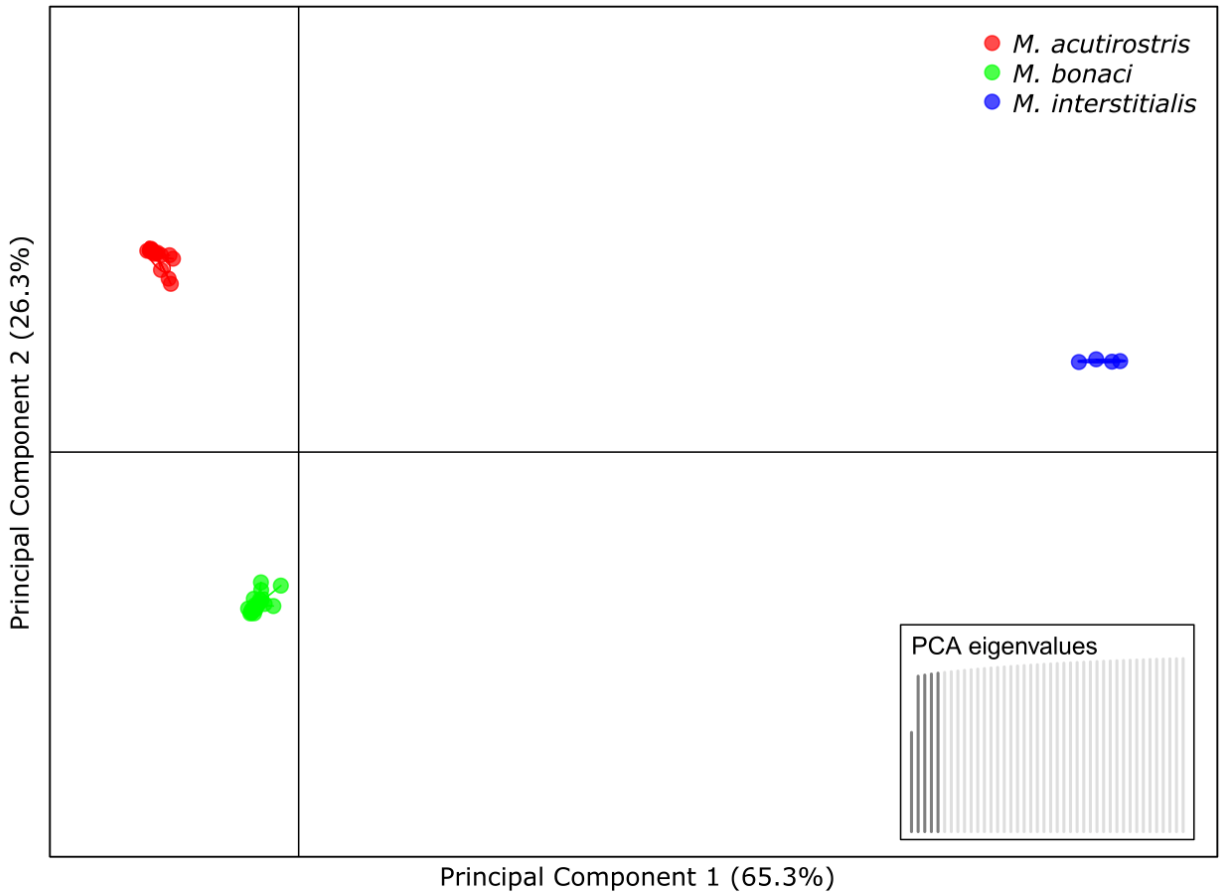
Genetics of groupers in the coast of Brazil

GBS libraries produced 150,458,819 raw reads for the 96 groupers, and 139,429,799 reads were retained following "process_radtags" filtering. After applying the Stacks pipeline to each genus and additional filtering, 1313 SNP loci were identified in 43 individuals of *Mycteroperca* (*M. acutirostris* = 16, *M. bonaci* = 23 and *M. interstitialis* =4). For *Epinephelus*, 3528 SNP loci were identified in 45 individuals (*E. marginatus* = 27 and *E. morio* =18) (Figs 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. The analysis based on SNPs with neutral behavior explained almost 92% of the total variation in the first two components and clearly showed that samples of *M. acutirostris***,** *M. bonaci* and *M. interstitialis* were highly distinct from each other (Fig 5). In Fig. 5, the DAPC scatter plot of *Mycteroperca acutirostris*, *M. bonaci*, and *M. interstitialis* that were collected in five Brazilian States (e.g., Rio de Janeiro, Bahia, Paraiba, Rio Grande do Norte), which were performed using 1,275 SNPs is shown.

Fig 5. DAPC Plot of *Mycteroperca acutirostris*, *M. bonaci*, and *M. interstitialis*. Individual *Mycteroperca* species are represented by colored symbols, and the PCA eigenvalues show that the first two principal components explain more than 91% of the genetic variability.

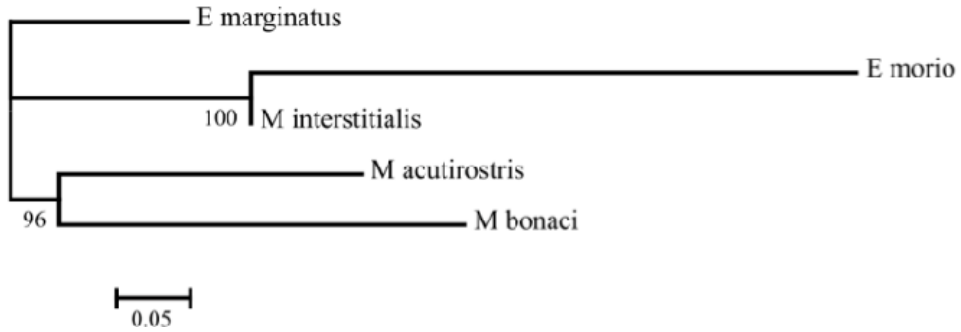


The same clustering patterns were observed for the neighbor-joining trees using both the neutral and outlier datasets (Fig A1 Appendix), although individuals of *M. interstitialis* appeared to be less divergent to those of *M. acutirostris* than to those of *M. bonaci*.

The genetic distances between the five grouper species (Fig 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, except for another species from the same genus (*E. marginatus*) (Fig 6). In Fig. 6 neighbor-joining trees based on Nei's genetic distances were produced using the set of

817 SNP loci identified with Stacks pipeline considering all the five Grouper species simultaneously.

Fig 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.



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, and a decreasing pattern for comb grouper, for

309 the Copacabana small-scale fishery. We added genetics to better understand the species and to
310 gain insights into their conservation and biology. With this study, we hope to fill some gaps in
311 Brazil and contribute to management efforts.
312
313 By comparing catches of dusky grouper and comb grouper, contrasting patterns are found
314 in the catches of the studied groupers: while we found relative stability for the catches of dusky
315 grouper, we found very irregular and decreasing catches in terms of number and weight for comb
316 groupers. Even considering that comb grouper is of LC (least concern, IUCN Red List), there is
317 an information gap concerning its diet and reproduction [11]. Thus, an optimistic prognosis is
318 offered from the studies of dusky grouper and Copacabana [16], but there is a pessimistic
319 prognosis for comb grouper due to its decreasing catches when the fishing effort is maintained at
320 a constant level, as was observed for the Copacabana fishery.
321 The results for the genetics of groupers reveal that *E. morio* shows the greatest genetic
322 distance from *Epinephelus*; however, the *Mycteroperca* subfamily has a relatively short genetic
323 distance.
324 How could these data aid in the conservation of species? Such information can help
325 directly and indirectly.
326 Directly, information regarding the reproduction period is helpful for managing fish
327 resources, as fishing closures for comb grouper in the spring could help maintain stocks.

328 Information on diet, in addition to its importance in maintaining available prey, could provide
329 insights for possible aquaculture, which exists in SE Brazil for dusky grouper [29,30].

330 Indirectly, grouper genetics provides basic information for aquaculture; it engenders
331 information that could aid in conservation by finding bottlenecks at some points, as was found
332 for dusky grouper [31]. Here, genetic information could also be helpful for taxonomic analysis of
333 the subfamily *Epinephelus*. Grouper systematics has been reevaluated, restricting the genus
334 *Epinephelus* and expanding *Mycteroperca* when several species of Epinephelus were included in
335 Mycteroperca, including *E. marginatus* [7,32].

336

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344 fish.

345

346

347 ## Compliance with ethical standards

348 This research was approved and signed by B. R. Martins dos Santos, Comitê de Ética,
349 Universidade Santa Cecília, number 1.747.889 on September 27, 2016 (Plataforma Brasil). It is
350 approved under number 53824 at the SISBIO and is registered under number AB53669 at the
351 SISGEN, MMA (Ministério do Meio Ambiente, Brasil).
352

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Appendix

Fig 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. acutirostis* (red dots), *M. bonaci* (green) and *M. interstitialis* (blue).

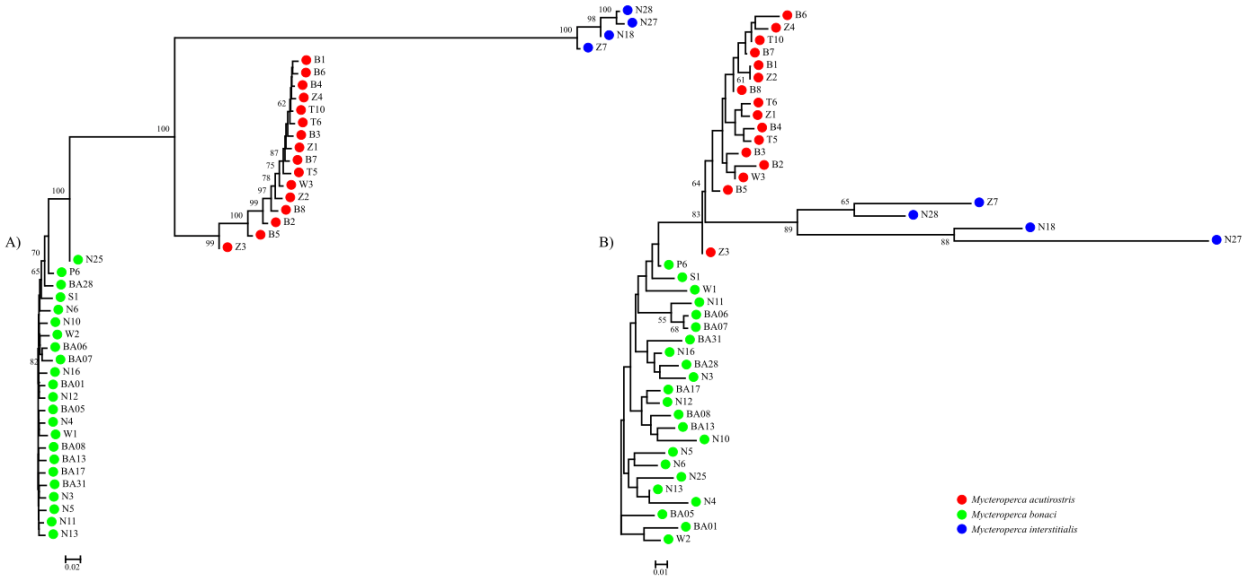
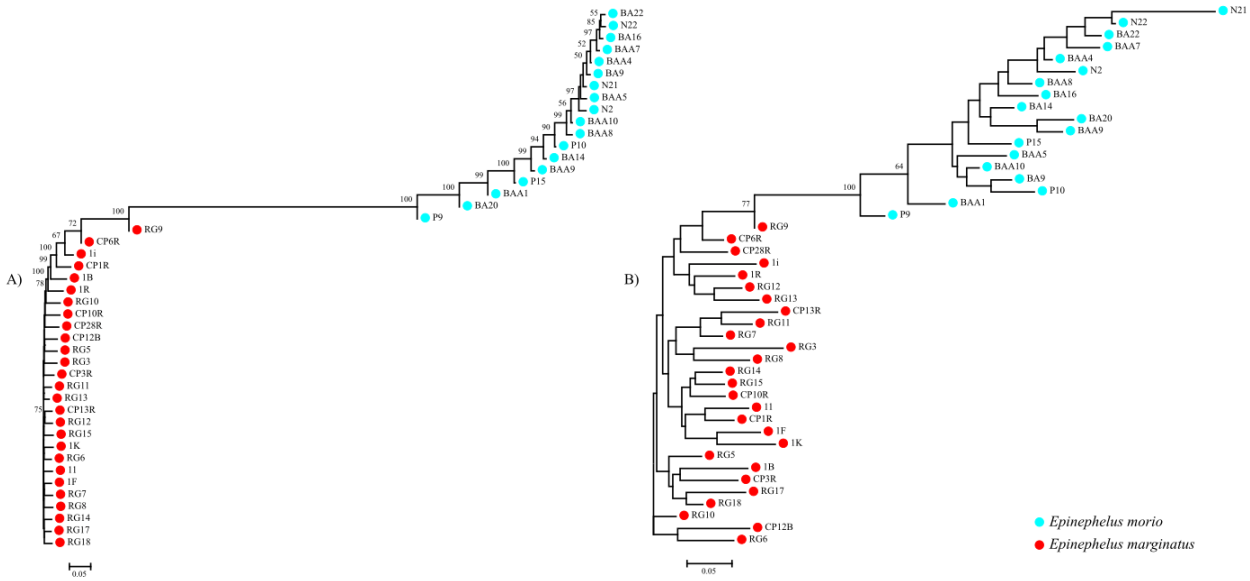
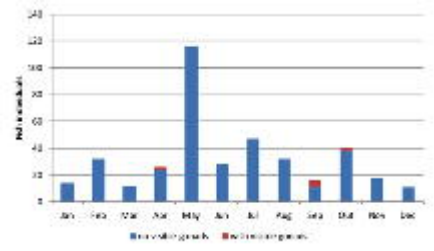


Fig A2. Neighbor-joining Trees Based on Nei's Genetic Distances Using the Following Sets of Loci: (A) a panel of 3,490 putatively neutral SNPs and (B) a panel of 38 putatively adaptive SNPs. Branch nodes are denoted as the percentage of bootstrap support that was generated with 1,000 replicates. Collection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



451


Rio de Janeiro
Ilha Cagarras
Ilha das Palmas
Ilha Redonda
Ilha Rasa
Ilha Pedra de Guaratiba


Jan
Feb
Mar
Apr
May
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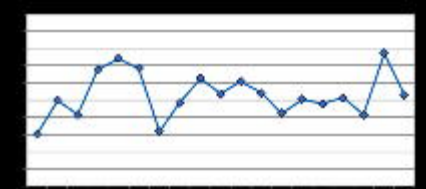
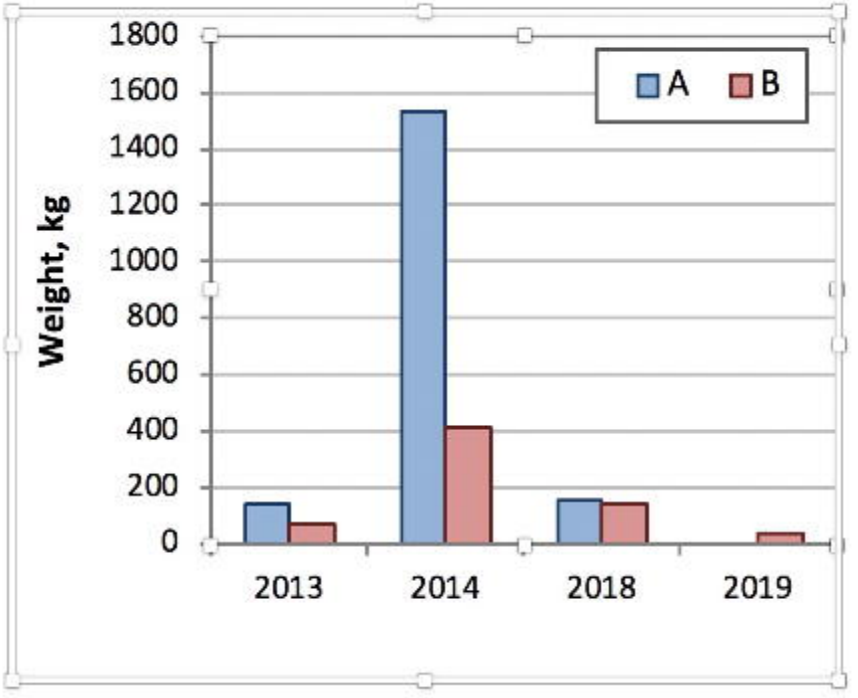
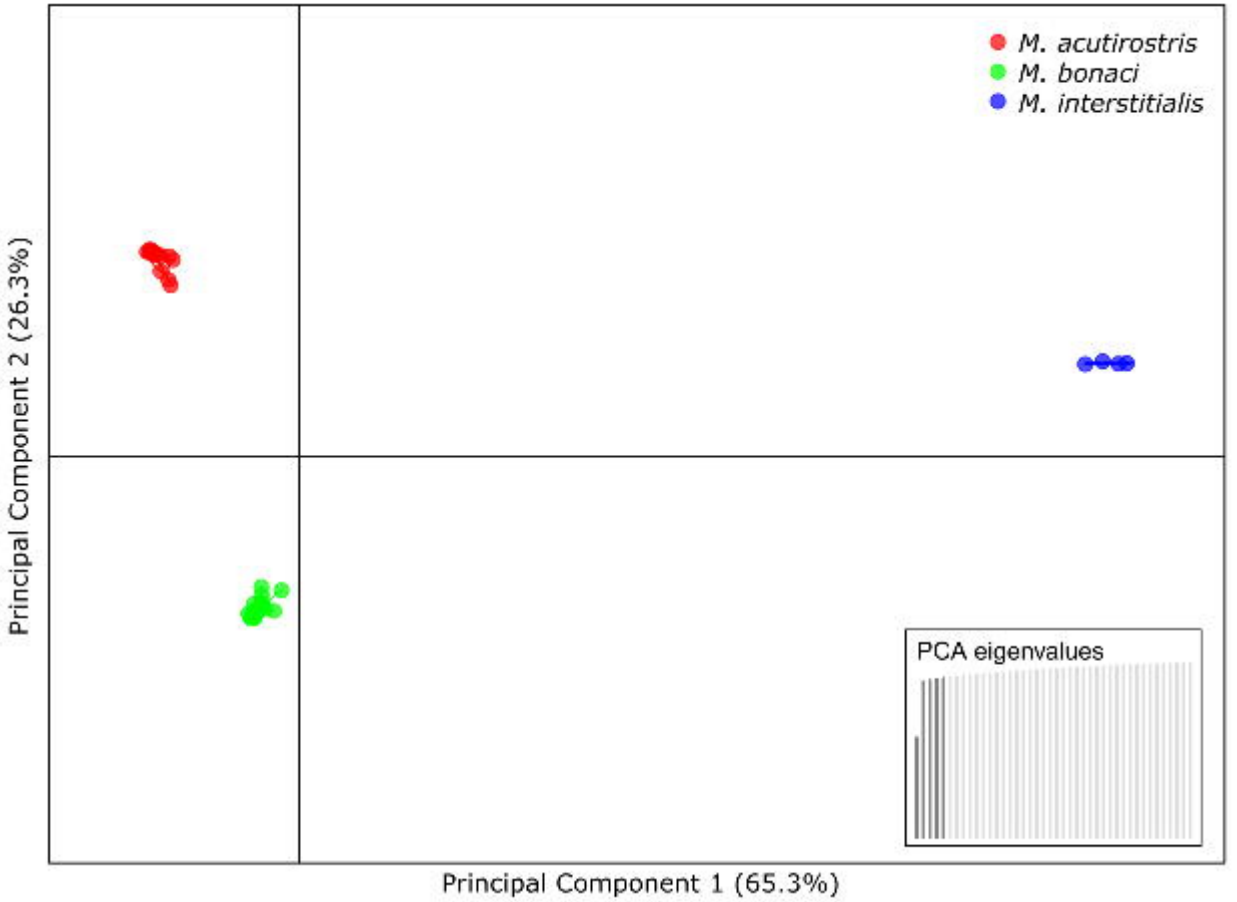
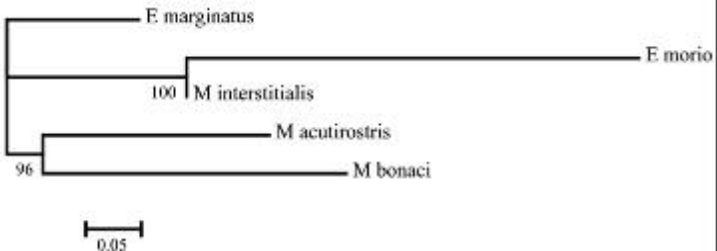



1800
1600
1400
1200
1000
800
600
400
200
0
Weight, kg
A
B
2013
2014
2018
2019


M. acutirostris
M. bonaci
M. interstitialis
Principal Component 2 (26.3%)
PCA eigenvalues
Principal Component 1 (65.3%)


E marginatus
E morio
100
M interstitialis
M acutirostris
96
M bonaci
0.05