STOCK STRUCTURE AND VULNERABILITY OF COMMERCIALLY EXPLOITED FISHES IN BRAZIL

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Statement of Originality

This thesis entitled **Stock structure and vulnerability of commercially exploited fishes in Brazil** is being submitted to Macquarie University and Universidade Federal do Rio Grande do Norte in accordance with the Cotutelle agreement dated 5th of March 2019. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

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Statement on the contribution of others

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SUMMARY

Sustainable management of fish stocks is critical to the conservation of marine biodiversity, but is often hindered by knowledge gaps, which include the spatial distribution of fish stocks, how this distribution is influenced by environmental characteristics and the consequences of connectivity to genetic variation. In this thesis I describe the conservation status, effective population size, genetic structure and connectivity of commercially important marine fishes in Brazil. The first chapter presents an analysis of catch data for 132 species over 61 years. The results indicate that the magnitude of population decline is influenced by the type of exploitation and complex interactions between climatic and biological factors. The second chapter analyzes mitochondrial DNA (mtDNA) at COI and CytB and describes the genetic structure and historical demography for 17 species of commercially exploited marine fish. Here, the genetic structure implies a lack of environmental barriers to gene flow along the Brazilian coast for 15 of these species. The exceptions were the mtDNA structure identified for *Pomatomus saltatrix* and *Cynoscion jamaicensis*, which indicate genetic discontinuity due to temperature changes and isolation by distance, respectively. The goal of the third chapter was to, through the analysis of single nucleotide polymorphisms (SNPs), characterize the effective population size of two reef species – *Lutjanus jocu* and *Sparisoma axillare* – and examine the influence of past climatic variability and habitat suitability on changes on the effective population size. The population of *Sparisoma axillare* expanded until five thousand years ago due to increased habitat suitability, and has been mostly stable since then. The population of *L. jocu* has been stable in the last one million years, although an expansion was detected prior to this period. Although *L. jocu* currently finds less suitable habitats than in the past, this change does not seem related to population size estimates. The distribution of *L. jocu*, from south Brazil to Caribe, is larger than that of *S. axillare*, which is endemic to Brazil, so the first is likely less susceptible to regional climate change. In the fourth chapter, I identified which seascape characteristics explain patterns of genetic connectivity for *S. axillare*, by analyzing samples collected at six locations on the Brazilian coast and also at the islands of Fernando de Noronha, Abrolhos and Trindade, which are located at various distances from the coast. The results indicate that only individuals at Trindade Island, the most distant one, are genetically distinct. I found that seascape factors, such as bathymetry and oceanic currents are better at explaining genetic variation than geographical distance. The results of this thesis suggest that some environmental variables affect species conditions: temperature can positively affect some stocks, but species vulnerable to warmer temperatures have a higher chance of collapse; *Sparisoma axillare*, a species with vulnerable characteristics, seems to be sensitive to past changes in habitat suitability, whereas depth and oceanic currents influence its population connectivity, increasing the risks of its isolated population.

Key words: Catch-based analysis, fishery, fish stock, effective population size, species distribution modelling, population connectivity

GENERAL INTRODUCTION

Genetic tools applied to fishery science

In the current biodiversity crisis, the importance of applied science is paramount. Scientific data are helping to identify high priority areas and more vulnerable species to be protected, in addition to pointing out more sustainable ways of using natural resources. Marine fisheries support millions of people worldwide, but their unsustainable exploitation has resulted in the decline or collapse of fish stocks, threatening the human communities that depend on them, especially in developing countries (FAO, 2016). Fishery science is engaged in finding sustainable solutions for the maintenance of both peoples' livelihood and biodiversity. Among several scientific tools, genetic-related ones have been developed and applied in fishery management in the last decades (Ovenden et al., 2013). Recent technological developments have increased the array of genetic applications in fisheries and lowered their costs, opening new doors to fill the knowledge gaps that especially plague developing countries.

Fish stocks are defined as groups of individuals of a same species demographically independent from others (Carvalho & Hauser, 1994) and stock structure can be characterised using genetic tools. Mitochondrial DNA was used for years, but these markers were not powerful enough and identified a very low level of structure in marine organisms. On the other hand, the use of microsatellite markers is considered a better approach to identify structure because they can resolve more recent differentiation (e.g. Xue et al., 2014). Consequently, different genetic markers can result in contrasting results, for example, mitochondrial DNA may not identify population structure, whereas microsatellites do (Xue et al., 2014). Currently, new sequencing-based methods, called Single Nucleotide Polymorphism (SNPs), identify a much larger number of markers, which allows testing for population structure more accurately with a lower sample size. In addition, large SNP-based datasets open a new door to investigate local adaptation. Usually, mtDNA markers and microsatellites are considered to be selectively neutral. With large SNP datasets, it is possible to identify both neutral loci and those under selection.

Brazilian Marine Environment

The large latitudinal range (4ºN to 33ºS), across almost 8 thousand kilometers of the Brazilian coastline results in an environmental gradient. The northern region, with the Amazonas River outflow, is rich in nutrients that result in high primary productivity, which, on its turn, translates into high fishery production (McGlinley, 2008). This region is marked by two special

environments, the deep sponge reefs near the Amazonas outflow, one of the most unique reef formations, and the reefs that grow at giant pinnacles, from 30 m to sea level, at Parcel Manuel Luiz (Leão et al., 2016). The less productive northeast region is marked by high species richness and reef environments (Miloslavich et al., 2011). Reef formations from the northeast coast up to the large São Francisco River outflow (10ºS of latitude) are characterised as coastal, connected to the coast or extending in parallel to the coastline (Leão et al., 2016). South of the São Francisco outflow there is an especially high density of reefs (Leão et al., 2016). In this region, on an enlargement of the continental shelf lies the Abrolhos Bank, the most biodiversity rich area in the South Atlantic (Miloslavich et al., 2011). Abrolhos reef formations are of great interest because of their unique characteristics of large abundance of corals and high animal diversity (Leão & Kikuchi, 2001). The area from Bahia to Santa Catarina has the highest species richness and is considered an independent biogeographical province by Pinheiro et al. (2018). The Southeastern region is subtropical, and has a high general fish abundance (Miloslavich et al., 2011), although with a low reef species biomass compared to the oceanic islands and the northeast coast (Morais et al., 2017). Its high productivity is due to environmental conditions, such as the presence of an upwelling system that positively affects biomass and biodiversity, especially near Cabo Frio (Brandini, 2006). The South region is characterised by environmental conditions including low sea temperature, absence of reefs, sparse mangrove occurrence, and lower species richness (Miloslavich et al., 2011).

In addition to its high coastal biodiversity, Brazil also has high levels of endemism and reef fish biomass in its oceanic islands. These include the archipelagos of Fernando de Noronha and Atol das Rocas, the archipelago of São Pedro e São Paulo, and Trindade e Martin Vaz islands (Morais et al., 2017). Fernando de Noronha and Atol das Rocas are located less than 400 km from the coast (Rio Grande do Norte State), between 3ºS and 4ºS of latitude. Atol das Rocas is the only atoll of the Southwest Atlantic and presents a large diversity of reef formation, despite its relatively small size (Leão et al., 2016). Fernando de Noronha and Trindade e Martin Vaz are volcanic islands that can be considered as connected to the coast by seamount chains. However, Trindade e Martin Vaz islands are located further southern, at 20ºS of latitude, and over one thousand kilometers from the nearest coast (Espírito Santo State). The archipelago of São Pedro e São Paulo consists of small rocky islands, located at latitude 3ºN of latitude, more than a thousand kilometers from the coast (Rio Grande do Norte state).

Natural changes in marine environment

The idea that oceans are homogeneous areas without barriers raises questions about how marine diversity arises. Characteristics of marine organisms such as life histories that include large and open populations, and at least one pelagic phase, make it even harder to answer this question (Carr et al., 2003). The speciation of organisms that share these characteristics are thought to be shaped by five mechanisms: i) barriers; ii) isolation by distance; iii) dispersal limiting behavior; iv) selection; v) recent species demographic and evolutionary history (Palumbi, 1994).

Both barriers to gene flow and local environmental variability can lead to diversification (Rundle & Nosil, 2005). Some clear barriers are responsible for speciation, such as the Panama Isthmus uplift that split marine populations from the Atlantic and the Pacific 15 million years ago (Coates & Stallard, 2013). Other types of barriers can be more permeable, such as river outflows or deep open oceanic areas. However, "invisible barriers" can also play an important role in diversification. Oceanic currents are one example, because they can limit and direct individual dispersal during pelagic phases and influence gene flow (Palumbi, 1994). Temperature, salinity and productivity gradients can also have an "invisible barrier" effect.

However, diversification can also occur without barriers. Although pelagic larvae disperse over large distances, most will establish in closer areas, creating an isolation by distance pattern (Palumbi, 1994). The extent to which geographically distant populations will be genetically isolated strongly depends on the environment and species biology (Palumbi, 1994). Around 50% of studies testing geographic distance as one of the factors influencing genetic divergence found significant relationships (Selkoe et al., 2016).

Selective pressures can also play an important role in diversification. Occasionally, different populations can occupy different niches that lead to genetic differentiation caused by adaptation to local conditions (e.g. Schroth et al., 2002). Thus, widely distributed species can have populations occupying different environments, and these differences can lead to diversification. These adaptive processes can play an even more important role in genetic differentiation between populations than barriers or geographic distance (Chen et al., 2019; Frankham, 1997).

Climate can also have species-specific effects on survival (Pörtner & Farrell, 2008). Negative effects of a warming climate have already been reported for adult fish (Pörtner & Knust, 2007), impacting their reproductive capacity (Takasuka et al., 2008), and on larval survival (O'Connor et al., 2007). Although adaptation might be possible for some species, the climate is changing fast, perhaps faster than the adaptive capacity of some populations or species (Pievani, 2014).

Distribution (or niche) models can contribute to an understanding of these patterns (e.g. Milanesi et al., 2018). With technological advances in Geographical Information Systems it is now routine to predict distributions and identify high or low environmental suitability. These niche models now also allow the use of past and expected future climate models to predict or estimate changes in species distribution (e.g. Martínez et al., 2018).

The Anthropocene

The current epoch is called by many as the Anthropocene because of the human impact on the natural environment, including an enormous biodiversity crisis referred to as the sixth mass extinction event (Corlett, 2015; Dirzo et al., 2014; Pievani, 2014). In America, for example, human communities were responsible for the extinction of 72% of the large mammals in the past, and the impact is currently increasing (Barnosky et al., 2004). Threatening processes include natural habitat degradation, pollution and overexploitation. The same processes have impacted all marine ecosystems (Halpern et al., 2007). It is predicted that, if greenhouse gas emissions continue unabated, climate change will threaten one sixth of all species, especially in South America and Oceania (Urban, 2015). This rate is the same for all ecosystems, including the marine environment.

Global climatic change increases the temperature, changes pluviosity, and increases ocean acidity and extreme event frequency (IPCC, 2014). Because the oceanic temperature typically varies only slightly, it is possible that marine organisms have a lower capacity to deal with large changes in comparison with terrestrial organisms (McCauley et al., 2015). Although tropical species might be supposed to be pre-adapted to sea warming, small increases in temperature can be disastrous, as reported for tropical corals (McCauley et al., 2015; Parmesan, 2006). Coral bleaching has been shown to be more intense in tropical regions, in intermediate latitudes, between 15º and 20º (Sully et al., 2019).

In Brazil, climate change has affected pluviosity, with drought intensification, affecting mainly semi-arid regions, and extreme flood events being seen in several regions (da Silva, 2004). Mangrove areas, important for the recruitment of several fish species, are predicted to disappear in the Southeast region due to increases in temperature and sea level (Godoy & Lacerda, 2015). Increased sea temperature has been reported for the Brazilian coast (Bernardino et al., 2015) causing coral bleaching in some areas (Leão et al., 2016).

Despite the climatic effects on marine biodiversity, there is a consensus that most damage is caused by unmanaged fisheries (Costello et al., 2010), especially on the more easily accessed coastal species (Halpern et al., 2007). For decades researchers have been warning about fish stock

overexploitation, especially marine stocks (Hauser et al., 2002; Hoarau et al., 2005; Hutchinson et al., 2003). Some estimates suggest that 60% of the marine stocks evaluated were already fully exploited in 2011 (FAO, 2016). A majority of the affected organisms are the k-selected species that are often at the top of the food chain, such as rays, sharks, groupers, snappers and other large body carnivorous fishes (Pauly et al., 1998; Bender et al., 2013). However, smaller species are also declining due to fishing pressure and other factors, such as climate variability sensitivity (Pinsky et al., 2011).

Brazilian fisheries catch around 585 thousand tons annually (MPA, 2011) and is associated with the employment of 3.5 million people (WWF-Brasil, 2016). Fishing occurs more frequently in the coastal regions and targets a large diversity of fish (WWF-Brasil, 2016). The northeastern coast provides 37% of the country's catch, mostly through small-scale fisheries exploiting a large diversity of species. On the South and Southeast regions, the main fleet is industrial, species diversity is lower, and productivity is higher, and represents almost 50% of all the national fishery production (WWF-Brasil, 2016). The high fish diversity, types of fishery, large coastline and decentralized landings, besides the lack of funding and management, result in poor or, currently, no fishery statistics at all in Brazil (WWF-Brasil, 2016). For example, there is no reliable information on fishing effort, especially in the Northeast region. In data-poor regions, the use of catch data to analyze species compositions is an alternative. Also, fishery reconstruction data, which is based on a scientific effort to congregate different sorts of data and model missing information, are extremely useful to fill information gaps and refine the data (Freire et al., 2015).

Fishery management and spatial planning

Despite the increase in marine protected areas on the Brazilian coast (Ervin et al., 2010; Nagelkerken et al., 2015; Araújo & Bernard, 2016), the lack of enforcement and inappropriate management minimize their effectiveness (Maretti, 2001; Santos & Schiavetti, 2014). Marine nursery areas, for example, are of great ecological value, and are also among the most impacted environments (Ervin et al., 2010; Nagelkerken et al., 2015). Moreover, the biased distribution of protected areas worsens the situation, frequently excluding vulnerable areas due to other interests and ignoring experts' opinions (Magris et al., 2013; Giglio et al., 2017). New strategies for selecting protected areas should now account for climate change (Green et al., 2014), but this is still far from the Brazilian reality. Still, the Brazilian government has proposed initiatives to establish and monitor protected areas, including an analysis of the conservation priority area along the coast (MMA, 2012), although no concrete decisions have been made.

Globally, marine conservation strategies lag behind those adopted for the terrestrial environment. Less than 4% of marine areas are protected and those that tend to be smaller than many home ranges of animals that should benefit from this protection (McCauley et al., 2015). Moreover, the combination of natural and anthropogenic effects on marine ecosystems affects habitat change and biodiversity loss in Brazilian marine ecoregions (Bernardino et al., 2015; Lana et al., 2018). However, unlike in the terrestrial environment, only recently has the importance of connectivity between marine protected areas really been included in the conservation debate (Botsford et al., 2009; Burgess et al., 2014). Indirect measures, such as the use of genetic tools, are of great use to better understand connectivity in marine environments, given the difficulty of directly observing dispersal in all levels.

Thesis structure

In Chapter 1, we analysed a large database of fishing catches from 1950 to 2010 of 132 species in order to identify which factors, including fishing, climate and biological traits, increase species vulnerability. In the second chapter, we used mitochondrial DNA data from 17 species of commercial importance in Brazil. The objective was to identify if there is a general pattern of genetic structure and historical demography along the Brazilian coast. In Chapter 3, we aimed to identify the effective population size variability of two reef fish species – *Lutjanus jocu* and *Sparisoma axillare* – and to test the correlation between past climate and population size. In Chapter 4, we identified which features of the seascape explain *Sparisoma axillare* genetic connectivity patterns by analyzing samples collected from six coastal sites and three islands.

REFERENCES
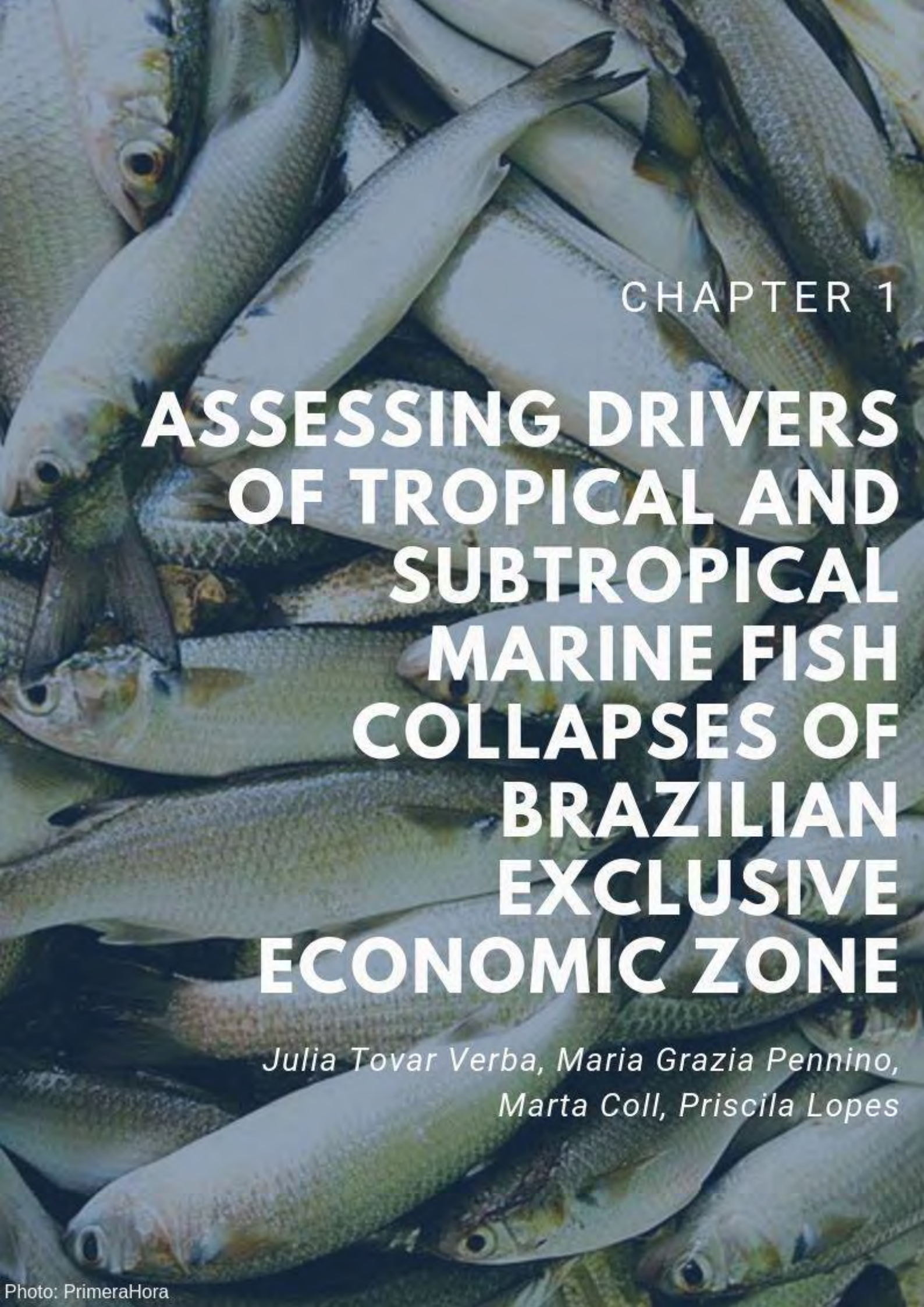
Araújo, J. L., & Bernard, E. (2016). Management effectiveness of a large marine protected area in Northeastern Brazil. *Ocean & Coastal Management*, *130*, 43–49. https://doi.org/10.1016/j.ocecoaman.2016.05.009

Barnosky, A. D., Koch, P., Feranec, R., Wing, S., & Shabel, A. (2004). Assessing the causes of late pleistocene extinctions on the continents. *Science*, *306*(5693), 70–75. https://doi.org/10.1126/science.1101476

Bender, M. G., Pie, M. R., Rezende, E. L., Mouillot, D., & Floeter, S. R. (2013). Biogeographic, historical and environmental influences on the taxonomic and functional structure of Atlantic reef fish assemblages. *Global Ecology and Biogeography*, *22*(11), 1173-1182. https://doi.org/10.1111/geb.12099

Bernardino, A. F., Netto, S. A., Pagliosa, P. R., Barros, F., Christofoletti, R. A., Rosa Filho, J. S.,

… Lana, P. C. (2015). Predicting ecological changes on benthic estuarine assemblages through decadal climate trends along Brazilian Marine Ecoregions. *Estuarine, Coastal and Shelf Science*, *166*, 74–82. https://doi.org/10.1016/j.ecss.2015.05.021

Botsford, L. W., Brumbaugh, D. R., Grimes, C., Kellner, J. B., Largier, J., O'Farrell, M. R., … Wespestad, V. (2009). Connectivity, sustainability, and yield: bridging the gap between conventional fisheries management and marine protected areas. *Reviews in Fish Biology and Fisheries*, *19*(1), 69–95. https://doi.org/10.1007/s11160-008-9092-z

Brandini, F. P. (2006). Hidrografia e produção biológica na região sudeste-sul do Brasil, no contexto do Programa REVIZEE. In *O Ambiente oceanografico da plataforma continental e do talude na regiao sudeste-sul do Brasil* (pp. 447–460).

Burgess, S. C., Nickols, K. J., Griesemer, C. D., Barnett, L. A. K., Dedrick, A. G., Satterthwaite, E. V., … Botsford, L. W. (2014). Beyond connectivity: how empirical methods can quantify population persistence to improve marine protected-area design. *Ecological Applications*, *24*(2), 257–270. https://doi.org/10.1890/13-0710.1

Carr, M. H., Neigel, J. E., Estes, J. A., Andelman, S., Warner, R. R., & Largier, J. L. (2003). Comparing marine and terrestrial ecosystems: implications for the design of coastal marine reserves. *Ecological Applications*, *13*(1), 90–107. https://doi.org/10.1890/1051-0761

Carvalho, G. R., & Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries*, *4*(3), 326–350. https://doi.org/10.1007/BF00042908

Chen, C.-T., Robitzch, V., Sturaro, N., Lepoint, G., Berumen, M. L., & Frédérich, B. (2019). 'Homemade': the phenotypic diversity of coral reef damselfish populations is driven by the local environment. *Biological Journal of the Linnean Society*. https://doi.org/10.1093/biolinnean/blz049

Coates, A. G., & Stallard, R. F. (2013). How old is the Isthmus of Panama? *Bulletin of Marine Science*, *89*(4), 801–813. https://doi.org/10.5343/bms.2012.1076

Corlett, R. T. (2015). The Anthropocene concept in ecology and conservation. *Trends in Ecology & Evolution*, *30*(1), 36–41. https://doi.org/10.1016/j.tree.2014.10.007

Costello, M. J., Coll, M., Danovaro, R., Halpin, P., Ojaveer, H., & Miloslavich, P. (2010). A census of marine biodiversity knowledge, resources, and future challenges. *PLoS ONE*, *5*(8), e12110. https://doi.org/10.1371/journal.pone.0012110

da Silva, V. P. R. (2004). On climate variability in Northeast of Brazil. *Journal of Arid Environments*, *58*(4), 575–596. https://doi.org/10.1016/j.jaridenv.2003.12.002

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014). Defaunation in the Anthropocene. *Science*, *345*(6195), 401–406. https://doi.org/10.1126/science.1251817

Ervin, J., Mulongoy, K. J., Lawrence, K., Game, E., Sheppard, D., Bridgewater, P., … Bos, P. (2010). *Making Protected Areas Relevant: A guide to integrating protected areas into wider landscapes, seascapes and sectoral plans and strategies*. Montreal.

FAO. (2016). The State of World Fisheries and Aquaculture. In *Contributing to food security and nutrition for all*. https://doi.org/10.5860/CHOICE.50-5350

Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? Heredity, 78(3), 311–327. https://doi.org/10.1038/hdy.1997.46

Freire, K. M. F., Aragão, J. A. N., Araújo, A. R. R., Ávila-da-Silva, A. O., Bispo, M. C. S., Canziani, G. V., … Divovich, E. (2015). Reconstruction of catch statistics for Brazilian marine waters (1950-2010). In Kátia Meirelles Felizola Freire & D. Pauly (Eds.), *Fisheries catch reconstructions for Brazil's mainland and oceanic islands*. Fish. Centre Res. Rep.

Giglio, V. J., Bender, M. G., Zapelini, C., & Ferreira, C. E. L. (2017). The end of the line? Rapid depletion of a large-sized grouper through spearfishing in a subtropical marginal reef. *Perspectives in Ecology and Conservation*, *15*(2), 115–118. https://doi.org/10.1016/j.pecon.2017.03.006

Godoy, M. D. P., & Lacerda, L. D. de. (2015). Mangroves Response to Climate Change: A Review of Recent Findings on Mangrove Extension and Distribution. *Anais Da Academia Brasileira de Ciências*, *87*(2), 651–667. https://doi.org/10.1590/0001-3765201520150055

Green, A. L., Fernandes, L., Almany, G., Abesamis, R., McLeod, E., Aliño, P. M., … Pressey, R. L. (2014). Designing marine reserves for fisheries management, biodiversity conservation, and climate change adaptation. *Coastal Management*, *42*(2), 143–159. https://doi.org/10.1080/08920753.2014.877763

Halpern, B. S., Selkoe, K. A., Micheli, F., & Kappel, C. V. (2007). Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. *Conservation Biology*, *21*(5), 1301–1315. https://doi.org/10.1111/j.1523-1739.2007.00752.x

Hauser, L., Adcock, G. J., Smith, P. J., Bernal Ramirez, J. H., & Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences*, *99*(18), 11742–11747. https://doi.org/10.1073/pnas.172242899

Hoarau, G., Boon, E., Jongma, D. N., Ferber, S., Palsson, J., Van der Veer, H. W., … Olsen, J. L. (2005). Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proceedings of the Royal Society B: Biological Sciences*, *272*(1562), 497–503. https://doi.org/10.1098/rspb.2004.2963

Hutchinson, W. F., Oosterhout, C. van, Rogers, S. I., & Carvalho, G. R. (2003). Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1529), 2125–2132. https://doi.org/10.1098/rspb.2003.2493

IPCC. (2014). *Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernamental Panel on Climate Change.* (R. K. P. and L. A. M. Core Writing Team, Ed.). Geneva.

Lana, P., Christofoletti, R., Gusmão Jr, J. B., Barros, T. L., Spier, D., Costa, T. M., … dos Santos, C. S. G. (2018). *Benthic Estuarine Assemblages of the Southeastern Brazil Marine Ecoregion (SBME)*. https://doi.org/10.1007/978-3-319-77779-5_5

Leão, Z. M. A. N., & Kikuchi, R. K. P. (2001). The Abrolhos reefs of Brazil. In *Coastal marine ecosystems of Latin America* (pp. 83-96). Springer, Berlin, Heidelberg.

Leão, Z. M. A. N., Kikuchi, R. K. P., Ferreira, B. P., Neves, E. G., Sovierzoski, H. H., Oliveira, M. D. M., … Johnsson, R. (2016). Brazilian coral reefs in a period of global change: A synthesis. *Brazilian Journal of Oceanography*, *64*(spe2), 97–116. https://doi.org/10.1590/S1679-875920160916064sp2

Magris, R. A., Mills, M., Fuentes, M. M. P. B., & Pressey, R. L. (2013). Analysis of progress

towards a comprehensive system of Marine Protected Areas in Brazil. *Natureza & Conservação*, *11*(1), 81–87. https://doi.org/10.4322/natcon.2013.013

Maretti, C. (2001). Comentários sobre a situação das unidades de conservação no Brasil. *Revista de Direitos Difusos*, *5*, 633–652.

Martínez, B., Radford, B., Thomsen, M. S., Connell, S. D., Carreño, F., Bradshaw, C. J. A., … Wernberg, T. (2018). Distribution models predict large contractions of habitat-forming seaweeds in response to ocean warming. *Diversity and Distributions*, *24*(10), 1350–1366. https://doi.org/10.1111/ddi.12767

McCauley, D. J., Pinsky, M. L., Palumbi, S. R., Estes, J. A., Joyce, F. H., & Warner, R. R. (2015). Marine defaunation: Animal loss in the global ocean. *Science*, *347*(6219), 1255641–1255641. https://doi.org/10.1126/science.1255641

McGlinley, M. (2008). East Brazil Shelf large marine ecosystem. In *Encyclopedia of Earth*. Retrieved from http://editors.eol.org

Milanesi, P., Caniglia, R., Fabbri, E., Puopolo, F., Galaverni, M., & Holderegger, R. (2018). Combining Bayesian genetic clustering and ecological niche modeling: Insights into wolf intraspecific genetic structure. *Ecology and Evolution*, *8*(22), 11224–11234. https://doi.org/10.1002/ece3.4594

Miloslavich, P., Klein, E., Díaz, J. M., Hernández, C. E., Bigatti, G., Campos, L., … Martín, A. (2011). Marine biodiversity in the Atlantic and Pacific Coasts of South America: knowledge and gaps. *PLoS ONE*, *6*(1), e14631. https://doi.org/10.1371/journal.pone.0014631

MMA. (2012). *Panorama da Conservação dos Ecossistemas Costeiros e Marinhos no Brasil. Page (Gerência de Biodiversidade Aquática e Recursos Pesqueiros., ed.) Gerência de Biodiversidade Aquática e Recursos Pesqueiros* (MMA, Ed.). Brasília.

Morais, R. A., Ferreira, C. E. L., & Floeter, S. R. (2017). Spatial patterns of fish standing biomass across Brazilian reefs. *Journal of Fish Biology*, *91*(6), 1642-1667. https://doi.org/10.1111/jfb.13482

MPA. (2011). *Boletim estatístico da pesca e aquicultura*.

Nagelkerken, I., Sheaves, M., Baker, R., & Connolly, R. M. (2015). The seascape nursery: a novel spatial approach to identify and manage nurseries for coastal marine fauna. *Fish and Fisheries*, *16*(2), 362–371. https://doi.org/10.1111/faf.12057

O'Connor, M. I., Bruno, J. F., Gaines, S. D., Halpern, B. S., Lester, S. E., Kinlan, B. P., & Weiss, J. M. (2007). Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proceedings of the National Academy of Sciences*, *104*(4), 1266–1271. https://doi.org/10.1073/pnas.0603422104

Ovenden, J. R., Berry, O., Welch, D. J., Buckworth, R. C., & Dichmont, C. M. (2015). Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. *Fish and Fisheries*, *16*(1), 125-159. https://doi.org/10.1111/faf.12052

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, *25*, 547–572. https://doi.org/10.1146/annurev.es.25.110194.002555

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual*

Review of Ecology, Evolution, and Systematics, *37*(1), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., & Jr, F. T. (1998). Fishing down marine food webs. *Science*, *279*(5352), 860–863. https://doi.org/10.1126/science.279.5352.860

Pievani, T. (2014). The sixth mass extinction: Anthropocene and the human impact on biodiversity. *Rendiconti Lincei*, *25*(1), 85–93. https://doi.org/10.1007/s12210-013-0258-9

Pinsky, M. L., Jensen, O. P., Ricard, D., & Palumbi, S. R. (2011). Unexpected patterns of fisheries collapse in the world's oceans. *Proceedings of the National Academy of Sciences*, *108*(20), 8317–8322. https://doi.org/10.1073/pnas.1015313108

Pörtner, H. O., & Farrell, A. P. (2008). Ecology: Physiology and climate change. *Science*, *322*(5902), 690–692. https://doi.org/10.1126/science.1163156

Pörtner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, *315*(5808), 95–97. https://doi.org/10.1126/science.1135471

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Santos, C. Z., & Schiavetti, A. (2014). Spatial analysis of Protected Areas of the coastal/marine environment of Brazil. *Journal for Nature Conservation*, *22*(5), 453–461. https://doi.org/10.1016/j.jnc.2014.05.001

Schroth, W., Jarms, G., Streit, B., & Schierwater, B. (2002). Speciation and phylogeography in the cosmopolitan marine moon jelly, *Aurelia* sp. *BMC Evolutionary Biology*, *2*(1), 1. https://doi.org/10.1186/1471-2148-2-1

Selkoe, K., D'Aloia, C., Crandall, E., Iacchei, M., Liggins, L., Puritz, J., … Toonen, R. (2016). A decade of seascape genetics: contributions to basic and applied marine connectivity. *Marine Ecology Progress Series*, *554*, 1–19. https://doi.org/10.3354/meps11792

Sully, S., Burkepile, D. E., Donovan, M. K., Hodgson, G., & van Woesik, R. (2019). A global analysis of coral bleaching over the past two decades. *Nature communications*, *10*(1), 1264. https://doi.org/10.1038/s41467-019-09238-2

Takasuka, A., Oozeki, Y., & Kubota, H. (2008). Multi-species regime shifts reflected in spawning temperature optima of small pelagic fish in the western North Pacific. *Marine Ecology Progress Series*, *360*, 211–217. https://doi.org/10.3354/meps07407

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573. https://doi.org/10.1126/science.aaa4984

WWF-Brasil. (2016). *Situação atual e tendências da pesca marinha no brasil e o papel dos subsídios*. São Paulo.

Xue, D. X., Wang, H. Y., Zhang, T., & Liu, J. X. (2014). Population genetic structure and demographic history of *Atrina pectinata* based on mitochondrial DNA and microsatellite markers. *PLoS ONE*, *9*(5). https://doi.org/10.1371/journal.pone.0095436

ASSESSING DRIVERS OF TROPICAL AND SUBTROPICAL MARINE FISH COLLAPSES OF BRAZILIAN EXCLUSIVE ECONOMIC ZONE

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ASSESSING DRIVERS OF TROPICAL AND SUBTROPICAL MARINE FISH COLLAPSES OF BRAZILIAN EXCLUSIVE ECONOMIC ZONE

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ABSTRACT

Overfishing is a concerning threat that can lead to the collapse of fish stocks. We assessed combinations of factors, including biological traits, types of exploitation and responses to sea temperature and salinity changes, that drive species to collapse in the Brazilian Exclusive Economic Zone (EEZ) tropical and subtropical regions. We applied a catch-based method of stock classification and a catch time series of 61 years from 132 exploited fish species. Species were categorized as Collapsed, Overexploited, Fully Exploited or in Development, and we used a Generalized Additive Model (GAM) analysis to understand their categorization over time. Furthermore, a Redundancy Analysis was developed to assess the species characteristics that best predicted each exploitation category. Twelve species were classified as Collapsed, 55 as Overexploited, 46 as Fully Exploited and 19 as in Development. Tropical and subtropical exploited species collapses in Brazil were best explained by a complex combination of a negative impact of warmer sea temperatures, fishery exploitation and specific life-history traits. A synergistic interaction between these factors could bring species to collapse. We hypothesize that the exploitation of species with vulnerable traits may alter how these species respond to temperature and, therefore, lead them to collapse given that intense exploitation may affect their ability to respond to temperature increases. Measures to mitigate climate change impacts should take into consideration incentives to decrease the exploitation of vulnerable species and, specifically, consider species with more sensitive biological traits. Such measures are also important to minimize the socioeconomic impacts on the people that depend on these species.

1. INTRODUCTION

The Anthropocene, characterized by a wide variety of anthropic stressors, is strongly transforming the natural environments on Earth (Costello et al., 2012; Lewis & Maslin, 2015). Overexploitation, species extinction, habitat degradation, pollution and climate change are putting the resilience of entire biological communities at risk, leading to dramatic changes in populations of marine and terrestrial species, including declines and collapses. The impact on the oceans is of particular concern, given that more than 38 million people, mostly in tropical low-income countries, are directly or indirectly dependent on aquatic resources (FAO, 2018). Therefore, fish population declines, evidenced by the overexploitation of more than 30% of fish stocks (FAO, 2018) and the global loss of more than 90% of large predatory fish biomass (Myers & Worm, 2003), can have a notable impact on human well-being.

The decline in marine populations is attributed to many causes, with overexploitation being an important one resulting from the fishing industry. Although industrial fisheries are largely to blame for practicing unsustainable levels, small-scale or artisanal fisheries can also reduce richness and abundance of target species (e.g. Alfaro-Shigueto et al., 2010; Goetze et al., 2011; Hawkins & Roberts, 2004). In low-income tropical countries, small-scale fisheries can account for more than 50% of the total catch (Vasconcellos et al., 2007), and are generally poorly or unmanaged both in high- and low-income countries (Berkes et al., 2001). Small-scale fisheries tend to be more restricted to coastal regions, which also happen to be more negatively impacted by cumulative anthropic activities (Jackson et al., 2001). Even selective small-scale practices, such as spearfishing, can negatively affect fish stocks, especially when targeting vulnerable species, such as groupers (Giglio et al., 2017; Lindfield et al., 2014).

Both large- and small-scale exploitation can affect species' capacity to respond to environmental changes through several mechanisms. For example, the excessive extraction of fish can result in

the removal of some population units, which reduces species' capacity to adapt to a changing environment through the "rescue effect" (Gonzalez et al., 1998; Jennings & Blanchard, 2004). The decrease in total abundance can also exclude populations from less suitable areas, leading to a distribution contraction (Blanchard et al., 2005; Ciannelli et al., 2013). Exploitation can affect species' responses to climate variability through size truncation when individuals of a specific body size are removed, due to the preference of fisheries for larger–and older–individuals (Pinsky et al., 2011). Even in moderate levels of exploitations (Berkeley et al., 2004), size truncation can have several effects, such as a decrease in a population's average body size, a reduction in egg-to-recruit survival (because smaller produce less and lower quality eggs, e.g. Hislop, 1988), and a loss of learning capacity due to the loss of older individuals (Planque et al., 2010). Increased mortality due to fishing can also alter growth rates and decrease maturity at age (Enberg et al., 2012) with the potential of affecting the speed at which species respond to a changing environment (Perry et al., 2005).

Changes in sea temperature due to global climate change have been affecting species composition and fish production worldwide (Cheung et al., 2013; Free et al., 2019). The effect of environmental shifts, especially temperature, were detected in nearly seven out of every ten stocks analysed by Vert-pre et al. (2013), with consequences on species occurrence or abundance due to changes in mortality rates and distributions (Blanchard et al., 2005). Nevertheless, there is still much to be learned about how, when and why a warmer sea will affect fish stocks, especially for tropical species, for which there is less information available on ecosystems, communities, populations and individuals (Cheung et al., 2012; Cheung et al., 2016; Free et al., 2019). The response to changes in the environment will depend on the magnitude of climate change and the species adaptation capacity (Torda et al., 2017), which, among other factors, depends on species physiology, dispersion capacity, and genetic diversity (Post & Palkovacs, 2009). Even within the same species, the individual response can vary depending on its life stage, with some stages (e.g., eggs and

larvae) being more vulnerable to temperature changes (Pörtner & Farrell, 2008). Recruits can be more severely impacted by environmental changes, and a decrease in their abundance has a large impact on a population's size (Brander, 2005; Lindegren & Checkley, 2013). On the other hand, warmer oceans can benefit fish production in some areas (Blanchard et al., 2012) or benefit some large herbivores due to effects on food web dynamics (Smith-Ramesh et al., 2017).

It has been suggested that exploitation and climate variability interaction affect demographic dynamics and conservation of fish species (Cheung et al., 2018; Planque et al., 2010). The responses to these two factors, however, vary according to the species and region. For example, in the North Sea, changes in climate affected the primary production while fishing impacted demersal fish abundance, increasing the abundance of macro-invertebrates and unbalancing the whole food web (Heath, 2005). In the western English Channel, a long-term time series of demersal fisheries showed that small body-sized species abundance is more affected by climate, whereas larger bodied species abundance is more influenced by exploitation (Genner et al., 2010). For sardines and anchovy, climate affects long-term population variability, but fisheries can induce higher-frequency variability (Lindegren et al., 2013). Identifying those biological traits that make species respond differently to the same climate and exploitation conditions can help predict future responses of marine communities to climate change (Sunday et al., 2015).

Although there is no consensus (e.g. Pinsky et al., 2013; Schuetz et al., 2018), biological traits play an important role in species vulnerability to climate changes and exploitation (Cheung et al., 2018; Pörtner & Farrell, 2008). Some evidence suggests that large bodied marine fish are more likely to collapse, both because of their own life history traits and the selective harvesting to which they are exposed (Cheung et al., 2018; Dulvy et al., 2003; Olden et al., 2007). Large body species can present higher value for fishers, increasing its exploitation. The time it takes for a species to grow is also relevant, with some evidence suggesting that slow-growth species, such as sharks, are more affected by fisheries and also have a delayed recovery of overexploitation, as well as trophic level,

because top predators can be more sensitive to food web disequilibrium (Quetglas et al., 2016). However, fast-growth species seem to be more affected by climate (Quetglas et al., 2016). Moreover, short-lived species, which tend to grow faster and are positioned at low trophic levels, are also likely to decline due to both climate and exploitation (Pikitch et al., 2018; Pinsky et al., 2011; Pinsky & Byler, 2015). Other traits, such as dispersion capacity, region and distribution range affect the likelihood of species collapse due to fishing or climate variability. Among sharks and rays, more rare and specialized species living in estuarine or coastal areas are more vulnerable to climate change (Chin et al., 2010), whereas species mobility also seems to contribute (Sunday et al., 2015).

Synergistic interactions between climate, fishing and biological traits have been identified in temperate regions (Botsford et al., 2014; Lindegren et al., 2013). Here, we identify how these interactions are related to tropical and subtropical fish stock statuses, and which combinations of biological (habitat, size, trophic level) and external (temperature, salinity, type of fishery and price) are more likely to put species on the brink of collapse We used 61 years of reconstructed data on the fisheries catches of 132 marine species exploited over more than 8,000 km along the Brazilian coastline. To define stock status, we applied a catch-based classification method (Froese & Kesner-reyes, 2002; Kleisner et al., 2013), which is adequate for data-poor areas like Brazil. This is the first assessment that attempts to analyze the responses of a large set of tropical and subtropical fish facing a combination of threats, including overexploitation and climate variability. We expect that larger species, in high trophic levels, exploited by industrial fisheries and with higher prices will be the ones with higher collapse chance.

2. MATERIAL AND METHODS

2.1 Catch data

Brazilian EEZ catch data for 132 studied species were extracted from the Sea Around Us website (www.seaaroundus.org) for the period 1950 to 2010 (Fig. 1). This reconstruction data is a result of a long-term effort project to re-estimate global catch combining reported data from FAO and estimates of other unreported sources of catch (Pauly & Zeller, 2016). The analysed dataset includes industrial and small-scale fisheries catches (excluding discarded fish due to the lack of reliable information) expressed as wet weight equivalent of landings per year for each species.

Species were selected based on their distribution range limited within West Atlantic waters, so stock declines and collapses can be attributed mostly to Brazilian factors (local environmental variables, exploitation and economic factors). Species were representative of 43 families, with a wide range of life history traits: maximum body size varied between 6.8 and 250 cm (average of 64.64±45.87), and trophic level ranged from 2 to 4.5. Of 132 species, 63 were demersal, 26 pelagic and 43 reef species (*Supplementary Material S1*). For 110 of selected species, the exploitation is mainly by small-scale fleet, while for only 22 species the most important exploitation was the industrial fishery (*Supplementary Material S1*).
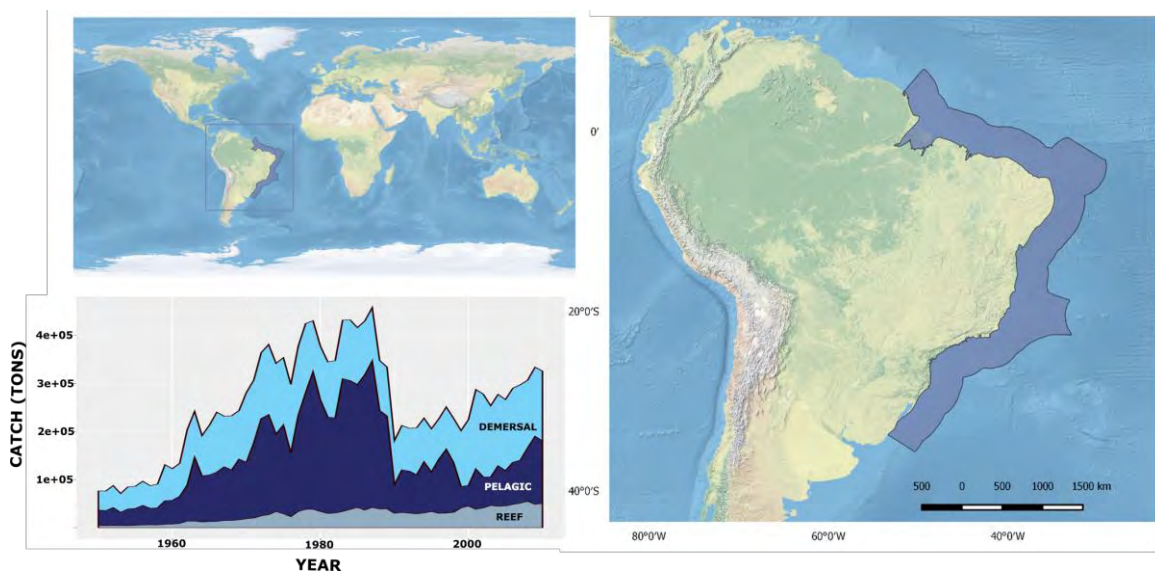
Figure 1: a) Map showing Brazilian Exclusive Economic Zone along a more that 8,000 km of coastline; b) Temporal catch (in tons) of 132 analysed species, separated by habitat (demersal, pelagic or reef).

2.2 Ecological Traits

Four different ecological traits were selected as possible predictors of vulnerability to species collapse: trophic level, maximum body size, resilience category and type of habitat (*Supplementary material Table S1*). All of these features, individually or aggregated, have been shown to affect the vulnerability of species to exploitation or climate change (e.g. Cheung et al., 2018; Dulvy et al., 2003; Olden et al., 2007; Pinsky et al. 2011; Pinsky & Byler, 2015; Quetglas et al., 2016). Information on traits was extracted from the online database FishBase; all species has the necessary information on traits available (Froese & Pauly, 2017).

Trophic level represents the position that each species occupies in the food web, where higher values represent carnivorous species (Froese & Pauly, 2015). Maximum body size is the maximum size recorded for a species. The resilience index aggregates several biological features (growth parameter von Bertalanffy K, the intrinsic rate of population growth, fecundity, life span, and age of first maturity) of a species' life history and is expressed on a four categories scale that varies from high to very low resilience values (Musick, 1999). Finally, the type of habitat preferred by a fish was divided into three levels: demersal, pelagic and reefs.

2.3 External Drivers

2.3.1 Environmental Variables

As a proxy for climate variability, we used annual data of Sea Surface Temperature (SST) and Sea Surface Salinity (SSS) (*Supplementary Material Figure S2*). Data were obtained from the NEMO climatology model (https://www.nemo-ocean.eu/) for Brazil for the time series 1950-2010. The two variables were explored for correlation, outliers, and missing data before including them in the analyses (Zuur et al., 2009). The Pearson correlation test ($r=0.8$, p-value=0.06) identified high correlation between these two variables.

2.3.2 Economic and Fishery Variables

We considered the type of fishery exploitation (industrial or small-scale) and two economic factors (the ex-vessel price and the landed value of the species) as economic and fishery variables (*Supplementary material in Table S1*). The fishery exploitation type was computed as the proportion (from 0 to 100%) caught by small-scale fisheries for each species per year (1950-2010). The classification in small-scale or industrial fishery was provided by Sea Around Us. The ex-vessel price, the value fishers receive when they first sell the catch (in USD), was extracted from the Sea Around Us website and included the period between 1950 and 2010. This quantitative variable was available for only 56 out of the 132 species (Table 2); thus, the analyses that included price and landed value (kg of landing multiplied by ex-vessel price) as one independent variable were only performed for these species. Statistical models for species without economic information available were performed using only the other variables. For the RDA, we also used a qualitative price information variable (very low, low, medium, high, very high price categories), available from FishBase for all the species.

2.4 Stock Status Definition: Exploitation Index (EI) and Exploitation Status (ES)

Species Exploitation Status (ES) was obtained by calculating the Exploitation Index (EI) using the catch-based method of stock classification (Froese & Kesner-Reyes, 2002). It is important to emphasize some important caveats of catch-only methods. First, they do not include any effort data (Walsh et al., 2018). We opted for a catch-only method because effort data is not available for Brazilian fisheries, which suffer from a lack of fisheries monitoring (Ruffino et al., 2016). Second, the occurrence of an unusual high catch in one year (an outlier) could strongly influence the classification of a collapsed stock. Aside from its limitations, this method provides an adequate

indicator of stock status and is frequently the only option available for use in data-poor fisheries, such as Brazil and most developing tropical countries (Chrysafi & Kuparinen, 2015; Froese & Kesner-Reyes, 2002; Ghosh et al., 2015; Kleisner et al., 2013; Thorson et al., 2013; Tsikliras et al., 2015).

Firstly, the EI was defined annually based on the relationship between the catch in a given year and the maximum catch of the historical landing data (catch of the year divided by the maximum catch). The EI ranges from 0 to 1, where values closer to 0 suggest that the species is closer to collapse, and values closer to 1 suggest that the species is at a low exploitation level. Using these values, species were classified into one of the four ES categories: values from 0 to 0.10 are Collapsed stocks, from 0.10-0.50 are Overexploited stocks, from 0.50 to 0.80 are Fully Exploited, and from 0.80-1 are Developing stocks (*Table 1*). To classify the investigated species in each category of ES, we considered the most frequent corresponding value in the last ten years of catches (time series from 2001 to 2010).

Table 1: Definition of the Exploitation Status of a stock using the catch-based method. This method is based on the relationship between the catches (C_Y) of a given year (Y_C) and the year (Y_{Cmax}) of historical maximum catch (C_{max}).

Exploitation status	Criterion applied
Collapsed	$Y_C > Y_{Cmax}$ and $C_Y < 0.1\, C_{max}$
Overexploited	$Y_C > Y_{Cmax}$ and $0.1\, C_{max} < C_Y < 0.5\, C_{max}$
Fully exploited	$C_Y > 0.5\, C_{max}$
Developing	$Y_C < Y_{Cmax}$ and $0.1\, C_{max} < C_Y < 0.5\, C_{max}$

2.5 Statistical Analysis

2.5.1 Generalized Additive Models to assess External Drivers over time

Generalized Additive Models (GAMs; Hastie & Tibshirani, 1987) were used to test the influences of External Drivers on the species EI. In order to normalize the EI a logarithmic transformation was applied to these variables and a Gaussian distribution was used to fit it in the GAMs. Specifically, we tested the percentage of small-scale fisheries per year (considering an inverse relationship between small-scale and industrial fisheries), annual climate variability (sea surface temperature–SST and sea surface salinity–SSS) and economic factors (ex-vessel prices and landed values) on the species EI. The annual EI was used as the response variable. As explicative variables in GAMs cannot be highly correlated among them, SSS and SST (Pearson correlation, r=0.8, p-value=0.06) and ex-vessel price and landed value (Pearson correlation, r=0.85, p-value=0.08) were included in the model separately with the rest of variables.

GAMs are often used for their ability to deal with non-linear and non-monotonic relationships between response variables and explanatory variables (Hastie & Tibshirani, 1987; Wood, 2006). Separated GAMs for each of the 132 species were fitted using a Gaussian distribution.

Models by species were run for each of the possible combinations of terms. Variables were selected with forward and backward stepwise procedures based on three different criteria including Akaike Information Criterion (AIC), Un-Biased Risk Estimator (UBRE) and deviance explained (D^2). The best (and most parsimonious) model was ultimately chosen based on the compromise between low AIC and UBRE values, high D^2 values, and significant predictors. GAMs were performed using the "*mgcv*" package (Wood, 2011) of the R software (R Core Team, 2015).

2.5.2 Redundancy Analysis to identify Ecological Traits and External Drives related to EI

A Redundancy Analysis (RDA, Anderson, 2001) was applied to explore links between the species' ES and intrinsic characteristics of the species (ecological traits) and external parameters. Specifically, the ecological traits used were trophic level, maximum body size, resilience category (very low, low, medium, high) and the type of habitat (demersal, pelagic, reef), whereas the external traits were the percentage of catch by small-scale fisheries (considering total catch) and the ex-vessel price (very low, low, medium, high, very high).

In particular the response variable $y=(y_1, \ldots y_n)$ was the EI matrix of the entire time-series (1950-2010) by species, while the set of explanatory variables $x=(x_1, \ldots x_n)$ was represented by a mean of the ecological and external factors by species.

In RDA it is possible to assess the influence of each co-variables (partial RDA), which allows testing the effect of a particular explanatory variable after removing the variation explained by the co-variables. This technique was used to verify how much of the total variation of the EI was explained by the different variables. The significance of each RDA model was tested using Monte Carlo permutation tests (Manly, 1991) to retain the ten variables that best explained the variation in EI. For this purpose, the "*vegan*" package (Oksanen et al., 2011) of the R software was used.

3. RESULTS
3.1 Exploitation Status

Of the 132 species analysed, 12 species were classified as Collapsed, 55 as Overexploited, 46 as Fully Exploited and 19 as in Development (*Table 2*). External and intrinsic variables tested affected species in each category differently (more details below).

Table 2: Summary of the main results of the multi-step analytical approach. Results of the GAMs refer to the final selected model for each species, based on the compromise between low AIC and UBRE values, high D2 values, and significant predictors. Ex-vessel price and landed value were only available for the species indicated by asterisks. In order to summarize the overall trends of the explicative variable seen all the GAMs fitted, we reported in Table 2 these trends as (+) if the functional curve of the GAM was mainly with an increasing trend and (-) if was mainly a decreasing pattern.

Species	Family	Exploitation status	GAMs significant predictors	GAMs D^2 explained (%)
Amphiarius rugispinis	Ariidae	Collapsed	SST (-)	18
Anchoa januaria	Engraulidae	Fully exploited	SST (+)	15
Anchoa spinifer*	Engraulidae	Collapsed	SSS (+) and Price (-)	45
Anchovia clupeoides	Engraulidae	Developing	SST (+)	15
Anchoviella lepidentostole	Engraulidae	Developing	No significance	
Anchoviella vaillanti	Engraulidae	Fully exploited	SSS (-)	14
Anisotremus surinamensis	Haemulidae	Collapsed	SSS (-)	26
Anisotremus virginicus	Haemulidae	Fully exploited	SST (+)	38
Archosargus rhomboidalis*	Sparidae	Fully exploited	Price (-)	15
Aspistor luniscutis	Ariidae	Overexploited	SST (+)	37
Aspistor quadriscutis*	Ariidae	Fully exploited	SST (+)	42
Astroscopus sexspinosus	Uranoscopidae	Overexploited	SSS (+)	20
Bagre bagre*	Ariidae	Fully exploited	Price (-)	32
Bairdiella ronchus*	Scianidae	Fully exploited	No significance	
Batrachoides surinamensis	Batrachoididae	Fully exploited	SST (+)	45
Brevoortia pectinata*	Clupeidae	Collapsed	SST (-) and Price (+)	42
Calamus pennatula	Sparidae	Collapsed	SSS (-) and Artisanal fishery (-)	88
Carangoides bartholomaei	Carangidae	Developing	SST (+)	16
Cathorops spixii	Ariidae	Overexploited	No significance	
Caulolatilus chrysops	Malacanthidae	Developing	SST (+)	29
Centropomus ensiferus	Centropomidae	Overexploited	SSS (+)	13
Centropomus parallelus	Centropomidae	Fully exploited	SST (+)	15
Centropomus pectinatus	Centropomidae	Overexploited	SSS(+)	13
Centropomus undecimalis*	Centropomidae	Developing	SST (+)	15
Cephalopholis fulva*	Serranidae	Developing	SST (+)	22
Cetengraulis edentulus*	Engraulidae	Overexploited	SSS (+)	21
Chaetodipterus faber	Ephippidae	Developing	SST (+)	23
Chaetodon ocellatus	Chaetodontidae	Overexploited	No significance	
Conodon nobilis*	Haemulidae	Overexploited	Artisanal fishery (+)	48
Cryptotomus roseus	Scaridae	Overexploited	No significance	
Ctenosciaena gracilicirrhus*	Scianidae	Fully exploited	SST (+)	15
Cynoscion acoupa*	Scianidae	Developing	SST (+) and Artisanal fishery (-)	69

Cynoscion guatucupa	Scianidae	Fully exploited	SST (+)	30
Cynoscion jamaicensis	Scianidae	Fully exploited	SST (+)	36
Cynoscion leiarchus	Scianidae	Overexploited	SSS (+) and Artisanal fishery (+)	49
Cynoscion microlepidotus	Scianidae	Fully exploited	SST (+)	39
Cynoscion virescens	Scianidae	Fully exploited	SST (+) and Artisanal fishery (+)	53
Dasyatis americana	Dasyatidae	Overexploited	SST (+)	31
Dasyatis guttata*	Dasyatidae	Overexploited	SST (+)	31
Diapterus auratus	Gerreidae	Developing	Artisanal fishery (-)	34
Diapterus rhombeus*	Gerreidae	Overexploited	SST (+) and Price (-)	27
Elops saurus*	Elopidae	Developing	SST (+) and Price (-)	48
Epinephelus itajara*	Serranidae	Overexploited	SST (+)	22
Epinephelus morio*	Serranidae	Collapsed	Artisanal fishery (-)	47
Eugerres brasilianus	Gerreidae	Developing	SST (+)	44
Genidens barbus	Ariidae	Collapsed	SST (-) and Artisanal fishery (-)	46
Genyatremus luteus*	Haemulidae	Overexploited	SSS (+) and Price (+)	32
Genypterus brasiliensis	Ophidiidae	Fully exploited	SST (+)	14
Gobioides broussonnetii	Gobiidae	Fully exploited	SST (+)	17
Gobionellus oceanicus	Gobiidae	Overexploited	SST (+)	28
Haemulon aurolineatum	Haemulidae	Overexploited	Artisanal fishery (-)	40
Haemulon chrysargyreum	Haemulidae	Fully exploited	SST (+)	20
Haemulon flavolineatum	Haemulidae	Fully exploited	SST (+)	23
Haemulon melanurum	Haemulidae	Overexploited	No significance	
Haemulon parra	Haemulidae	Overexploited	No significance	
Haemulon plumierii	Haemulidae	Developing	SST (+)	33
Haemulon squamipinna	Haemulidae	Developing	SST (+)	52
Harengula clupeola*	Clupeidae	Overexploited	SST (+)	22
Harengula jaguana*	Clupeidae	Overexploited	SST (+)	12
Hexanematichthys herzbergii	Ariidae	Fully exploited	SST (+)	39
Hyporhamphus unifasciatus	Hemiramphidae	Overexploited	SST (+) and Artisanal fishery (-)	51
Hyporthodus niveatus*	Serranidae	Fully exploited	SST (+)	37
Isopisthus parvipinnis	Scianidae	Overexploited	Artisanal fishery (-)	50
Larimus breviceps	Scianidae	Collapsed	SST (+)	27
Lile piquitinga	Clupeidae	Developing	SST (+)	38
Lophius gastrophysus*	Lophiidae	Overexploited	SST (+)	11
Lopholatilus villarii	Malacanthidae	Overexploited	SST (+) and Artisanal fishery (-)	57
Lutjanus alexandrei	Lutjanidae	Fully exploited	SST (+)	29
Lutjanus analis*	Lutjanidae	Fully exploited	SST (+)	33
Lutjanus cyanopterus	Lutjanidae	Collapsed	No significance	

*Lutjanus jocu**	Lutjanidae	Fully exploited	SST (+) and Price (-)	55
*Lutjanus purpureus**	Lutjanidae	Fully exploited	SST (+) and Price (-)	59
*Lutjanus synagris**	Lutjanidae	Developing	No significance	
*Lutjanus vivanus**	Lutjanidae	Fully exploited	SST (+) and Price (-)	25
Lycengraulis grossidens	Engraulidae	Fully exploited	SST (+)	20
*Macrodon ancylodon**	Scianidae	Fully exploited	SST (+) and Artisanal fishery (-)	33
Macrodon atricauda	Scianidae	Collapsed	SST (-)	32
Malacanthus plumieri	Malacanthidae	Fully exploited	SST (+) and Artisanal fishery (+)	27
*Menticirrhus americanus**	Scianidae	Fully exploited	SST (+) and Price (-)	33
*Merluccius hubbsi**	Merluccidae	Collapsed	SST (-) and Artisanal fishery (+)	42
*Micropogonias furnieri**	Scianidae	Fully exploited	SSS (-) and Price (-)	23
Mugil gaimardianus	Mugilidae	Overexploited	SST (+)	14
Mugil incilis	Mugilidae	Developing	SST (+)	37
*Mugil liza**	Mugilidae	Collapsed	SST (-) and Artisanal fishery (-)	46
Mugil trichodon	Mugilidae	Fully exploited	SST (+)	14
*Mullus argentinae**	Mullidae	Fully exploited	Artisanal fishery (-) and Price (-)	68
*Mustelus schmitti**	Triakidae	Collapsed	SST (+), Price (-) and Artisanal fishery (+)	75
*Mycteroperca bonaci**	Serranidae	Overexploited	SST (+) and Artisanal fishery (-)	45
Myrichthys breviceps	Ophichthidae	Fully exploited	No significance	
*Nebris microps**	Scianidae	Collapsed	SST (-) and Price (-)	23
*Notarius grandicassis**	Ariidae	Overexploited	SST (+) and Price (-)	30
*Ocyurus chrysurus**	Lutjanidae	Developing	SST (+)	19
Odontesthes argentinensis	Atherinopsidae	Collapsed	SST (+)	22
Odontoscion dentex	Scianidae	Fully exploited	No significance	
Oligoplites palometa	Carangidae	Fully exploited	SST (+)	29
Oligoplites saliens	Carangidae	Fully exploited	Artisanal fishery (-)	36
*Opisthonema oglinum**	Clupeidae	Fully exploited	SST (+) and Price (-)	43
Orthopristis ruber	Haemulidae	Fully exploited	SST (+)	24
Paralichthys orbignyanus	Paralichthyidae	Fully exploited	SST (+)	34
*Paralonchurus brasiliensis**	Scianidae	Overexploited	SST (+), Price (-) and Artisanal fishery (-)	38
*Parona signata**	Carangidae	Fully exploited	SSS (+) and Price (-)	53
*Peprilus paru**	Stomateidae	Overexploited	SSS (+) and Artisanal fishery (+)	70

Percophis brasiliensis *	Percophidae	Fully exploited	SST (+) and Price (-)	35
Pogonias cromis *	Scianidae	Overexploited	SSS (-)	23
Polydactylus virginicus	Polynemidae	Overexploited	SST (+)	32
Polyprion americanus *	Polyprionidae	Collapsed	SST (-)	22
Pomacanthus paru	Pomacanthidae	Developing	SST (+) and Price (-)	47
Prionotus punctatus	Triglidae	Developing	SST (+)	18
Pseudopercis numida	Pinguipedidae	Collapsed	SSS (-) and Artisanal fishery (-)	78
Pseudupeneus maculatus	Mullidae	Developing	SST (+)	18
Pterengraulis atherinoides	Engraulidae	Overexploited	SST (+)	23
Rhinobatos horkelii	Rhinobatidae	Collapsed	SST (+) and Artisanal fishery (-)	48
Rhizoprionodon lalandii	Carcharhinidae	Fully exploited	SST (+)	41
Rhizoprionodon porosus	Carcharhinidae	Fully exploited	SST (+)	40
Rhomboplites aurorubens *	Lutjanidae	Developing	SST (+) and Price (-)	28
Sardinella brasiliensis *	Clupeidae	Overexploited	SST (+)	27
Sciades couma *	Ariidae	Fully exploited	SST (+)	15
Sciades herzbergii	Ariidae	Overexploited	SST (+) and Artisanal fishery (+)	30
Sciades parkeri *	Ariidae	Fully exploited	SST (+)	25
Sciades proops *	Ariidae	Fully exploited	SST (+)	20
Scomberomorus brasiliensis *	Scombridae	Fully exploited	SST (+)	23
Scorpaena plumieri	Scorpaenidae	Developing	SST (+)	18
Selene setapinnis *	Carangidae	Overexploited	Artisanal fishery (+) and Price (+)	52
Selene vomer *	Carangidae	Collapsed	SSS (-)	14
Sparisoma axillare	Scaridae	Fully exploited	SST (+)	23
Sphyraena tome	Sphyraenidae	Overexploited	SST (+)	33
Stellifer brasiliensis	Scianidae	Developing	SST (+)	36
Trachinotus carolinus *	Carangidae	Fully exploited	SSS (+) and Artisanal fishery (-)	88
Trachinotus falcatus	Carangidae	Overexploited	SSS (-)	15
Trachurus lathami	Carangidae	Overexploited	SST (+)	24
Umbrina canosai *	Scianidae	Fully exploited	SST (+)	38
Urophycis brasiliensis *	Phycidae	Fully exploited	SST (+)	28

3.2 Explaining the Exploitation Index and Status – External Drivers

3.2.1 Collapsed species

The collapsed species models (GAMs) explained between 14% and 78% of the EI variation (deviance D^2) (*Table 2, Supplementary Material Figure S3a*). The Ground croaker *Bairdiella ronchus* was the only species without a significant predictor. EI of seven species (Argentine menhaden *Brevoortia pectinata,* White sea catfish *Genidens barbus,* Argentine hake *Merluccius hubbsi,* Black drum *Pogonias cromis,* Wreckfish *Polyprion americanus,* Sandperch *Pseudopercis numida,* Lookdown *Selene vomer*) had a mostly negative relationship with the SST or SSS; *i.e.,* these species were captured less under conditions of higher temperatures or salinity (*Fig. 2a*).

For five species the proportion of exploitation by small-scale fisheries was a significant predictor. For two species (Argentine hake *Merlucchius hubbsi* and Narrownose Smoothhound *Mustelus schmitti*), the relationship was positive, meaning that when the species were more exploited by small-scale fishery, the EI was closer to 1 (less collapsed); whereas for three other species (White sea catfish *Genidens barbus,* Namorado sandperch *Pseudopercis numida,* Brazilian guitarfish *Rhinobatos horkelii)* the relationship was negative, higher levels of exploitation by small-scale fisheries meant lower EI (*Fig. 2b*). Two species had their exploitation affected by the ex-vessel price in opposite ways: the Narrownose Smoothhound *Mustelus schmitti* was negatively related to it, whereas the Argentine menhaden *Brevoortia pectinata* was positively related to ex-vessel price (*Fig. 2c*).

3.2.2 Overexploited species

The GAM models explained between 11% and 88% of the EI variability for 34 species (*Table 2*). In only six of the 55 species (Madamango sea catfish *Cathorops spixii,* Spotfin butterflyfish *Chaetodon ocellatus,* Bluelip parrotfish *Cryptotomus roseus,* Cottonwick grunt *Haemulon melanurum,* Sailor's grunt *Haemulon parra,* Cubera snapper *Lutjanus cyanopterus*), the variables

used in the model could not significantly explain the EI variability (*Table 2, Supplementary material Figure S3b*). Out of the 42 species affected by the climatic variables, 34 showed a positive relationship, meaning that when either SST or SSS was higher, the EI was higher, meaning that the catch was higher, and the risk of collapsing was lower (*Fig. 2a*). Conversely, for seven species (Softhead sea catfish *Amphiarius rugispinis,* Black margate *Anisotremus surinamensis,* Pluma porgy *Calamus pennatula,* Southern king weakfish *Macrodon atricauda,* Lebranche mullet *Mugil liza,* Smalleye croaker *Nebris microps,* Permit *Trachinotus falcatus*), data suggested that higher temperatures were associated with a lower Exploitation Index, indicating a higher risk of collapse (*Fig. 2a*). Climatic variables were not relevant in seven species (Western Atlantic seabream *Archosargus rhomboidalis,* Barred grunt *Conodon nobilis,* Red grouper *Epinephelus morio,* Tomtate grunt *Haemulon aurolineatum,* Bigtooth corvina *Isopisthus parvipinnis,* Argentine goatfish *Mullus argentinae,* Atlantic moonfish *Selene setapinnis*).

3.2.3 Fully Exploited species

The GAM models were significant for 44 of the 47 species with a Fully Exploited status, which explained between 14% and 69% of the EI variation (*Table 2*, *Supplementary material Figure S3c*). Whereas one species (Vaillant's anchovy *Anchoviella vaillanti*) showed a mostly negative relationship between salinity and the EI, the other 39 showed a positive relationship: with higher temperatures or salinity, their risk of collapse decreased (*Fig. 2a*). For eight of these species (Coco sea catfish *Bagre bagre,* Dog snapper *Lutjanus jocu,* Southern red snapper *Lutjanus purpureus,* Silk snapper *Lutjanus vivanus,* Southern kingcroaker *Menticirrhus americanus,* Whitemouth croaker *Micropogonias furnieri,* Atlantic thread herring *Opisthonema oglinum,* Parona leatherjacket *Parona signata*), price was also important: in years when these species were more expensive, the EI was lower (*Fig. 2c*). For five species, the type of fishery was important: for three of them (Acoupa weakfish *Cynoscion acoupa,* King weakfish *Macrodon ancylodon,* Castin leatherjacket *Oligoplites saliens*) when the proportion of fish caught by small-scale fisheries was

higher, the species EI were lower, whereas for two (Green weakfish *Cynoscion virescens,* Sand tilefish *Malacanthus plumieri*), the EI were higher (*Fig. 2b*).

3.2.4 Developing species

The GAM models explained between 15% and 52% of the developing status variation of species, except for one species (Lane snapper *Lutjanus synagris*) (*Table 2, Supplementary material Figure S3d*). Seventeen species were positively affected by SST or SSS, meaning that when temperature or salinity was higher, so was the EI, whereas the risk of collapse was smaller (*Fig. 2a*). One species (Irish mojarra *Diapterus auratus*) was negatively affected by small-scale fisheries (*Fig. 2b*). Three species (Ladyfish *Elops saurus,* French angelfish *Pomacanthus paru,* Vermilion snapper *Rhomboplites aurorubens*) EI presented negative relationship with their prices (*Fig. 2c*).
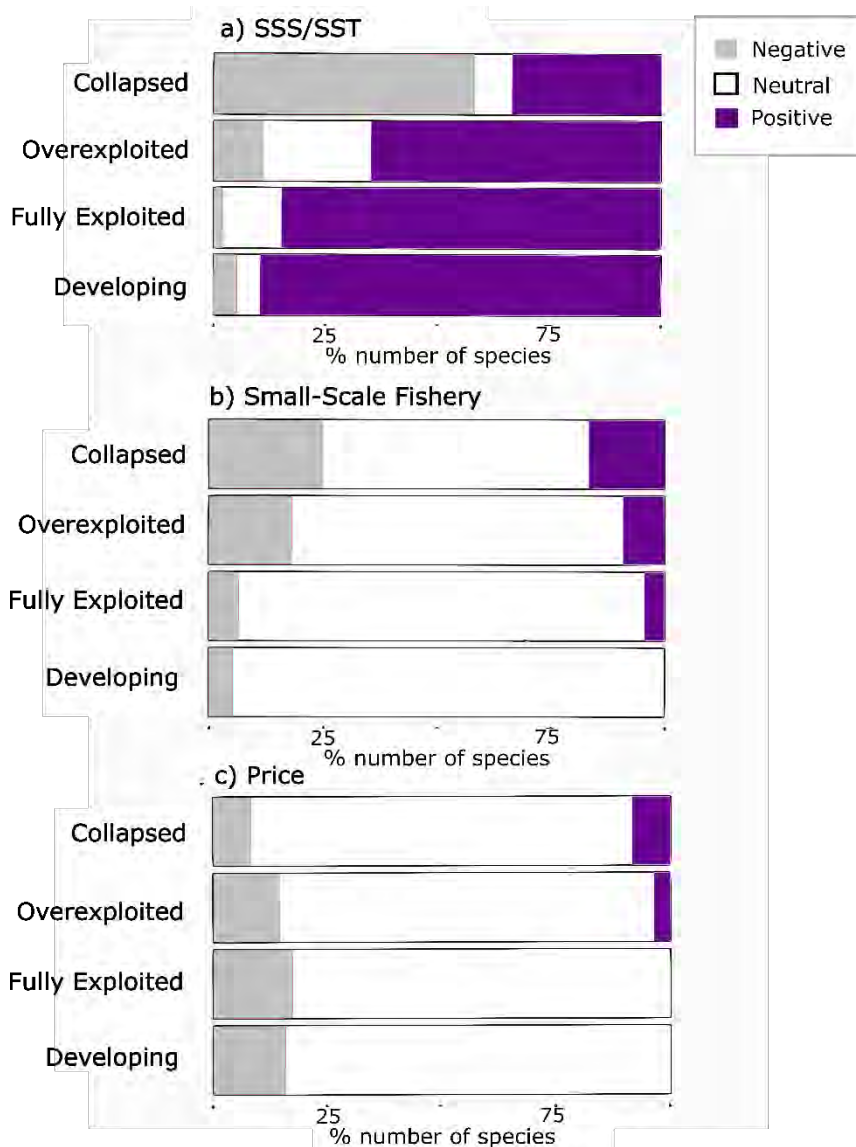
Figure 2: Relationship between temporal Exploitation Index and a) Sea Surface Temperature (SST) or Sea Surface Salinity (SSS), b) Small-scale fishery and c) ex-vessel price, based on Generalized Additive Model results. Colors show current Exploitation Status. Negative values imply that the Exploitation Index was negatively related to the variable, positive values imply that the relationship was positive, and neutral means that the variable was not selected as an important predictor for the species' Exploitation Index variability.

3.3 Biological and fishery effects on Exploitation Index (RDA)

The full RDA model (all factors together, Fig. 3) explained 61% of the species ES (i.e. constrained variance) (t=1.50, df=6, p-value < 0.001). Particularly, the main fishing fleet used to exploit species was the principal factor (34% conditioned variance of the partial RDA; Monte Carlo: *F =*

3.08, p-value<0.001), followed by price (12% conditioned variance of the partial RDA; Monte Carlo: $F = 2.12$, p-value <0.001), resilience (10% conditioned variance of the partial RDA; Monte Carlo: $F = 1.58$, p-value <0.001) and maximum body size (10% conditioned variance of the partial RDA; Monte Carlo: $F = 2.47$, p-value <0.001). Habitat and trophic level were the less relevant variables (8% and 6% conditioned variance of the partial RDAs; Monte Carlo: $F = 2.11$, p-value <0.001 and Monte Carlo: $F = 1.95$, p-value <0.001 respectively). Fully Exploited and in Development species were more affected by type of fishery and price, with a negative relationship, meaning that when species were more exploited by Small-Scale fisheries and presented a higher price, they were in a worst situation. These variables were not important to explain de EI variation for Overexploited and Collapsed species. Maximum size had some effect in Overexploited and Collapsed species, meaning that species with larger body sizes are in a better situation than smaller ones. Resilience was important to explain ES for the four categories, meaning that higher Resilience values were related with higher EI, and therefore a better situation.
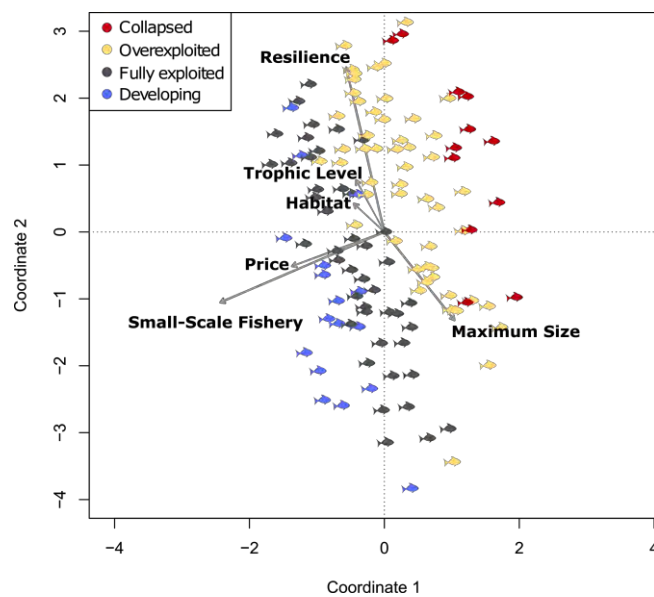
Figure 3: Two-dimensional RDA plot of the Exploitation Status groups for the 132 Brazilian species studied.

4. DISCUSSION

Most studies that address the vulnerability of marine fish stocks rely on "data-rich" fisheries, using quantitative methods such as stock assessment models (Fernandes et al., 2017). However, in tropical developing countries such as Brazil, fisheries tend to be poorly documented and inadequately managed due to a lack of research funding and official statistics for stock monitoring and analyses (Honey et al., 2010), compromising comprehensive assessments. For example, there is no effort data for the analysed area. Therefore, to measure the state of fish stocks, it is essential to adapt or develop flexible and reliable alternative analytical tools that can perform well with limited available information (Fonseca et al., 2017; Rufener et al., 2017).

The collapse of marine fishes is a result of myriad factors and cannot be assessed in a univariate approach. Our results provide evidence that tropical and subtropical exploited species collapse in Brazil is best explained by a complex combination of a negative impact of climate, fishery exploitation type (industrial fisheries versus small-scale fisheries) and specific ecological traits – here, smaller body size. These factors have been shown to explain fish population fluctuation in other contexts, including sardine, salmon, cod and herring, mostly from temperate regions (Botsford et al., 2014; Cheung et al., 2018; Lindegren et al., 2013; Rouyer et al., 2011).

Temperature and salinity affect different species in opposite ways: whereas some apparently flourish in higher temperatures and/or salinity concentrations, as demonstrated by higher catches, others struggle under these same conditions, showing decreased catches. Even within the same species, stocks located in different environmental conditions can present antagonistic effects to changes in temperature (Ottersen et al., 2013). Tropical species are frequently considered to have a lower capacity to adapt to warmer temperatures because they evolved in stable environments (Stillman, 2003; Tewksbury et al., 2008). In this study, however, results showed that more than 70% of tropical and subtropical species seemed to benefit from warmer temperatures at first glance. Each species presents an optimal thermal window in which its fitness is higher, and its

physiological functions performances are maintained (Ern et al., 2016). Small increases in temperature, within a species thermal tolerance, can increase its metabolic rate and, then, be positive for species production (Thresher et al., 2007).

Yet, when only the collapsed species are considered, we found that almost 60% of them presented a negative relationship with higher temperatures or salinity Intrinsic traits can explain how species respond to variability in the environment. This depends on species' thermal tolerance, physiology and behavior (Pörtner, 2006). Temperature affects marine organisms' metabolic rates by influencing water oxygen availability (Holt & Jorgensen, 2015). When an increase in temperature is higher than the species thermal tolerance, the effect on physiological performance will be negative. Warmer temperatures are known to increase growth rate in early stages, which can reduce pelagic larval duration and, then, affect larval dispersion (O'Connor et al., 2007), decrease body size in late stages due to limitations in oxygen acquisition by gills (Pauly & Cheung, 2018; Atkinson, 1994), reduce reproduction (Miller et al., 2015), and cause a general decrease in aerobic performance (Pörtner & Knust, 2007).

We found that, among already overexploited species, smaller body size species are more at risk than larger ones. This result suggests that overexploited smaller species have a higher chance of collapsing in our study area. In fact, fast-growth and small-sized species can be more vulnerable to climate variability, making them more likely to collapse, when exploited (Pinsky & Byler, 2015). Small species with short generation times and early maturation can respond more quickly to climate variability (Botsford et al., 2014). One mechanism that may be at work is the negative effect of temperature on recruitment, which is particularly crucial for population maintenance of small species with short generation times (Checkley et al., 2009; Hamdoun & Epel, 2007; Van Der Lee et al., 2016; Pinsky et al., 2013). Three major hypotheses could explain this pattern: (i) species negatively affected by warmer temperatures are more likely to collapse due to intrinsic traits, (ii)

exploitation is focused on more sensitive species, and/or (iii) after a certain level of exploitation, species start to respond negatively to warmer temperatures.

The exploitation regime is also an important aspect to be considered to avoid collapse. In this study, the type of fishery was an important factor in explaining EI variation for species in better status (Fully Exploited and in Development), therefore reinforcing the hegemonic paradigm that attests that small-scale fisheries can be less impactful to marine fish populations than industrial fisheries. We found that some stocks classified as in Development or Fully Exploited had lower chances of becoming Overexploited or Collapsed when harvested by small-scale fisheries, while others had a higher chance of collapse when exploited by small-scale. However, it is important to emphasize that most species currently performing better are not exploited by industrial fisheries, only 50% of collapsed species were industrial targets. This combination of large effort and vulnerable species can explain why the Brazilian coast is one of the most vulnerable areas for commercial harvesting (Di Minin et al., 2019).

Moreover, climate change adds new threats to the system. We found that collapsed species are vulnerable to warmer sea temperatures. One possible explanation is that these species have already reached a level of exploitation that makes them respond negatively to warmer temperatures, thus increasing their vulnerability. It is known that industrial fisheries can have a degrading effect on fish stocks (Free et al., 2019), especially when the gear used has a low selective capacity for species or size, by overexploiting small-bodied species or juveniles. Also, it is known that the recruitment and biomass of exploited stocks in temperate climates (Northern Atlantic) respond to warmer temperatures (Ottersen et al., 2013), although the mechanisms behind this observation are complex – some stocks presented a positive relationship, while others showed a negative relationship. The effect of temperature on the recruitment of herrings, for example, seems to be related to stock location, in which cold water stocks being positively affected by warmer waters (Ottersen et al., 2013). Most of the species considered here, even the overexploited ones, tend to respond positively

to warmer temperatures. However, the impact of fisheries on collapsed species may have led them to respond negatively to warmer temperatures, thus increasing their vulnerability further.

The collapse of tropical and subtropical fishes is shown here to be a consequence of a combination of a biological trait (body size), and a negative response to warmer sea temperatures. Thus, marine fish species collapse in Brazil could be produced due to: (i) exploitation focus on more vulnerable species; (ii) a decrease in population size, age truncation and other population structure changes due to overexploitation (not measured here); and (iii) in turn, a higher sensitivity to warmer temperature. Therefore, besides analyzing the synergetic effect of exploitation and climate sensitivity, it is necessary to include specific vulnerable traits. A deeper understanding of which traits and how these traits can affect the relationship of tropical and subtropical species to climate and exploitation is crucial, especially when considering that climate change may be the main factor leading species to extinction in the future (Thomas et al., 2004). However, the results presented here can support management strategies to minimize the exploitation of species with higher levels of vulnerability.

Saving species from the brink of extinction is one of the most vital challenges of modern civilizations. Our results bring important information to understanding the collapse of tropical and subtropical marine fish species, but strategies to reverse population declines should be considered at different scales of decision-making. To start, tropical developing countries could consider adopting broader governance systems within fisheries, especially regarding species with vulnerable traits. The application of measures to mitigate climate change is urgently needed to prevent the loss of more species, especially because the economic impacts of the effects of climate change on fisheries are difficult to predict (Sumaila et al., 2011). Our results can contribute to designing better strategies and provide examples of how to mitigate climate variability effects through management and regulations of marine organisms (e.g. Le Bris et al., 2018; Gattuso et al., 2018; Kritzer et al., 2019).

5. ACKNOWLEDGEMENTS

6. REFERENCES

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral ecology*, *26*(1), 32-46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Alfaro-Shigueto, J., Mangel, J. C., Pajuelo, M., Dutton, P. H., Seminoff, J. A., & Godley, B. J. (2010). Where small can have a large impact: Structure and characterization of small-scale fisheries in Peru. *Fisheries Research*, *106*(1), 8–17. https://doi.org/10.1016/j.fishres.2010.06.004

Berkeley, S. A., Hixon, M. A., Larson, R. J., & Love, M. S. (2004). Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries*, *29*(8), 23–32.

Berkes, F., Mahon, R., McConney, P., Pollnac, R., & Pomeroy, R. (2001). *Managing Small-scale Fisheries*.

Blanchard, J. L., Jennings, S., Holmes, R., Harle, J., Merino, G., Allen, J. I., … Barange, M. (2012). Potential consequences of climate change for primary production and fish production in large marine ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1605), 2979–2989. https://doi.org/10.1098/rstb.2012.0231

Blanchard, J. L., Mills, C., Jennings, S., Fox, C. J., Rackham, B. D., Eastwood, P. D., & O'Brien, C. M. (2005). Distribution, abundance relationships for North Sea Atlantic cod (*Gadus morhua*): observation versus theory. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(9), 2001–2009. https://doi.org/10.1139/f05-109

Botsford, L. W., Holland, M. D., Field, J. C., & Hastings, A. (2014). Cohort resonance: a significant component of fluctuations in recruitment, egg production, and catch of fished populations. *ICES Journal of Marine Science*, *71*, 2158–2170.

Brander, K. M. (2005). Cod recruitment is strongly affected by climate when stock biomass is low. *ICES Journal of Marine Science*, *62*(3), 339–343. https://doi.org/10.1016/j.icesjms.2004.07.029

Checkley, D. M., Alheit, J., Oozeki, Y., & Roy, C. (2009). Climate change and small pelagic fish. In *Climate Change and Small Pelagic Fish*. https://doi.org/10.1017/CBO9780511596681

Cheung, W. W. L., Jones, M. C., Reygondeau, G., & Frölicher, T. L. (2018). Opportunities for climate-risk reduction through effective fisheries management. *Global Change Biology*, *24*(11), 5149–5163. https://doi.org/10.1111/gcb.14390

Cheung, W. W. L., Pinnegar, J., Merino, G., Jones, M. C., & Barange, M. (2012). Review of climate change impacts on marine fisheries in the UK and Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *22*(3), 368–388. https://doi.org/10.1002/aqc.2248

Cheung, W. W. L., Reygondeau, G., & Frölicher, T. L. (2016). Large benefits to marine fisheries of meeting the 1.5°C global warming target. *Science*, *354*(6319), 1591–1594. https://doi.org/10.1126/science.aag2331

Cheung, W. W. L., Watson, R., & Pauly, D. (2013). Signature of ocean warming in global fisheries catch. *Nature*, *497*(7449), 365–368. https://doi.org/10.1038/nature12156

Chin, A., Kyne, P. M., Walker, T. I., & McAuley, R. B. (2010). An integrated risk assessment for climate change: Analysing the vulnerability of sharks and rays on Australia's Great Barrier Reef. *Global Change Biology*, *16*(7), 1936–1953. https://doi.org/10.1111/j.1365-2486.2009.02128.x

Chrysafi, A., & Kuparinen, A. (2015). Assessing abundance of populations with limited data: Lessons learned from data-poor fisheries stock assessment. *Environmental Reviews*, *24*, 1–14. https://doi.org/10.1139/er-2015-0044

Ciannelli, L., Fisher, J. A. D., Skern-Mauritzen, M., Hunsicker, M. E., Hidalgo, M., Frank, K. T., & Bailey, K. M. (2013). Theory, consequences and evidence of eroding population spatial structure in harvested marine fishes: A review. *Marine Ecology Progress Series*, *480*, 227–243. https://doi.org/10.3354/meps10067

Costello, C., Ovando, D., Hilborn, R., Gaines, S. D., Deschenes, O., & Lester, S. E. (2012). Status and solutions for the world's unassessed fisheries. *Science*, *338*(6106), 517–520. https://doi.org/10.1126/science.1223389

Di Minin, E., Brooks, T. M., Toivonen, T., Butchart, S. H. M., Heikinheimo, V., Watson, J. E. M., … Moilanen, A. (2019). Identifying global centers of unsustainable commercial harvesting of species. *Science Advances*, *5*(4), eaau2879. https://doi.org/10.1126/sciadv.aau2879

Dulvy, N. K., Sadovy, Y., & Reynolds, J. D. (2003). Extinction vulnerability in marine populations. *Fish and Fisheries*, *4*(1), 25–64. https://doi.org/10.1046/j.1467-2979.2003.00105.x

Enberg, K., Jørgensen, C., Dunlop, E. S., Varpe, Ø., & Boukal, D. S. (2012). *Fishing-induced evolution of growth: concepts, mechanisms and the empirical evidence*. (ii), 1–25. https://doi.org/10.1111/j.1439-0485.2011.00460.x

FAO. (2016). The State of World Fisheries and Aquaculture. In *Contributing to food security and nutrition for all*. https://doi.org/10.5860/CHOICE.50-5350

Fernandes, P. G., Ralph, G. M., Nieto, A., García Criado, M., Vasilakopoulos, P., Maravelias, C. D., … Carpenter, K. E. (2017). Coherent assessments of Europe's marine fishes show regional divergence and megafauna loss. *Nature Ecology & Evolution*, *1*(7), 0170. https://doi.org/10.1038/s41559-017-0170

Fonseca, V. P., Pennino, M. G., de Nóbrega, M. F., Oliveira, J. E. L., & de Figueiredo Mendes, L. (2017). Identifying fish diversity hot-spots in data-poor situations. *Marine Environmental Research*, *129*(June 2017), 365–373. https://doi.org/10.1016/j.marenvres.2017.06.017

Free, C. M., Thorson, J. T., Pinsky, M. L., Oken, K. L., Wiedenmann, J., & Jensen, O. P. (2019). Impacts of historical warming on marine fisheries production. *Science*, *983* (March), 979–983. https://doi.org/10.1126/science.aau1758

Froese, R., & Kesner-reyes, K. (2002). Impact of Fishing on the Abundance of Marine Species. In *ICES Council Meeting Report*.

Froese, R., & Pauly, D. (2017). Fish Base. World Wide Web electronic publication.

Gattuso, J.-P., Magnan, A. K., Bopp, L., Cheung, W. W. L., Duarte, C. M., Hinkel, J., … Rau, G. H. (2018). Ocean solutions to address climate change and its effects on marine ecosystems. *Frontiers in Marine Science*, *5*(October). https://doi.org/10.3389/fmars.2018.00337

Genner, M. J., Sims, D. W., Southward, A. J., Budd, G. C., Masterson, P., McHugh, M., … Hawkins, S. J. (2010). Body size-dependent responses of a marine fish assemblage to climate change and fishing over a century-long scale. *Global Change Biology*, *16*(2), 517–527. https://doi.org/10.1111/j.1365-2486.2009.02027.x

Ghosh, S., Muktha, M., Rao, M. V. H., Behera, P. R., Regional, V., & Fisheries, I. M. (2015). *Assessment of stock status of the exploited fishery resources in northern Bay of Bengal using landed catch data*. *62*(4), 23–30. https://doi.org/10.1136/sbmj.b5274

Giglio, V. J., Bender, M. G., Zapelini, C., & Ferreira, C. E. L. (2017). The end of the line? Rapid depletion of a large-sized grouper through spearfishing in a subtropical marginal reef. *Perspectives in Ecology and Conservation*, *15*(2), 115–118. https://doi.org/10.1016/j.pecon.2017.03.006

Goetze, J. S., Langlois, T. J., Egli, D. P., & Harvey, E. S. (2011). Evidence of artisanal fishing impacts and depth refuge in assemblages of Fijian reef fish. *Coral Reefs*, *30*(2), 507–517. https://doi.org/10.1007/s00338-011-0732-8

Gonzalez, A., Lawton, J. H., Gilbert, F. S., Blackburn, T. M., & Evans-Freke, I. (1998). Metapopulation dynamics, abundance, and distribution in a microecosystem. *Science*, *281*(5385), 2045–2047. https://doi.org/10.1126/science.281.5385.2045

Hamdoun, A., & Epel, D. (2007). Embryo stability and vulnerability in an always changing world. *Proceedings of the National Academy of Sciences*, *104*(6), 1745–1750. https://doi.org/10.1073/pnas.0610108104

Hastie, T., & Tibshirani, R. (1987). Generalized additive models: some applications. *Journal of the American Statistical Association*, *82*(398), 371–386.

Hawkins, J. P., & Roberts, C. M. (2004). Effects of artisanal fishing on Caribbean Coral Reefs. *Conservation Biology*, *18*(1), 215–226. https://doi.org/10.1111/j.1523-1739.2004.00328.x

Heath, M. R. (2005). Changes in the structure and function of the North Sea fish foodweb, 1973-2000, and the impacts of fishing and climate. *ICES Journal of Marine Science*, *62*(5), 847–868. https://doi.org/10.1016/j.icesjms.2005.01.023

Hislop, J. R. G. (1988). The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. *Journal of Fish Biology*, *32*(6), 923–930. https://doi.org/10.1111/j.1095-8649.1988.tb05435.x

Honey, K. T., Moxley, J. H., & Fujita, R. M. (2010). From rags to fishes: data-poor methods for fishery managers. In: Managing data-poor fisheries workshop: case studies, models and solutions. *California Sea Grant College Program. ISBN:978-1-888691-23-8*, *1*(978), 159–184.

Jackson, J. B. C., Michael X. Kirby, Berger, W. H., Karen A. Bjorndal, Louis W. Botsford, Bruce J. Bourque, … Warne. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science*, *293*(5530), 629–637. https://doi.org/10.1126/science.1059199

Jennings, S., & Blanchard, J. L. (2004). Fish abundance with no fishing: Predictions based on macroecological theory. *Journal of Animal Ecology*, *73*(4), 632–642. https://doi.org/10.1111/j.0021-8790.2004.00839.x

Kleisner, K., Zeller, D., Froese, R., & Pauly, D. (2013). Using global catch data for inferences on the world's marine fisheries. *Fish and Fisheries*, *14*(3), 293–311. https://doi.org/10.1111/j.1467-2979.2012.00469.x

Kritzer, J. P., Costello, C., Mangin, T., & Smith, S. L. (2019). Responsive harvest control rules provide inherent resilience to adverse effects of climate change and scientific uncertainty. *ICES Journal of Marine Science*. https://doi.org/10.1093/icesjms/fsz027

Le Bris, A., Mills, K. E., Wahle, R. A., Chen, Y., Alexander, M. A., Allyn, A. J., … Pershing, A. J. (2018). Climate vulnerability and resilience in the most valuable North American fishery. *Proceedings of the National Academy of Sciences*, (21), 201711122. https://doi.org/10.1073/pnas.1711122115

Lewis, S. L., & Maslin, M. A. (2015). Defining the Anthropocene. *Nature*, *519*(7542), 171–180. https://doi.org/10.1038/nature14258

Lindegren, M., & Checkley, D. M. (2013). Temperature dependence of Pacific sardine (*Sardinops sagax*) recruitment in the California Current Ecosystem revisited and revised. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(2), 245–252. https://doi.org/10.1139/cjfas-2012-0211

Lindegren, M., Checkley, D. M., Rouyer, T. A., MacCall, A. D., & Stenseth, N. C. (2013). Climate, fishing, and fluctuations of sardine and anchovy in the California Current. *PNAS*, *110*(33), 13672–13677. https://doi.org/10.1073/pnas.1305733110

Lindfield, S. J., McIlwain, J. L., & Harvey, E. S. (2014). Depth refuge and the impacts of SCUBA spearfishing on coral reef fishes. *PLoS ONE*, *9*(3), 1–12. https://doi.org/10.1371/journal.pone.0092628

Musick, J. A. (1999). Criteria to define extinction risk in marine fishes. *Fisheries*, *24*(6), 6–13. https://doi.org/10.1577/1548-8446(1999)024<0006

Myers, R. A., & Worm, B. (2003). Rapid worldwide depletion of large predatory fish communities. *Nature*, *423*(May), 280–283. https://doi.org/10.1038/nature01610

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., ..., & Wagner, H. (2011). *vegan: Community ecology package* (pp. 117–118).

Olden, J. D., Hogan, Z. S., & Zanden, M. J. Vander. (2007). Small fish, big fish, red fish, blue fish: Size-biased extinction risk of the world's freshwater and marine fishes. *Global Ecology and Biogeography*, *16*(6), 694–701. https://doi.org/10.1111/j.1466-8238.2007.00337.x

Ottersen, G., Stige, L. C., Durant, J. M., Chan, K. S., Rouyer, T. A., Drinkwater, K. F., & Stenseth, N. C. (2013). Temporal shifts in recruitment dynamics of North Atlantic fish stocks: Effects of spawning stock and temperature. *Marine Ecology Progress Series*, *480,* 205–225. https://doi.org/10.3354/meps10249

Pauly, D., & Zeller, D. (2016). Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. *Nature Communications*, *7*(10244), 1–9. https://doi.org/10.1038/ncomms10244

Perry, A. L., Low, P. J., Ellis, J. R., & Reynolds, J. D. (2005). Climate change and distribution in marine fishes. *Science*, *308*(June), 1912–1924.

Pikitch, E. K., Boersma, P. D., Boyd, I. L., Conover, D. O., Cury, P., Essington, T. E., … Steneck, R. S. (2018). The strong connection between forage fish and their predators: A response to Hilborn et al. (2017). *Fisheries Research*, *198*(August), 220–223. https://doi.org/10.1016/j.fishres.2017.07.022

Pinsky, M. L., & Byler, D. (2015). Fishing, fast growth and climate variability increase the risk of collapse. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1813), 1–9. https://doi.org/10.1098/rspb.2015.1053

Pinsky, M. L., Jensen, O. P., Ricard, D., & Palumbi, S. R. (2011). Unexpected patterns of fisheries collapse in the world's oceans. *Proceedings of the National Academy of Sciences*, *108*(20), 8317–8322. https://doi.org/10.1073/pnas.1015313108

Pinsky, M. L., Worm, B., Fogarty, M. J., Sarmiento, J. L., & Levin, S. A. (2013). Marine taxa track local climate velocities. *Science*, *341*(6151), 1239–1242. https://doi.org/10.1126/science.1239352

Planque, B., Fromentin, J. M., Cury, P., Drinkwater, K. F., Jennings, S., Perry, R. I., & Kifani, S. (2010). How does fishing alter marine populations and ecosystems sensitivity to climate? *Journal of Marine Systems*, *79*(3–4), 403–417. https://doi.org/10.1016/j.jmarsys.2008.12.018

Pörtner, H. O. (2006). Climate-dependent evolution of Antarctic ectotherms: An integrative analysis. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *53*(8–10), 1071–1104. https://doi.org/10.1016/j.dsr2.2006.02.015

Pörtner, H. O., & Farrell, A. P. (2008). Ecology: Physiology and climate change. *Science*, *322*(5902), 690–692. https://doi.org/10.1126/science.1163156

Post, D. M., & Palkovacs, E. P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: Interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1523), 1629–1640. https://doi.org/10.1098/rstb.2009.0012

Quetglas, A., Rueda, L., Alvarez-Berastegui, D., Guijarro, B., & Massutí, E. (2016). Contrasting responses to harvesting and environmental drivers of fast and slow life history species. *PLoS ONE*, *11*(2), 1–15. https://doi.org/10.1371/journal.pone.0148770

Rouyer, T., Ottersen, G., Durant, J. M., Hidalgo, M., Hjermann, D., Persson, J., … Stenseth, N. C. (2011). Shifting dynamic forces in fish stock fluctuations triggered by age truncation? *Global Change Biology*, *17*(10), 3046–3057. https://doi.org/10.1111/j.1365-2486.2011.02443.x

Rufener, M. C., Kinas, P. G., Nóbrega, M. F., & Lins Oliveira, J. E. (2017). Bayesian spatial predictive models for data-poor fisheries. *Ecological Modelling*, *348*(March), 125–134. https://doi.org/10.1016/j.ecolmodel.2017.01.022

Ruffino, M. L., Lima, L. H., & S'antana, R. (2016). *Situação e tendências da pesca marítima no Brasil e o papel dos subsídios*. WWF.

Schuetz, J. G., Mills, K. E., Allyn, A. J., Stamieszkin, K., Bris, A. Le, & Pershing, A. J. (2018). Complex patterns of temperature sensitivity, not ecological traits, dictate diverse species responses to climate change. *Ecography*. https://doi.org/10.1111/ecog.03823

Smith-Ramesh, L. M., Rosenblatt, A. E., & Schmitz, O. J. (2017). Multivariate climate change can favor large herbivore body size in food webs. *The American Naturalist*, *191*(3), 000–000. https://doi.org/10.1086/695768

Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, *301*(5629), 65. https://doi.org/10.1126/science.1083073

Sumaila, U. R., Cheung, W. W. L., Lam, V. W. Y., Pauly, D., & Herrick, S. (2011). Climate change impacts on the biophysics and economics of world fisheries. *Nature Climate Change*, *1*(9), 449–456. https://doi.org/10.1038/nclimate1301

Sunday, J. M., Pecl, G. T., Frusher, S., Hobday, A. J., Hill, N., Holbrook, N. J., … Bates, A. E. (2015). Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. *Ecology Letters*, *18*(9), 944–953. https://doi.org/10.1111/ele.12474

Team, R. C. (2015). *R: A language and environment for statistical computing*.

Tewksbury, J. J., Huey, R. B., & Deutsch, C. A. (2008). Putting the heat on tropical animals. *Science*, *320*(4), 1296–1297. https://doi.org/10.1126/science.1084296

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., … Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*, 145–148. https://doi.org/10.1038/nature02121

Thorson, J. T., Minto, C., Minte-Vera, C. V., Kleisner, K. M., Longo, C., & Jacobson, L. (2013). A new role for effort dynamics in the theory of harvested populations and data-poor stock assessment. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(12), 1829–1844. https://doi.org/10.1139/cjfas-2013-0280

Torda, G., Donelson, J. M., Aranda, M., Barshis, D. J., Bay, L., Berumen, M. L., … Munday, P. L. (2017). Rapid adaptive responses to climate change in corals. *Nature Climate Change*, *7*(9), 627–636. https://doi.org/10.1038/nclimate3374

Tsikliras, A. C., Dinouli, A., Tsiros, V. Z., & Tsalkou, E. (2015). The Mediterranean and Black Sea fisheries at risk from overexploitation. *PLoS ONE*, *10*(3), 1–19. https://doi.org/10.1371/journal.pone.0121188

Van Der Lee, A. S., Koops, M. A., & Shuter, B. (2016). Are small fishes more sensitive to habitat loss? A generic size-based model. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(4), 716–726. https://doi.org/10.1139/cjfas-2015-0026

Vasconcellos, M., Diegues, A. C., & Sales, D. R. (2007). Limites e possibilidades na gestão da pesca artesanal costeira. *Nas Redes Da Pesca Artesanal*, pp. 2–70.

Vert-pre, K. A., Amoroso, R. O., Jensen, O. P., & Hilborn, R. (2013). Frequency and intensity of productivity regime shifts in marine fish stocks. *Proceedings of the National Academy of Sciences*, *110*(5), 1779–1784. https://doi.org/10.1073/pnas.1214879110

Walsh, J. C., Minto, C., Jardim, E., Anderson, S. C., Dickey-collas, M., Kleisner, K. M., … Cooper, A. B. (2018). Trade-offs for data-limited fisheries when using harvest strategies based on catch-only models. *Fish and Fisheries*, *19*, 1130–1146. https://doi.org/10.1111/faf.12316

Wood, S. N. (2006). *Generalized additive models: an introduction with R*. Chapman and Hall/CRC.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*(1), 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R* (E. Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, Ed.). New York: Spring Science and Business Media.

7. SUPPLEMENTARY MATERIAL

Table S1: List of the ecological and external traits for the 132 studied species.

Species	Family	Exploitation status	Habitat	Maximum size	Trophic level	Resilience	Price category	Small-scale fishery exploitation (%)
Amphiarius rugispinis	Ariidae	Collapsed	demersal	45	3.3	medium	medium	100
Anchoa januaria	Engraulidae	Fully exploited	pelagic	7.5	2.9	high	na	100
Anchoa spinifer	Engraulidae	Collapsed	pelagic	24	4.1	high	medium	98.39049
Anchovia clupeoides	Engraulidae	Developing	pelagic	30	3.4	high	medium	100
Anchoviella lepidentostole	Engraulidae	Developing	pelagic	13.1	3.1	high	medium	99.85351
Anchoviella vaillanti	Engraulidae	Fully exploited	pelagic	6.8	3.2	high	na	100
Anisotremus surinamensis	Haemulidae	Collapsed	reef	76	3.6	low	medium	99.69557
Anisotremus virginicus	Haemulidae	Fully exploited	reef	40.6	3.6	medium	medium	99.93546
Archosargus rhomboidalis	Sparidae	Fully exploited	reef	33	2.9	high	very high	99.99451
Aspistor luniscutis	Ariidae	Overexploited	pelagic	120	3.8	very low	na	99.72214
Aspistor quadriscutis	Ariidae	Fully exploited	pelagic	50	3.5	medium	medium	99.29363
Astroscopus sexspinosus	Uranoscopidae	Overexploited	demersal	31	4.3	medium	na	5.148599
Bagre bagre	Ariidae	Fully exploited	demersal	55	4	low	medium	98.94126
Bairdiella ronchus	Scianidae	Fully exploited	demersal	35	3.5	high	medium	100
Batrachoides surinamensis	Batrachoididae	Fully exploited	demersal	57	3.7	low	na	99.87813
Brevoortia pectinata	Clupeidae	Collapsed	pelagic	35	3.4	high	low	45.18829
Calamus pennatula	Sparidae	Collapsed	reef	37	3.7	medium	medium	69.29762
Carangoides bartholomaei	Carangidae	Developing	reef	100	4.5	high	medium	97.89175
Cathorops spixii	Ariidae	Overexploited	demersal	30	3.5	medium	medium	99.91453
Caulolatilus chrysops	Malacanthidae	Developing	demersal	60	3.5	low	very high	99.51799

Centropomus ensiferus	Centropomidae	Overexploited	pelagic	36.2	4.2	medium	very high	100
Centropomus parallelus	Centropomidae	Fully exploited	demersal	72	4.2	medium	very high	99.37948
Centropomus pectinatus	Centropomidae	Overexploited	pelagic	56	4	high	very high	100
Centropomus undecimalis	Centropomidae	Developing	reef	140	4.2	medium	low	99.77485
Cephalopholis fulva	Serranidae	Developing	reef	41	4.1	low	very high	99.97666
Cetengraulis edentulus	Engraulidae	Overexploited	pelagic	18.2	2.1	medium	medium	71.19001
Chaetodipterus faber	Ephippidae	Developing	reef	91	4.5	medium	medium	93.3095
Chaetodon ocellatus	Chaetodontidae	Overexploited	reef	20	3.7	high	na	100
Conodon nobilis	Haemulidae	Overexploited	demersal	33.6	3.6	medium	low	90.58414
Cryptotomus roseus	Scaridae	Overexploited	reef	13	2	high	na	100
Ctenosciaena gracilicirrhus	Scianidae	Fully exploited	demersal	21	3.9	medium	medium	100
Cynoscion acoupa	Scianidae	Developing	demersal	110	4.1	medium	medium	95.53175
Cynoscion guatucupa	Scianidae	Fully exploited	pelagic	50	3.7	medium	na	3.221284
Cynoscion jamaicensis	Scianidae	Fully exploited	demersal	50	3.8	high	medium	11.98273
Cynoscion leiarchus	Scianidae	Overexploited	demersal	90.8	3.1	medium	medium	91.1822
Cynoscion microlepidotus	Scianidae	Fully exploited	demersal	92	4	low	medium	96.93048
Cynoscion virescens	Scianidae	Fully exploited	demersal	115	4	low	medium	94.17786
Dasyatis americana	Dasyatidae	Overexploited	reef	200	3.5	very low	low	98.73743
Dasyatis guttata	Dasyatidae	Overexploited	demersal	200	2.6	low	low	98.73743
Diapterus auratus	Gerreidae	Developing	demersal	34	2.4	high	medium	100

Species	Family	Status	Habitat	Length	Trophic			Value
Diapterus rhombeus	Gerreidae	Overexploited	demersal	40	3	high	medium	95.11321
Elops saurus	Elopidae	Developing	reef	100	3.5	medium	low	97.1187
Epinephelus itajara	Serranidae	Overexploited	reef	250	4.1	low	very high	96.25688
Epinephelus morio	Serranidae	Collapsed	reef	125	3.5	medium	medium	79.98057
Eugerres brasilianus	Gerreidae	Developing	demersal	50	3.4	medium	medium	99.91535
Genidens barbus	Ariidae	Collapsed	demersal	120	3.8	low	medium	67.39006
Genyatremus luteus	Haemulidae	Overexploited	demersal	37	3.5	medium	medium	99.36289
Genypterus brasiliensis	Ophidiidae	Fully exploited	demersal	68.8	4	low	medium	7.420273
Gobioides broussonnetii	Gobiidae	Fully exploited	demersal	55.3	3.7	low	very high	100
Gobionellus oceanicus	Gobiidae	Overexploited	demersal	15.4	3.4	medium	na	100
Haemulon aurolineatum	Haemulidae	Overexploited	reef	25	4.4	medium	medium	85.75917
Haemulon chrysargyreum	Haemulidae	Fully exploited	reef	23	3.5	high	medium	100
Haemulon flavolineatum	Haemulidae	Fully exploited	reef	30	3.5	medium	medium	100
Haemulon melanurum	Haemulidae	Overexploited	reef	33	2.2	medium	medium	100
Haemulon parra	Haemulidae	Overexploited	reef	41.2	3.5	medium	medium	100
Haemulon plumierii	Haemulidae	Developing	reef	53	3.8	medium	medium	98.67408
Haemulon squamipinna	Haemulidae	Developing	pelagic	11.5	3.4	high	na	100
Harengula clupeola	Clupeidae	Overexploited	reef	18	3.3	high	low	76.58062
Harengula jaguana	Clupeidae	Overexploited	reef	21.2	3.4	medium	low	98.53447
Hexanematichthys herzbergii	Ariidae	Fully exploited	demersal	94.2	3.3	medium	medium	100

Hyporhamphus unifasciatus	Hemiramphidae	Overexploited	reef	30	2	high	na	67.16993
Hyporthodus niveatus	Serranidae	Fully exploited	demersal	122	4	low	very high	65.85647
Isopisthus parvipinnis	Scianidae	Overexploited	demersal	25	3.6	high	medium	92.21827
Larimus breviceps	Scianidae	Collapsed	demersal	31	3.5	high	medium	89.33915
Lile piquitinga	Clupeidae	Developing	pelagic	15	3.1	high	low	100
Lophius gastrophysus	Lophiidae	Overexploited	demersal	60	4.5	medium	low	2.473732
Lopholatilus villarii	Malacanthidae	Overexploited	demersal	107	3.8	low	na	21.92815
Lutjanus alexandrei	Lutjanidae	Fully exploited	demersal	24.3	3.8	medium	na	86.85309
Lutjanus analis	Lutjanidae	Fully exploited	reef	94	3.9	low	high	82.98959
Lutjanus cyanopterus	Lutjanidae	Collapsed	reef	160	4.4	low	high	99.43184
Lutjanus jocu	Lutjanidae	Fully exploited	reef	128	4.4	low	high	91.16136
Lutjanus purpureus	Lutjanidae	Fully exploited	demersal	100	3.6	low	high	56.60694
Lutjanus synagris	Lutjanidae	Developing	reef	60	3.8	medium	medium	99.05092
Lutjanus vivanus	Lutjanidae	Fully exploited	reef	83	3.1	low	high	98.71344
Lycengraulis grossidens	Engraulidae	Fully exploited	pelagic	23.5	3.7	medium	medium	100
Macrodon ancylodon	Scianidae	Fully exploited	demersal	45	3.9	medium	medium	98.04558
Macrodon atricauda	Scianidae	Collapsed	demersal	46	4	high	na	24.13667
Malacanthus plumieri	Malacanthidae	Fully exploited	reef	70	3.7	low	very high	99.49777
Menticirrhus americanus	Scianidae	Fully exploited	demersal	50	3.5	medium	medium	94.81103
Merluccius hubbsi	Merluccidae	Collapsed	pelagic	95	4.2	low	medium	0.809073

Micropogonias furnieri	Scianidae	Fully exploited	demersal	60	3.1	medium	medium	63.96272
Mugil gaimardianus	Mugilidae	Overexploited	reef	67	na	low	na	100
Mugil incilis	Mugilidae	Developing	demersal	40	2	high	medium	100
Mugil liza	Mugilidae	Collapsed	demersal	80	2	medium	high	59.62887
Mugil trichodon	Mugilidae	Fully exploited	pelagic	46	2	medium	na	100
Mullus argentinae	Mullidae	Fully exploited	demersal	30	3.5	medium	high	36.76047
Mustelus schmitti	Triakidae	Collapsed	demersal	92	3.6	very low	medium	2.762541
Mycteroperca bonaci	Serranidae	Overexploited	reef	150	4.3	low	very high	84.76615
Myrichthys breviceps	Ophichthidae	Fully exploited	reef	102	3.5	medium	na	100
Nebris microps	Scianidae	Collapsed	demersal	40	3.6	high	medium	97.79816
Notarius grandicassis	Ariidae	Overexploited	demersal	63	4	low	medium	97.07656
Ocyurus chrysurus	Lutjanidae	Developing	reef	86.3	4	low	medium	85.99113
Odontesthes argentinensis	Atherinopsidae	Collapsed	pelagic	42.1	3.7	medium	na	100
Odontoscion dentex	Scianidae	Fully exploited	reef	30	3.5	high	medium	100
Oligoplites palometa	Carangidae	Fully exploited	pelagic	49.7	4.3	medium	medium	98.70636
Oligoplites saliens	Carangidae	Fully exploited	pelagic	50	3.8	medium	medium	77.03803
Opisthonema oglinum	Clupeidae	Fully exploited	reef	38	4.5	medium	low	35.50198
Orthopristis ruber	Haemulidae	Fully exploited	demersal	40	3.6	medium	medium	33.43073
Paralichthys orbignyanus	Paralichthyidae	Fully exploited	demersal	50	3.5	low	na	0
Paralonchurus brasiliensis	Scianidae	Overexploited	demersal	30	3.4	high	medium	64.46513

Parona signata	Carangidae	Fully exploited	demersal	60	3.4	medium	low	67.32392
Peprilus paru	Stromateidae	Overexploited	pelagic	30	4.5	high	high	56.90707
Percophis brasiliensis	Percophidae	Fully exploited	demersal	53.3	4.2	medium	low	21.41425
Pogonias cromis	Scianidae	Overexploited	demersal	170	3.9	medium	low	49.37303
Polydactylus virginicus	Polynemidae	Overexploited	demersal	33	3.7	high	very high	100
Polyprion americanus	Polyprionidae	Collapsed	demersal	210	4.1	low	low	5.856582
Pomacanthus paru	Pomacanthidae	Developing	reef	41.1	2.8	medium	high	97.28996
Prionotus punctatus	Triglidae	Developing	demersal	45	3.8	low	medium	75.74702
Pseudopercis númida	Pinguipedidae	Collapsed	demersal	120	3.9	very low	na	40.50387
Pseudupeneus maculatus	Mullidae	Developing	reef	30	3.7	high	medium	99.99892
Pterengraulis atherinoides	Engraulidae	Overexploited	pelagic	30	3.9	high	medium	100
Rhinobatos horkelii	Rhinobatidae	Collapsed	demersal	138	3.8	very low	low	53.02956
Rhizoprionodon lalandii	Carcharhinidae	Fully exploited	demersal	77	4.3	very low	medium	99.05499
Rhizoprionodon porosus	Carcharhinidae	Fully exploited	reef	110	4	very low	medium	99.05499
Rhomboplites aurorubens	Lutjanidae	Developing	demersal	60	4.4	medium	very high	99.97458
Sardinella brasiliensis	Clupeidae	Overexploited	pelagic	27	3.1	high	medium	7.779252
Sciades couma	Ariidae	Fully exploited	demersal	97	3.9	medium	medium	79.74402
Sciades herzbergii	Ariidae	Overexploited	demersal	94.2	3.3	medium	medium	99.0129
Sciades parkeri	Ariidae	Fully exploited	demersal	190	4.1	high	medium	95.05379
Sciades proops	Ariidae	Fully exploited	demersal	100	4.4	high	medium	98.70436

Scomberomorus brasiliensis	Scombridae	Fully exploited	reef	125	3.3	medium	high	95.78942
Scorpaena plumieri	Scorpaenidae	Developing	reef	45	3.6	low	low	99.23456
Selene setapinnis	Carangidae	Overexploited	pelagic	60	3.7	medium	medium	93.12563
Selene vómer	Carangidae	Collapsed	demersal	48.3	4.3	medium	medium	99.86845
Sparisoma axillare	Scaridae	Fully exploited	reef	37	2	medium	na	100
Sphyraena tome	Sphyraenidae	Overexploited	pelagic	45	4.1	medium	na	80.0052
Stellifer brasiliensis	Scianidae	Developing	demersal	14.5	3.4	high	na	100
Trachinotus carolinus	Carangidae	Fully exploited	pelagic	64	3.5	medium	very high	68.01927
Trachinotus falcatus	Carangidae	Overexploited	reef	122	4	medium	medium	99.99345
Trachurus lathami	Carangidae	Overexploited	reef	40	4	medium	low	25.44865
Umbrina canosai	Scianidae	Fully exploited	demersal	40	3.9	medium	low	2.26094
Urophycis brasiliensis	Phycidae	Fully exploited	demersal	40	3.8	medium	low	40.69418
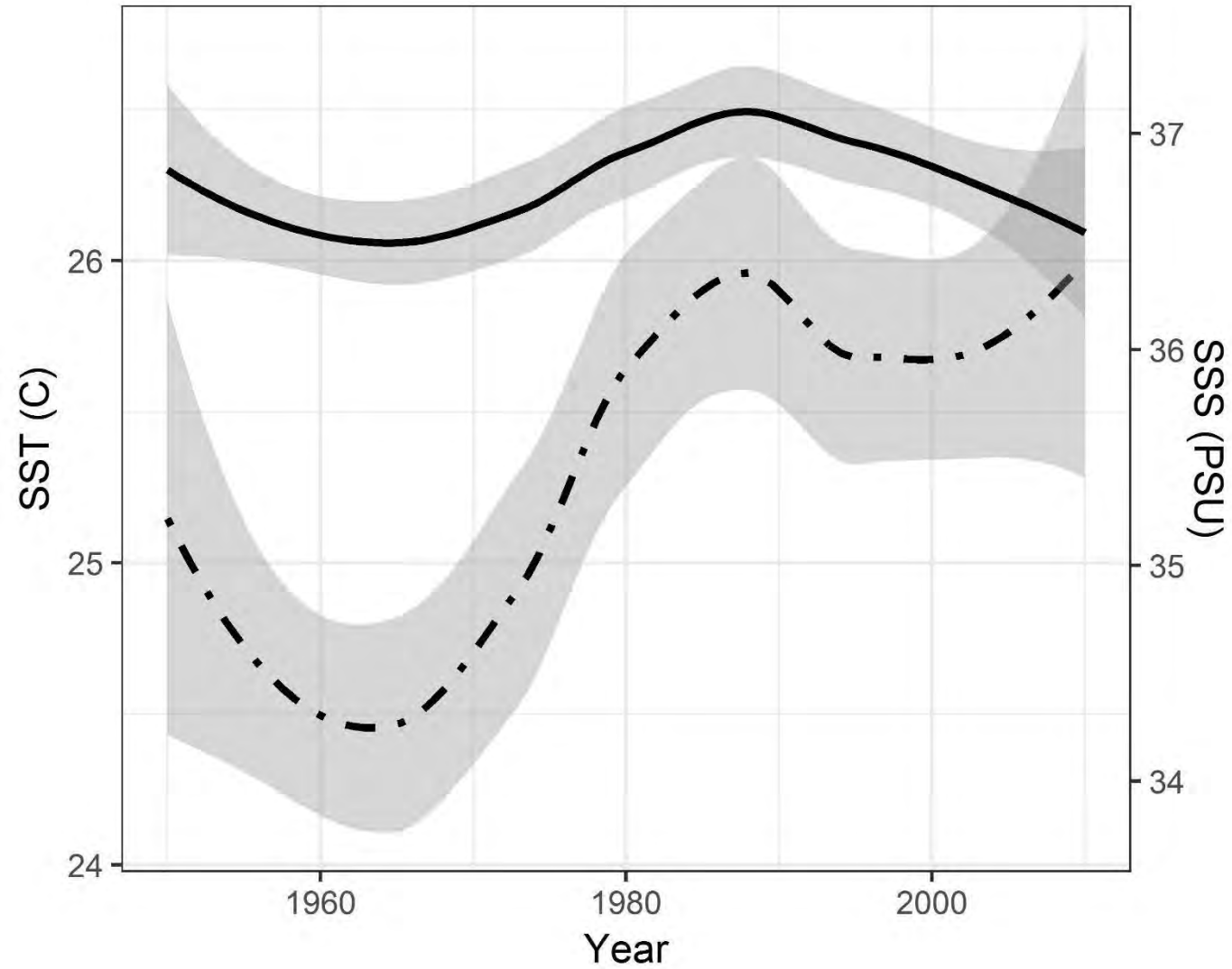
Figure S2. Time series of Sea Surface Temperature (SST), dashed line, and Sea Surface Salinity (SSS) in Brazil region extracted from NEMO climatology model (https://www.nemo-ocean.eu/).

a

Figure S3a. Results of General Additive Models for the significant variables for collapsed species

b1

Figure S3b1. Results of General Additive Models for the significant variables for overexploited species

b2

Figure S3b2. Results of General Additive Models for the significant variables for overexploited species

b3
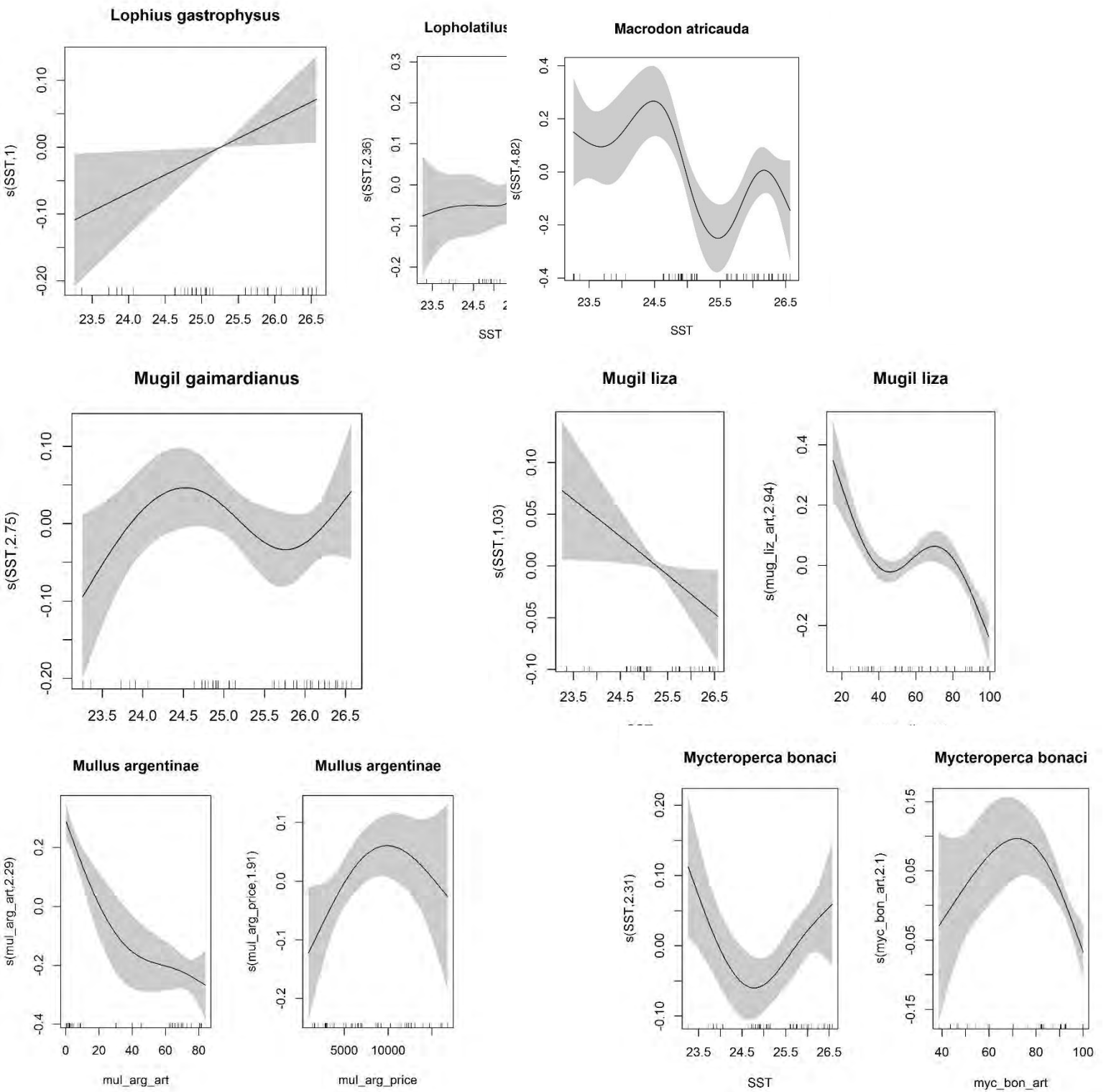
Figure S3b3. Results of General Additive Models for the significant variables for overexploited species

b4
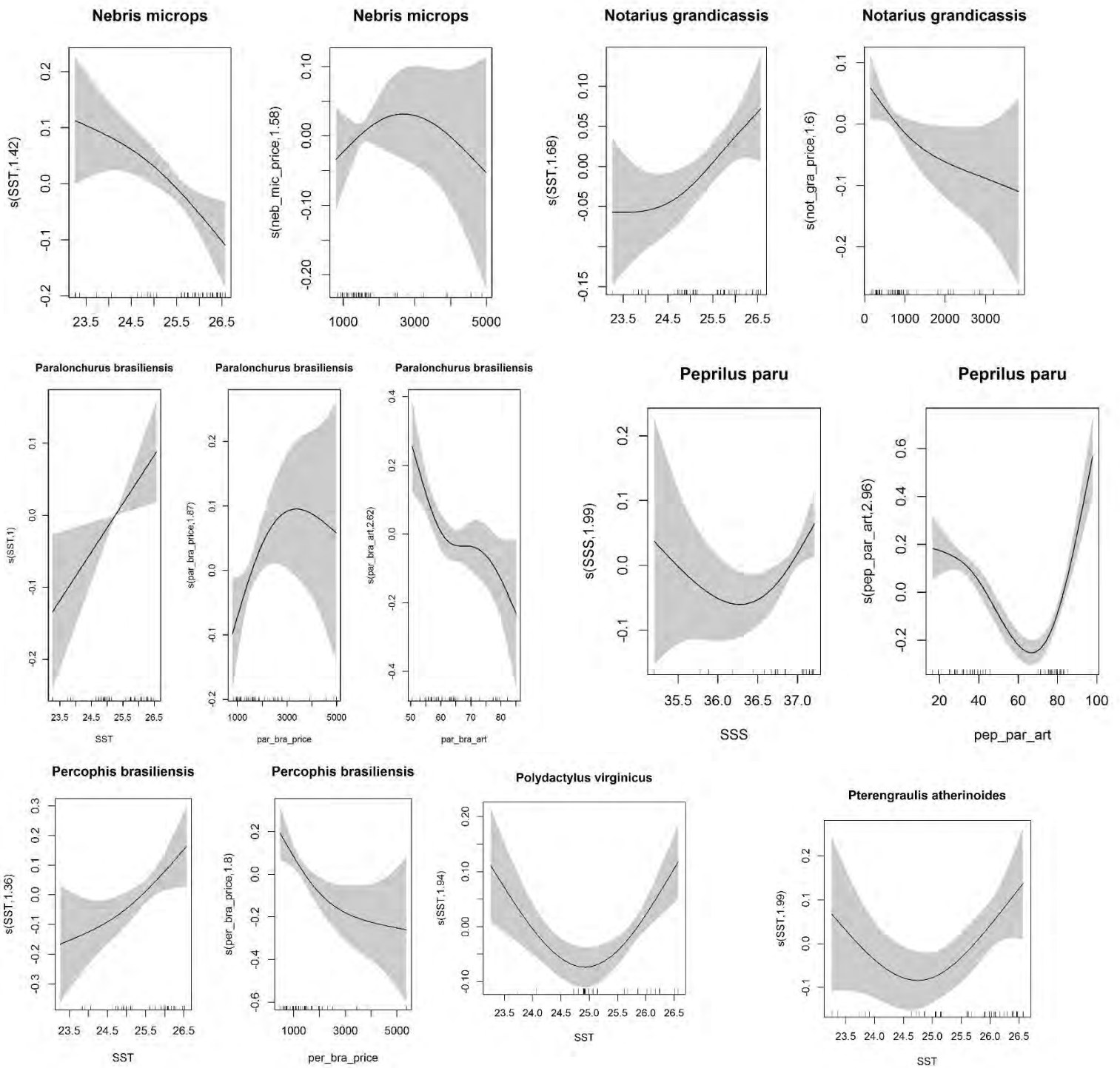
Figure S3b4. Results of General Additive Models for the significant variables for overexploited species
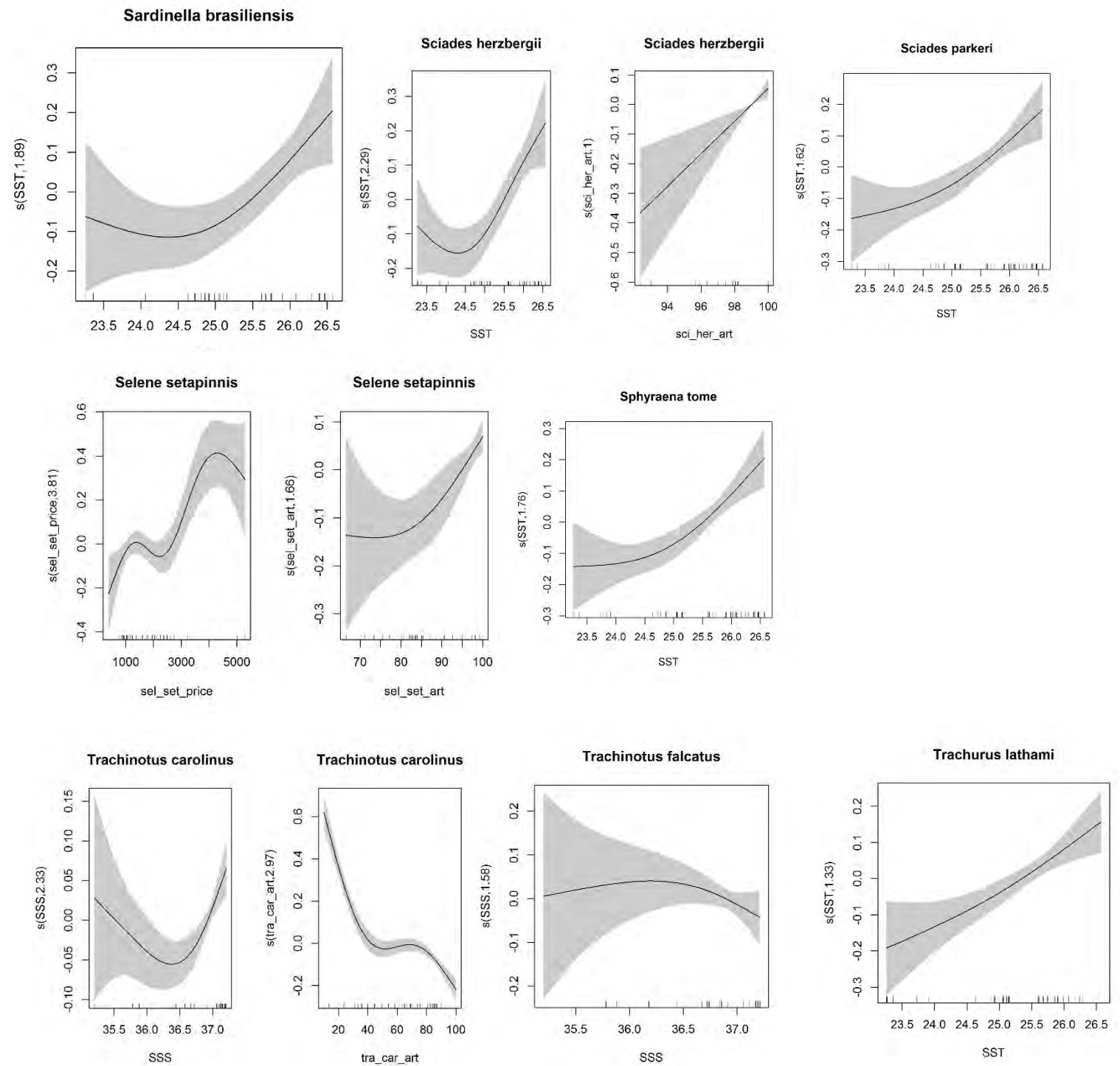
Figure S3b5. Results of General Additive Models for the significant variables for overexploited species

c1
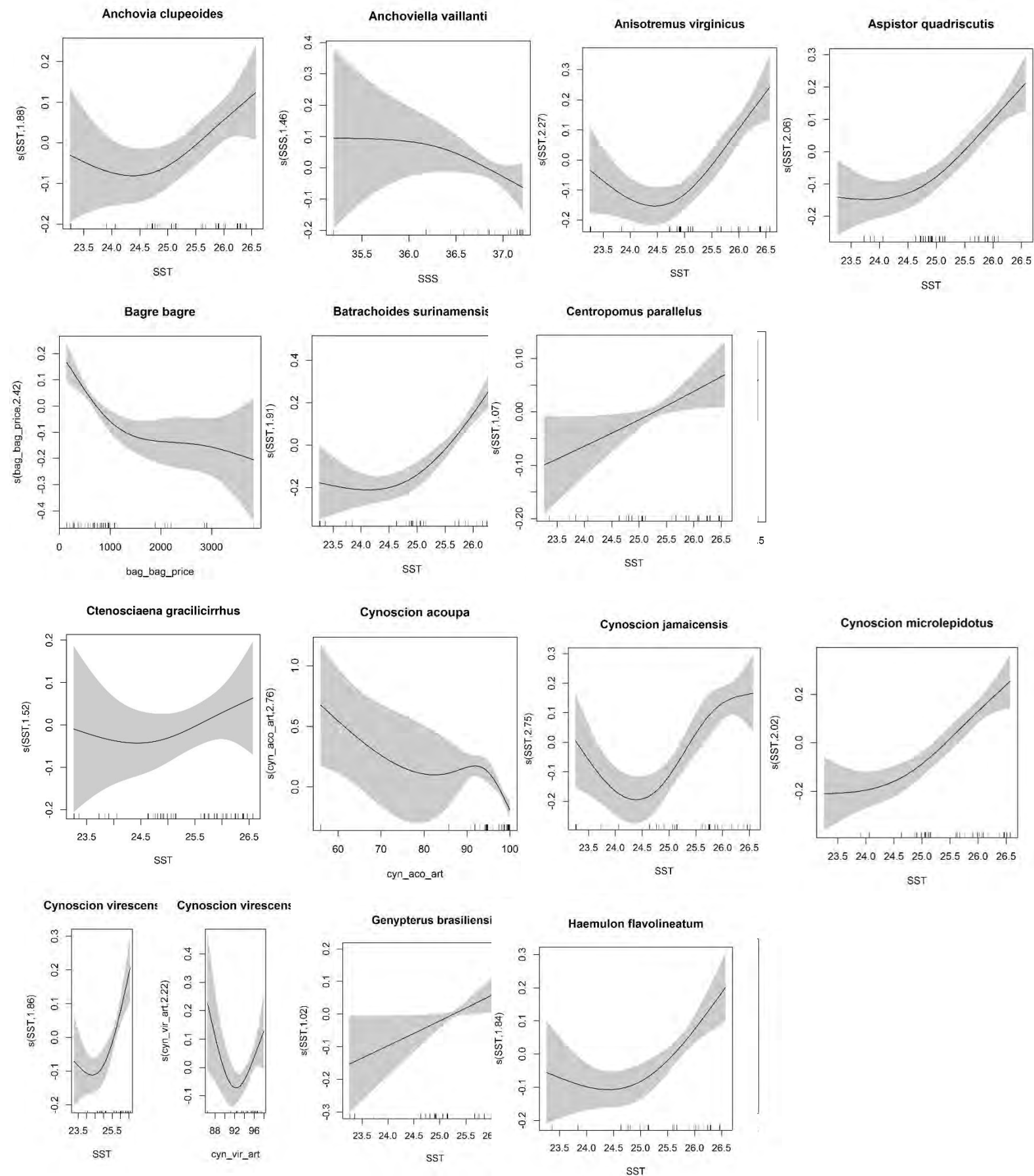
Figure S3c1. Results of General Additive Models for the significant variables for fully exploited species

c2
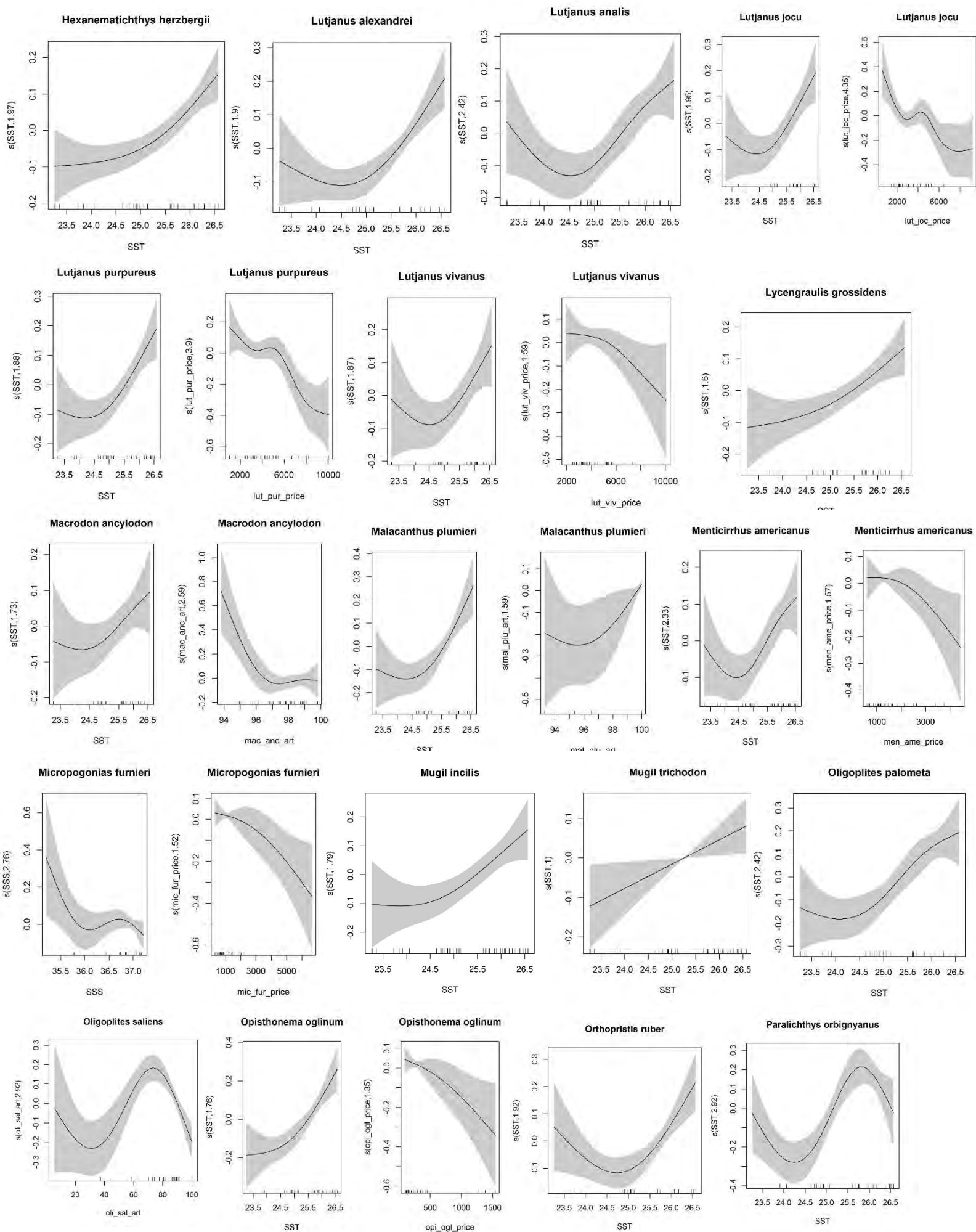
Figure S3c2. Results of General Additive Models for the significant variables for fully exploited species
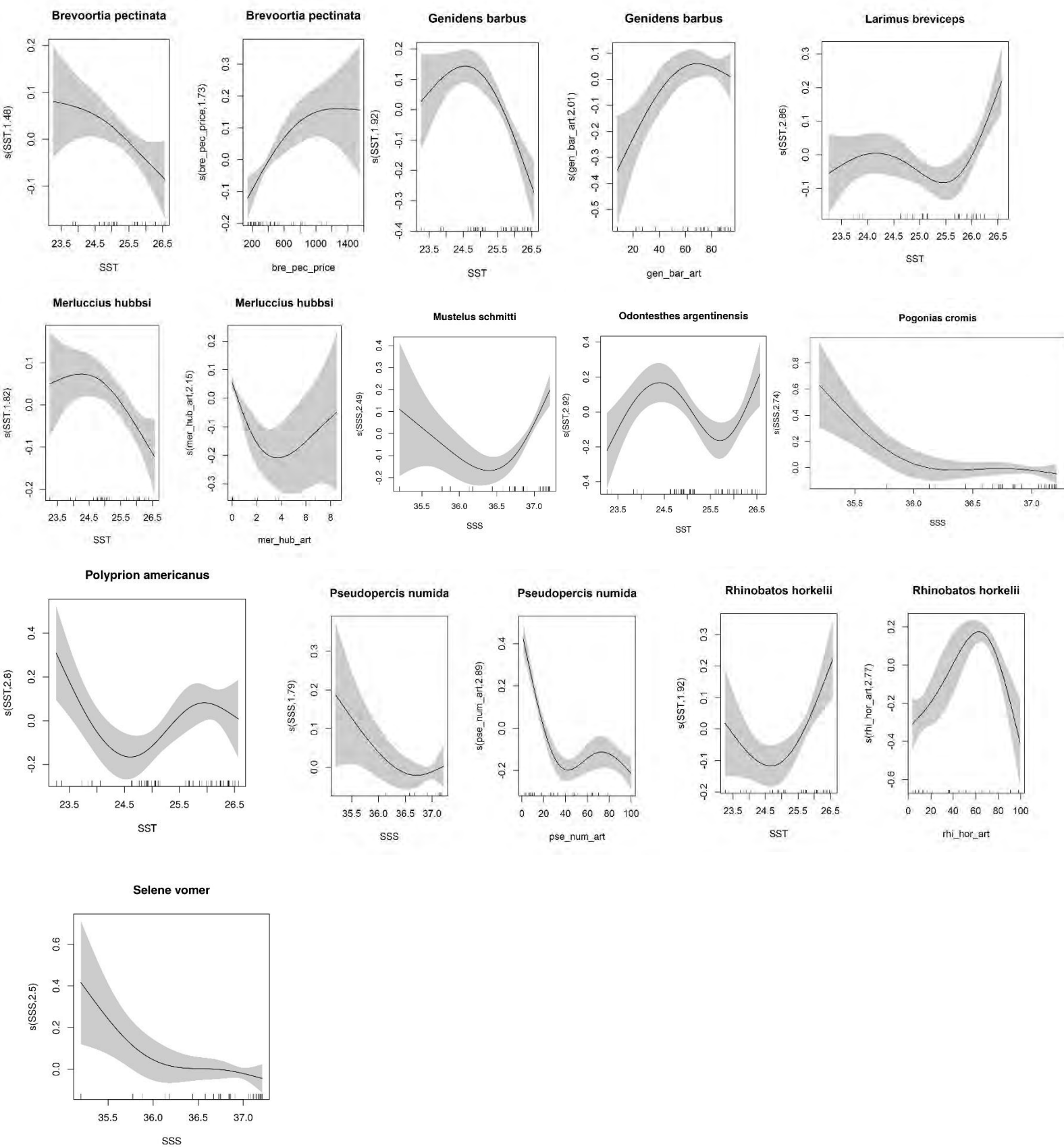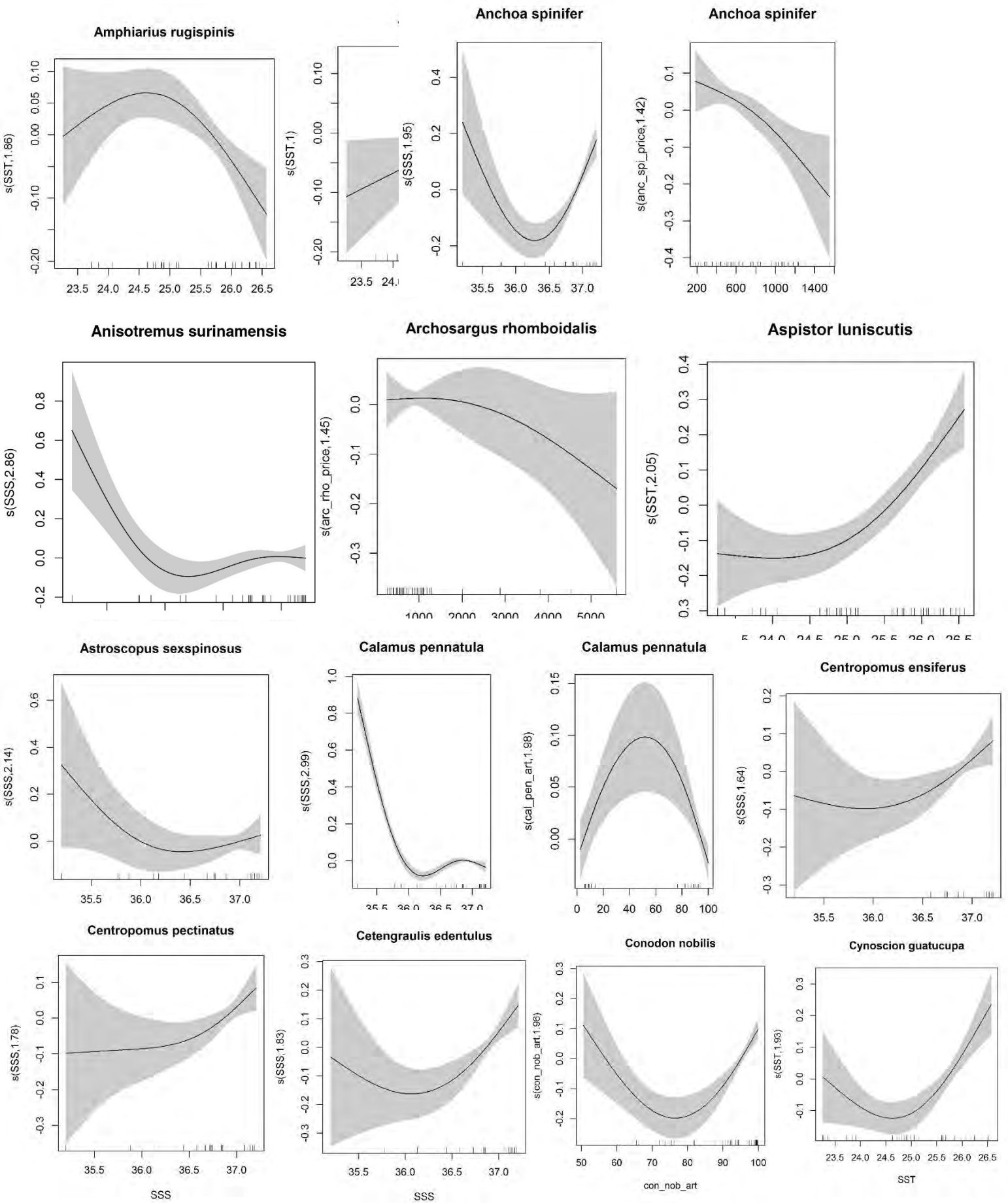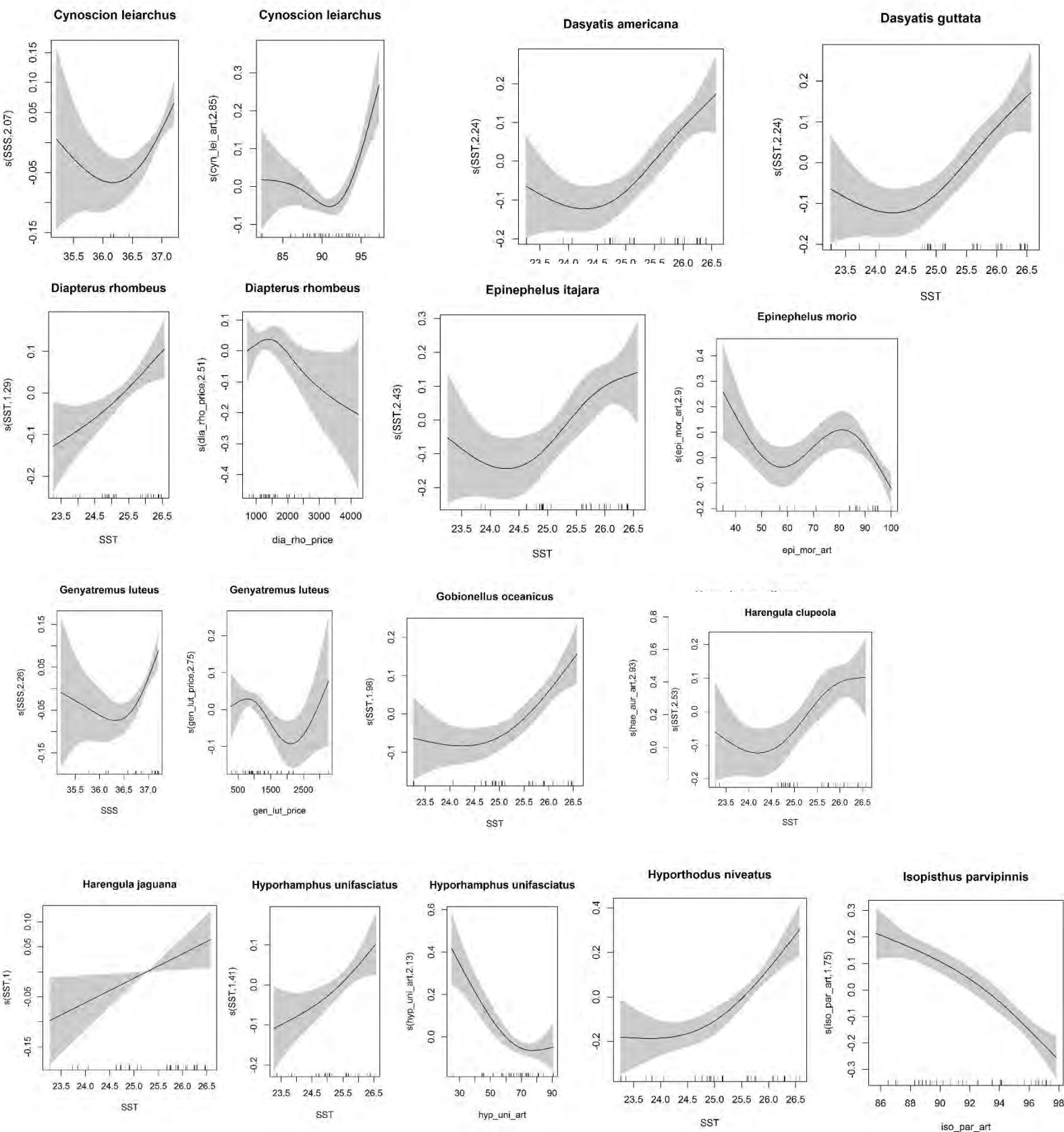
c3
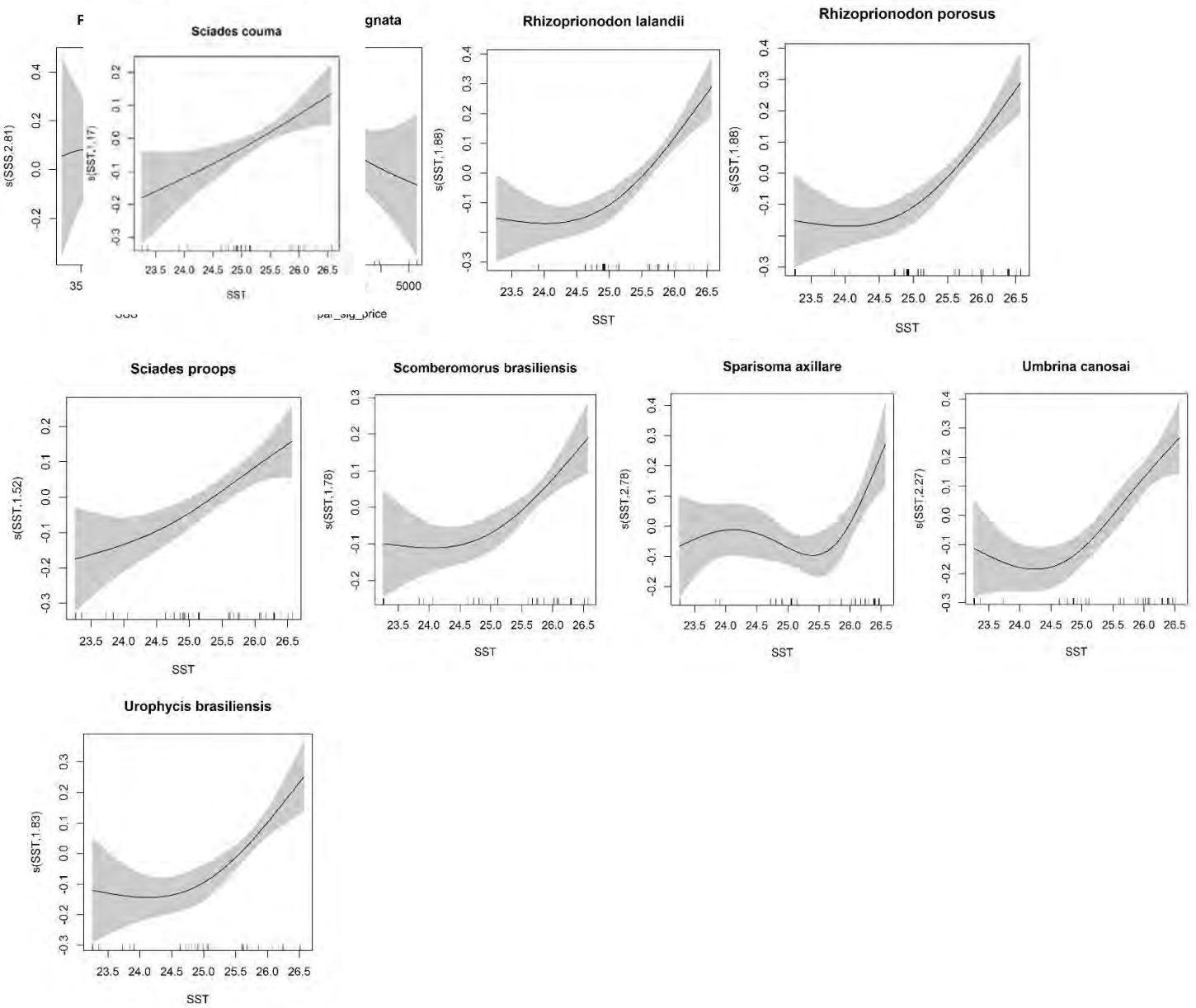
Figure S3c3. Results of General Additive Models for the significant variables for fully exploited species

d1
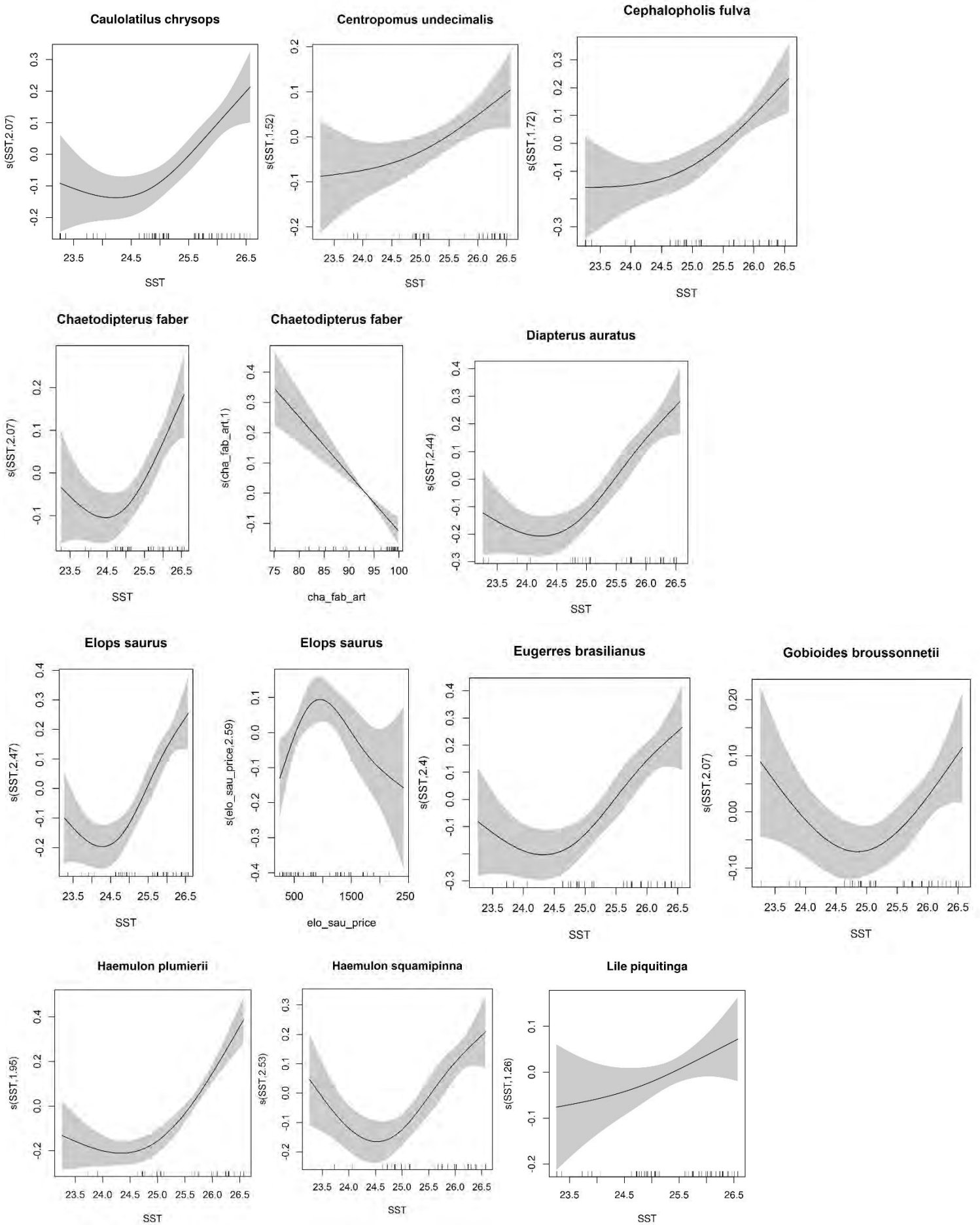
Figure S3d1. Results of General Additive Models for the significant variables for in development species

d2
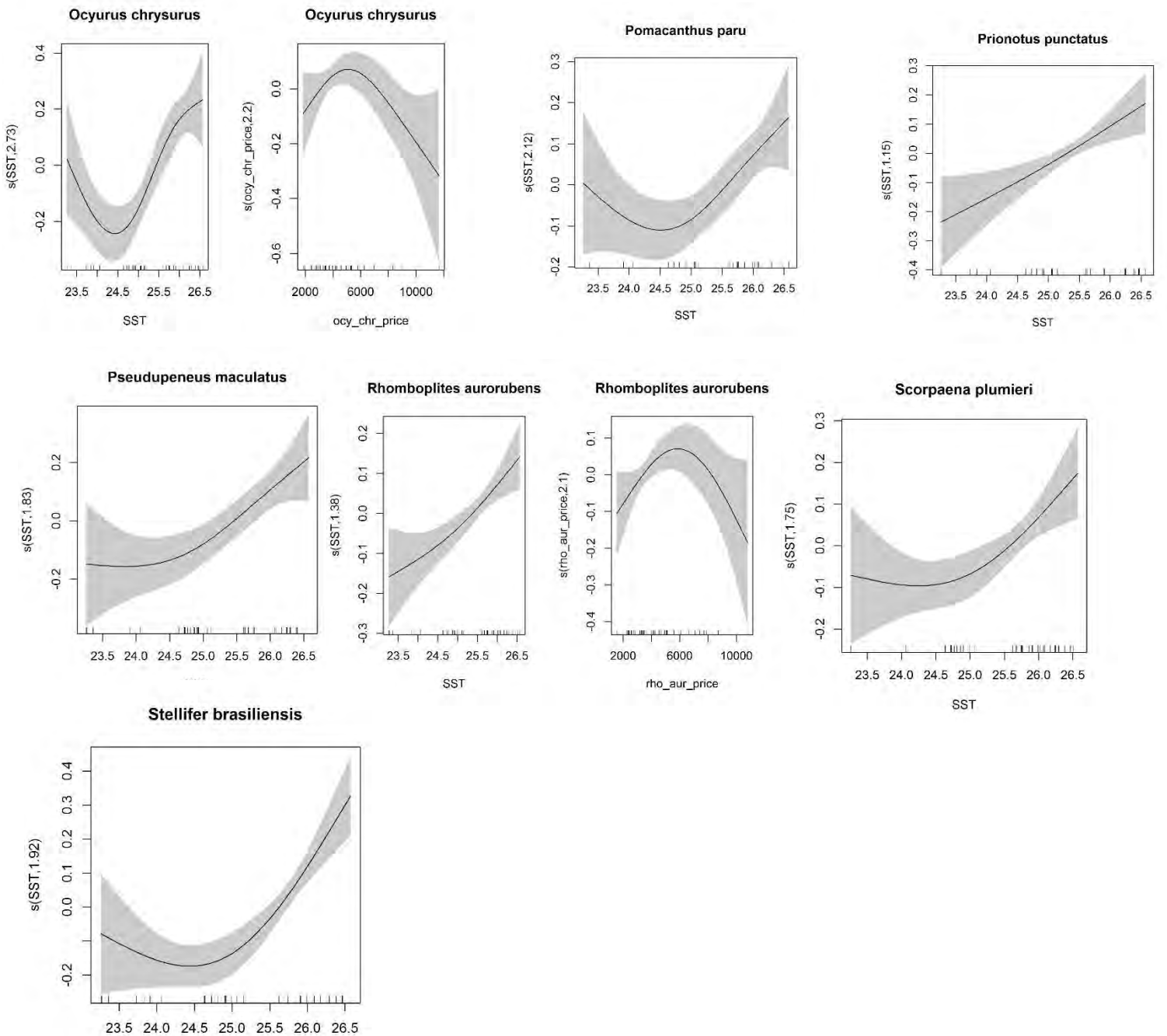
Figure S3d2. Results of General Additive Models for the significant variables for in development species

GENETIC STRUCTURE AND DEMOGRAPHIC HISTORY OF COMMERCIALLY EXPLOITED BRAZILIAN MARINE FISHES

Julia Tovar Verba, Adam Stow, Priscila Lopes, Maria Grazia Pennino, Antonio Sole-Cava, Frederico Henning, Sergio Maia Queiroz Lima

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ABSTRACT

Conservation of evolutionarily significant genetic lineages is considered crucial for the management of exploited species. We characterised the genetic structure and historical demography of 17 commercially important fish species using the mitochondrial COI and CytB sequence data. For most species, there was no evidence of gene flow barriers along the Brazilian coast, indicating that differences in the environment do not strongly affect their genetic structure. The only exception was *Pomatomus saltatrix*, for which we found significant population structure, with genetic discontinuity close to Rio de Janeiro, which is congruent with sudden changes in water temperature. In *Cynoscion jamaicensis*, significant isolation by geographic distance indicates that the dispersal of this species may be limited. The inference of past demographic changes using mtDNA sequence data suggested that most species have been keeping stable population sizes, potentially reflecting the stable environmental conditions found in the southwest Atlantic region.

KEYWORDS: Cytochrome Oxidase I, Cytochrome B, *Pomatomus saltatrix*, tropical fishes

1. INTRODUCTION

The influence of past climate change on species ecology and evolution may contribute to our understanding of current species distributions (Avise, 2000; Brown, 1995). Historical processes, including climate change, can influence connectivity through the formation of geographical barriers or corridors, thereby influencing dispersal rates or changes in habitat suitability that force species to move to other areas or adapt to new conditions (Brown & Lomolino, 1998; Brown et al., 2002). Genetic structuring may also occur due to habitat differences, for example populations occurring in less suitable locations may adapt genetically, leading to greater genetic divergence from the original population (Kawecki & Ebert, 2004).

In the marine environment, physical barriers to gene flow are less evident than those of terrestrial environments, and ecological boundaries may be more influential for patterns of diversity and distribution (Palumbi et al., 1997; Bowen et al., 2013). Changes in sea level, temperature, salinity and oceanic currents, especially during the last glacial maximum, have been shown to affect individual dispersal and, consequently, marine biodiversity patterns (O'Brien et al., 2013; Gaggiotti et al., 2009; O'Connor et al., 2007; Sjöqvist et al., 2015; White et al., 2010; Liu et al., 2006). Climatic changes during the Pleistocene may be responsible for population bottlenecks detected in some species, although the genetic signature that remains today depends on the severity of the bottleneck and the distribution and connectivity characteristics (Ludt & Rocha, 2015; Baggio et al., 2017). For example, during periods of low sea level, the importance of rivers outflow may increase (Rocha, 2003) and affect species that inhabit areas close to the coast (e.g. Liu et al., 2006).

Widely distributed marine fish stocks are common and can be explained by the lack of clear barriers in the marine environment (Hauser & Carvalho, 2008). However, a growing number of genetic studies shows that population structuring of marine species is more common than expected

(Hauser & Carvalho, 2008; Riginos & Nachman, 2001). Processes that drive genetic structure include environmental characteristics, such as large river mouths and sea currents, which can be effective barriers to dispersal and gene flow. In addition, climate effects, such as changes in sea surface temperature, may result in conditions outside the tolerance limits of an organism, determining its distribution (Allen et al., 2006; Castillo et al., 1996; Stuart-Smith et al., 2017).

Population size can also be affected by the past and present environment (e.g. Bucklin & Wiebe, 1998; Okello et al., 2008). One indirect genetic measure of the population size is the Effective Population Size (N_e), which is usually much smaller than the total population size, especially in high fecundity species, such as most marine ones (Frankham, 1995; Hauser et al., 2002; Ovenden et al., 2006). If a population has a large decline in effective size due to climatic factors, genetic diversity will be lost through random genetic drift (Hauser et al., 2002; Ovenden et al., 2006). Genetic diversity can be crucial as it influences the ability to adapt to changing environments (Reed et al., 2002).

The global status of fisheries suggests that overfishing is a major threat to the marine environment. More than 30% of all fish stocks are overexploited worldwide (FAO, 2016), which is of particular concern in developing tropical countries, where insufficient regulation and more vulnerable fishing communities are present (Bailey, 1988). Therefore, effective conservation and management planning are imperative to ensure fisheries sustainability and should be based on sound stock delimitation and evaluation of genetic variability (Cowen & Sponaugle, 2009). Genetic tools can offer some of the support needed for fisheries management (Waples et al., 2008).

For sound management strategies, some basic information is required, including the definition of fisheries stock structure (Ovenden et al., 2013). The term stock is used for commercially harvested fish and can be used as equivalent to the term population. A stock is a group of individuals of the same species, usually defined genetically, that share the same genetic characteristics and demographic history, and that respond distinctly to exploitation (Carvalho &

Hauser, 1994; Ovenden et al., 2013). Usually the limits of a stock are spatially defined, and these limits are used to support management measures (Ovenden et al., 2013). The analyses of stock limits for several species in a region can be used to indicate the most appropriate management scale (e.g. Lukoschek et al., 2016). This is especially useful in regions where the fishing is multispecific, such as in Brazil (WWF-Brasil, 2016). Another important measure for setting sustainable levels of fishing is fish abundance. However, it is difficult to count fish in the sea. An alternative is to use molecular tools to identify trends in effective population size. Decreases in effective population size can affect genetic diversity, a key measure of resilience.

We used a mitochondrial sequence dataset for 17 fish species exploited in tropical and subtropical regions of Brazil to investigate stock structure and effective population size variability. Brazil has a vast coastline, with a great heterogeneity in terms of temperature, productivity and freshwater outflows, which can affect species structure; yet this coastline does not have clear barriers. These species have different biological attributes, which may affect their individual genetic patterns. Here, we aim to: i) describe the genetic structure of these species; ii) describe changes in population effective size over time; and iii) relate the results to the current and past climate in the region. We expected coastal species to have higher population structure rates, mainly due to past changes in sea level. Such changes were expected to be especially pronounced where the continental shelf is narrower. Moreover, we also expected to find less population structure in pelagic species during their adult phase due to their dispersal capacity.

2. METHODOLOGY

2.1 Study area

In marine areas environmental changes may have differing impacts depending on location. The Brazilian coast extends over 8 thousand kilometers in the southwest Atlantic, covering both tropical and subtropical regions (Fig. 1). The wide latitudinal range represents a gradient of

different environments in terms of current and past climate, including temperature, salinity, oceanic currents, river mouths, continental shelf width and sea level fluctuation. The northern zone (north coast), highly affected by the Amazon River plume, has warmer sea temperatures and strong westward currents. The northeast area (northern region of the east coast) is characterised by warm sea temperatures and a narrow continental shelf, although its southern area is affected by the plume of the large São Francisco River. The southern coast of Brazil has relatively colder sea temperature, and a wide shelf, marked by an upwelling system around 30ºS of latitude (Coelho-Souza et al., 2012). These environmental and geographical differences along the coast, associated with past environmental changes, may explain the current distribution of marine species due to different habitat requirements for each species or populations.
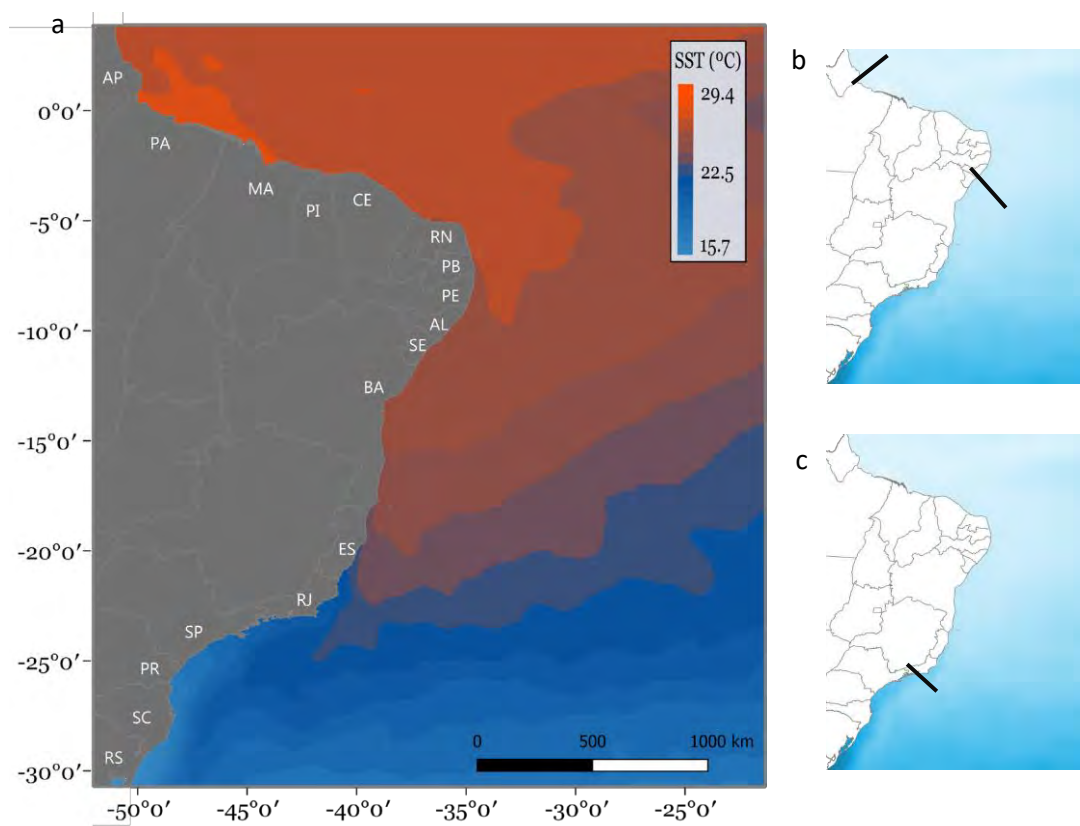
Fig. 1: a) Brazilian Coast with Sea Surface Temperature gradient and states location (AP – Amapá, PA – Pará, MA – Maranhão, PI – Piauí, CE- Ceará, RN- Rio Grande do Norte, PB – Paraíba, PE – Pernambuco, AL – Alagoas, SE – Sergipe, BA – Bahia, ES – Espírito Santo, RJ – Rio de Janeiro, SP – São Paulo, PR – Paraná, SC – Santa Catarina, RS – Rio Grande do Sul), and hypothesis tested in AMOVA: b) large rivers outflow (Amazonas and São Francisco) and c) temperature change .

Species selection and data collection

The 17 species (Supplementary Material Table S1) selected for this study represented more than 53,000 tons and almost 11% of the total fish catch in Brazil in 2010 (Freire et al., 2015), and belong to ten different families: five Lutjanidae, three Scombridae, three Sciaenidae, and one species of Serranidae, Ephippidae, Haemulidae, Malacanthidae, Clupeidae and Pomatomidae. In addition, these species have a wide diversity of biological traits. For example, their maximum body size ranges from 32.1 cm to 130 cm, their trophic level between 3.3 and 4.5, and there are species with demersal, pelagic and reef habitats (Froese & Pauly, 2019). They also show a variety of reproduction and developmental traits, including having some aggregation and estuarine spawners, species with pelagic and benthic larval stage and protogynous species. Most species are considered of 'least concern' by IUCN (N = 10), although five of those have their population trends unknown and two have been decreasing. Of the remaining seven, two species have not been evaluated by IUCN, two are considered 'data deficient' (with decreasing population), two are 'near threatened' and one is 'vulnerable'. Overall, the group of species analysed here is either of some conservation concern and/or important fishing targets.

These 17 species were also chosen due to number of sampling and spatial coverage available in the RENIMP (Rede Nacional de Identificação do Pescado; National Network for the Molecular Identification of Fisheries - UFRJ) project database and Genbank, as we needed species that had a minimum of four samples per "group" (see below). Genetic markers available in the dataset were cytochrome c oxidase subunit I (COI) and cytochrome b (CytB). Only sequences with a minimum length of 450 bp for COI and 700 bp for CytB were used. COI sequences were available for eight species, and CytB for 14 species. The RENIMP sequences analysed here will be submitted to Genbank.

2.2 Genetic analysis

The analyses involved three main steps: i) describing the genetic structure of each species; ii) analyzing demographic changes over time for each species; iii) looking for associations between demographic changes and the past environment.

2.3 Genetic population structure

The RENIMP and Genbank sequences were combined and aligned in MEGA4 (Tamura et al., 2007), using the MUSCLE function. Using DNAsp software (Librado & Rozas, 2009), populations sets were defined. To identify the population structure, Analysis of Molecular Variance (AMOVA) and φ_{ST} were performed using Arlequin 3.5 software (Excoffier & Lischer, 2010), with 10,000 permutations; differences were considered significant when $p < 0.05$. These parameters were calculated between some localities grouping combinations, based on possible barriers: i) large rivers (Rio São Francisco and Amazonas), temperature (based on Spalding et al., 2007 realms), endemism (Pinheiro et al., 2018), and location (coarse scale represented by Brazilian states due to location information availability). To visualize the haplotype diversity, networks were built using PopArt (Leigh & Bryant, 2015) using TCS network function. The figures were edited with InkScape software (Bah, 2009). After defining the population structure, some genetic diversity parameters were calculated: DNA Polymorphism, haplotype number, haplotype diversity, number of polymorphic sites, and nucleotide diversity. Aside from population structure due to barriers, genetic divergence may arise from geographic distance. Thus, we tested for isolation by distance. We used a pairwise Fst matrix between sites, calculated in Arlequin (Excoffier & Lischer, 2010), and geographic distance, calculated in Google Earth. The correlation between the two distance matrices was calculated using a Mantel test (mantel.rtest function from *ade4* package) in R for each species (R Core Team, 2019).

2.4 Historical Demography

We used three different and complementary approaches to identify population size tendencies (expansion, stability or decline). First, we applied two neutrality tests (Tajima's D and Fu's Fs), using Arlequin (considering significant p lower than 0.02 after Bonferroni correction). Tajima's D test uses information on mutation frequency to distinguish stable from expanding or contracting populations (Tajima, 1989). However, these changes in mutation frequency can be attributed to changes in population size change or positive selection (Ford, 2002). Fu's Fs uses haplotype distribution information to infer changes in population size (Fu, 1997). Fs can be sensitive to small sample size, but can be more powerful in identifying population expansion under some conditions (Ramos-Onsins & Rozas, 2002).

Because neutrality tests cannot use all signals of historical demography, we used an additional coalescent approach. We identified changes in effective population size over time using the Bayesian Skyline Plot, in Beast v1 and Beauti (Drummond, 2005). This analysis allows us to infer changes in population size over time. The parameters included in Beauti were clock rate = 1.2E-08, based on the mitochondrial gene substitution rate in fish (Bermingham et al., 1997). The chain length used was 10,000,000, but this number was increased whenever the Effective Sample Sizes of any parameter was less than 200. Convergence was assessed by the Bayesian Skyline. The reconstruction graphics were made using Tracer v1.7.1.

3. RESULTS

3.1 Genetic diversity

In total, 507 sequences from all the 17 species were analysed, 214 for eight species for COI and 293 for 14 species for CytB (Table 1). For COI, nucleotide diversity ranged from 0.00014 (*Chaetodipterus faber*) to 0.00563 (*Stellifer rastrifer*), and haplotype diversity ranged from 0.08 (*C. faber*) to 0.95 (*S. rastrifer*). For CytB, nucleotide diversity ranged from 0.00108 (*Lutjanus*

synagris) to 0.00497 (*L. purpureus*), and haplotype diversity ranged from 0.583 (*C. faber*) to 1.00 (*Auxis thazard*).

3.2 Population Structure

The only species that had significant geographic structuring was *Pomatomus saltatrix* (CytB Fst = 0.79467) (Fig. 2). For the other 16 species, the AMOVA and Fst analyses indicated no significant genetic differentiation between localities (Fig. 3, all haplotype networks are presented in Supplementary Material Fig. S1). Thus, for these species it was not possible to test the relationship between genetic structure and biological traits. The isolation by distance analysis found, for 16 species, that geographic distance is not correlated with genetic distance (p>0.05). *Cynoscion jamaicensis* was the only species with significant results for isolation by distance (p=0.0119), with genetic differentiation increasing with geographic distance.

Table 1: Results of number of analysed sequences and genetic diversity

Species	Marker	# sequences	Number of sites	Polymorphic sites	Haplotype number	Haplotype diversity	Nucleotide diversity Pi	Average number of nucleotide differences	Sites (sample number)
Auxis thazard	CytB	12	1022	22	12	1	0.0048	4.909	SE (7) PR+RJ (4)
Cephalopholis fulva	CytB	18	1043	7	8	0.752	0.00118	1.235	BA (11) PE+CE (5)
Chaetodipterus faber	COI	98	564	4	5	0.08	0.00014	0.082	SP (15) RJ (5) ES (21) MA (21) PB (8) PE (3) SC (21) AL (1) BA (1)
Chaetodipterus faber	CytB	9	725	4	4	0.583	0.00123	0.889	SC (4) PA (5)
Conodon nobilis	COI	13	517	1	2	0.154	0.0003	0.154	AL (5) RJ (5) SP (3)
Conodon nobilis	CytB	9	725	8	6	0.833	0.00245	1.778	SC (4) PA (5)
Cynoscion jamaicensis	COI	13	452	2	3	0.295	0.00068	0.308	SP (2) RJ (5) AL (5)
Cynoscion jamaicensis	CytB	21	722	14	11	0.895	0.00339	2.448	RS (8) SC (2) SP (3) RJ (5) ES (3)
Lopholatilus villarii	CytB	20	896	7	5	0.821	0.00238	2.132	RS (9) RJ (1) ES (7) BA (3)
Lutjanus analis	CytB	12	724	11	7	0.773	0.00291	2.106	PE (3) CE (2) BA (5) ES (2)
Lutjanus purpureus	CytB	51	779	40	44	0.995	0.00497	3.87	
Lutjanus synagris	CytB	17	725	5	6	0.588	0.00108	0.779	PE (7) CE (5) BA (4) PA (1)
Menticirrhus americanus	COI	23	565	5	5	0.644	0.00153	0.862	SP (11) RJ (5) AL (5)

Ocyurus chrysurus	COI	16	564	4	4	0.575	0.00137	0.775	SP (6) BA (1) PE (1) CE (8)
Ocyurus chrysurus	CytB	30	645	13	13	0.832	0.00231	1.49	PA (5) MA (3) CE (14) RN (2) PE (2) BA (2) ES (1)
Opisthonema oglinum	COI	13	600	11	8	0.91	0.00496	2.974	(3)
Pomatomus saltatrix	CytB	36	736	22	19	0.927	0.00259	1.908	RS (40) RJ (16) SC (1) SP (9) ES (2)
Scomber japonicus	CytB	20	1043	15	13	0.947	0.00256	2.674	SC (10) SE (8) RJ (2)
Scomberomorus brasiliensis	COI	22	625	11	11	0.913	0.00296	1.853	SP (13) RJ (4) AL (5)
Stellifer rastrifer	COI	16	627	12	11	0.95	0.00563	3.525	AL (1) SP (2) RJ (8) SC (1) PA (4)
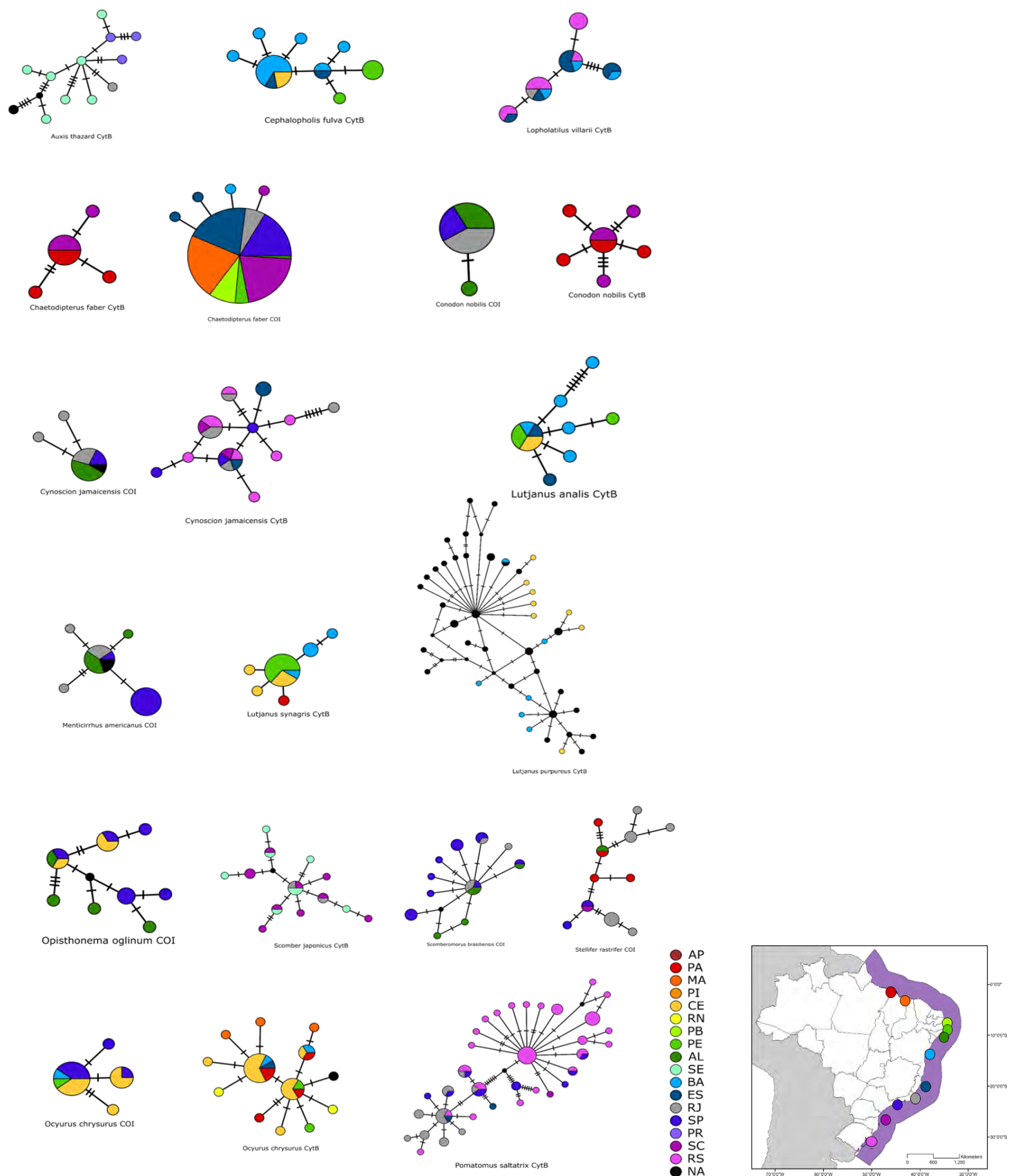
Fig. S1. Haplotype networks for each commercially marine fish species and mitochondrial marker. Abbreviations refer to Brazilian coastal states: AP – Amapá, PA – Pará, MA – Maranhão, PI – Piauí, CE – Ceará, RN – Rio Grande do Norte, PB – Paraíba, PE – Pernambuco, AL – Alagoas, SE – Sergipe, BA – Bahia, ES – Espírito Santo, RJ – Rio de Janeiro, SP – São Paulo, PR – Paraná, SC – Santa Catarina, RS – Rio Grande do Sul. NA is for the individuals without site information.

3.3 Historical Demography

According to Tajima's D, seven of the 17 species underwent population expansion. Fu's Fs provided evidence of population expansion in ten species, while signatures of expansion were common in the analyses of four species. However, selection may make some of the tests using mitochondrial DNA biased. Analysis of changes in population size over time, using Bayesian Skyline Plots, indicated that all but one species (*Lutjanus purpureus*) were stable (Table 1 and Supplementary material Fig. S2).

Table 1: Demography results for each species and marker to Tajima's D, Fu's Fs and Bayesian Skyline Plot (BSP) analyses. For Tajima's D and Fu's Fs, only significant results (p<0.02) were included.

Species	Marker	Tajima's D	Fu's Fs	BSP
Auxis thazard	CytB	Non-significant	**Expansion**	**Stability**
Cephalopholis fulva	CytB	Non-significant	**Expansion**	**Stability**
Chaetodipterus faber	COI	**Expansion**	**Expansion**	**Stability**
Chaetodipterus faber	CytB	Non-significant	Non-significant	**Stability**
Conodon nobilis	COI	Non-significant	Non-significant	**Stability**
Conodon nobilis	CytB	**Expansion**	Non-significant	**Stability**
Cynoscion jamaicensis	COI	Non-significant	**Expansion**	**Stability**
Cynoscion jamaicensis	CytB	Non-significant	**Expansion**	**Stability**
Lopholatilus villarii	CytB	Non-significant	Non-significant	**Stability**
Lutjanus analis	CytB	**Expansion**	Non-significant	**Stability**
Lutjanus jocu	CytB	**Expansion**	Non-significant	NA
Lutjanus purpureus	CytB	**Expansion**	**Expansion**	**Stable expansion through time**
Lutjanus synagris	CytB	Non-significant	**Expansion**	**Stability**
Menticirrhus americanus	COI	Non-significant	Non-significant	**Stability**
Ocyurus chrysurus	COI	Non-significant	Non-significant	**Stability**
Ocyurus chrysurus	CytB	**Expansion**	**Expansion**	**Stability**

Opisthonema oglinum	COI	Non-significant	Not significant	**Stability**
Pomatomus saltatrix north pop	CytB	Non-significant	**Expansion**	**Stability**
Pomatomus saltatrix south pop	CytB	**Expansion**	**Expansion**	**Stability**
Scomber japonicus	CytB	Non-significant	**Expansion**	**Stability**
Scomberomorus brasiliensis	COI	Non-significant	**Expansion**	**Stability**
Stellifer rastrifer	COI	Non-significant	**Expansion**	**Stability**

4. DISCUSSION

Our results indicate low levels of genetic structure and large effective population size variability for a large dataset of commercially exploited marine fish in Brazil. The pattern seems to be independent of the biological traits and distribution of the species. Low F_{ST} values and high connectivity are common in marine species, mainly due to lack of effective barriers, large population sizes and dispersal capacity (e.g. Momigliano et al., 2017). It is important to consider the differences in spatial coverage for each species and the low number of samples for some populations, which may influence the results of the genetic structure. In addition, it must be recognized that effective population size analysis using data from only a few mitochondrial loci is not ideal.

4.1 Genetic Structure

Despite relatively large environmental differences along the Brazilian coast, such as the existence of sea surface temperature gradients, there was no detectable genetic structure for 16 species. The outflow of the Amazon River, which has been considered a strong barrier, especially for reef species, is now seen as no more than a filter for some species (Rocha, 2003), including for the four species with samples taken north and south of the Amazonas river. Most previous studies in the same area found a similar pattern, although in general these studies focused only on one or

two species (e.g. da Silva et al., 2015; Silva-Oliveira et al., 2008). The lack of genetic structure in marine organisms can also be attributed to their pelagic larval phase, which allows the mixing of young individuals in early stages of their lives (Palumbi, 1994). There is, however, some evidence that this characteristic may still not explain the dispersal capacity across large marine barriers (Luiz et al., 2012). In addition, most species analysed here are large-bodied (76.2 cm in average), which facilitates the crossing of what might be otherwise considered a marine barrier (Luiz et al., 2012).

For species not restricted to coastal waters, such as *Lutjanus purpureus* and *Ocyurus chrysurus*, a lack of structure was previously reported using different genetic markers (da Silva et al., 2015; Gomes et al., 2012), which was confirmed by our findings. Surprisingly, coastal species, such as *Cephalopholis fulva, Chaetodipterus faber, Lutjanus jocu, Menticirrhus americanus, Opisthonema oglinum* and *Stellifer rastrifer*, which are usually found in waters less than 30-40m of depth, have also shown a lack of genetic structure (Froese & Pauly, 2017). A previous study indeed reported no genetic structure for *C. fulva*, even when comparing samples from the coast with oceanic islands (Souza et al., 2015). One coastal species, *Cynoscion jamaicensis,* was characterised by isolation by distance. The juveniles of this species depend on brackish waters, and usually inhabit estuaries and mangroves (FAO, 2019). This fact potentially limits the species dispersal and could explain the correlation between genetic and geographic distance.

Pomatomus saltatrix, a species that inhabits deeper waters up to 200m, was the only one to show some population structure, with one population identified in the southern Brazilian coast and another in the southeast coast, which is consistent with temperature differences (Fig. 2). Genetic divergence, however, is not explained by distance or by obvious aspects of this species' biology. *Pomatomus saltatrix* is a large bodied species and is globally distributed (Froese & Pauly, 2017), which are characteristics that suggest high dispersal, thus inconsistent with the presence of genetic structure. However, in some cases, environmental factors may be more important than biological traits in explaining genetic structure (Jenkins et al., 2018; Lehnert et al., 2019). The

genetic discontinuity happens close to an area of upwelling and temperature change (Coelho-Souza et al., 2012). This change in temperature has been suggested as an important determinant of species condition and distribution (Coelho-Souza et al., 2012). Local adaptations can also lead to unexpected patterns of genetic diversity (Rocha et al., 2005).

4.2 Historical Demography

We combined three different methods to identify demographic history (Eytan & Hellberg, 2010). Each of the tests used presents some caveats and responds differently to sample size, population size and departures from neutrality (selection) (Domingues et al., 2018; Grant, 2015). Despite some contrasting results between different analyses, most species showed stable or expanding populations. The high connectivity across sites, together with the West Atlantic climate stability, could explain the population stability found for 16 species (BSP results). Climatic stability presumably supports stable populations through time. Despite some debate, different models suggest that oceanic temperature varied less in low latitude areas.

Coastal dependent species may have been subject to more population bottlenecks followed by population expansion in response to past sea level declines (Domingues et al., 2018; Ludt & Rocha, 2015). However, the only species that showed consistent evidence of population expansion was *Lutjanus purpureus*, which is not dependent on coastal habitats and is found in waters more than 300m deep (Froese & Pauly, 2017). The distribution of *L. purpureus* is mostly tropical, and is associated with especially warm waters (Froese & Pauly, 2017). Its expansion, contrary to expectations, could have been due to changes in the environment, related to the effect of sea level changes and increased habitat availability given by the expanded continental shelf (Hoareau et al., 2013).

4.3 Management implications and future directions

Here we provided some useful information for the management of marine resources in Brazil. Overall, the results suggest that the Brazilian marine environment can potentially be managed as a single unit for most of its fished species (despite some exceptions, such as *Pomatomus saltatrix* and *Cynoscion jamaicensis*). We found that most species' populations have been stable or expanding over the last thousand years, although the methods used do not allow any inference about short time changes in population size that could be caused by recent anthropic impacts. Integrating these types of genetic data with fishery monitoring could improve the prediction of sustainable catch rates for commercially exploited species.

It is important to bear in mind that low levels of genetic structure may not have been detected (Mariani et al., 2005). Subtler levels of differentiation could be detected using markers that have higher resolving markers (e.g. microsatellites, SNPs) (e.g. Drinan et al., 2018; Xue et al., 2014). Mitochondrial DNA is traditionally used to identify management units, but the application of different markers provides finer grained information on genetic connectivity (e.g. Knutsen et al., 2003; Nielsen et al., 2004). Although our study presents some methodological caveats (e.g., low sample size and the spatial arrangement of the data), this is the first available information on genetic structure for a large number of species along the Brazilian coastline. This is the type of information that could be applied in evolutionary and molecular systematics studies, in addition to management and conservation measures. Following this publication, new RENIMP sequences will be publicly available to help build a strong database that can aid future phylogeographical, molecular systematic, and fish forensics studies.

5. ACKNOWLEDGEMENTS

6. REFERENCES

Allen, A. P., Gillooly, J. F., Savage, V. M., & Brown, J. H. (2006). Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences*, *103*(24), 9130–9135. https://doi.org/10.1073/pnas.0603587103

Avise, J. C. (2000). *Phylogeography: the history and formation of species*. Harvard University Press.

Baggio, R. A., Stoiev, S. B., Spach, H. L., & Boeger, W. A. (2017). Opportunity and taxon pulse: the central influence of coastal geomorphology on genetic diversification and endemism of strict estuarine species. *Journal of Biogeography*, *44*(7), 1626–1639. https://doi.org/10.1111/jbi.12934

Bah, T. (2009). *Inkscape: Guide to a vector drawing program*.

Bailey, R. (1988). Third world fisheries: Prospects and problems. *World Development*, *16*(6), 751–757.

Bermingham, E., McCafferty, S., & Martin, A. (1997). Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. *Molecular Systematic of Fishes*.

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology and Evolution*, *28*(6), 359–366. https://doi.org/10.1016/j.tree.2013.01.018

Brown, J H, & Lomolino, M. V. (1998). *Biogeography*. Sunderland.

Brown, James H., Stevens, G. C., & Kaufman, D. M. (2002). The Geographic Range: Size, Shape, Boundaries, and Internal Structure. *Annual Review of Ecology and Systematics*, *27*(1), 597–623. https://doi.org/10.1146/annurev.ecolsys.27.1.597

Brown, James H. (1995). *Macroecology*. University of Chicago Press.

Bucklin, A., & Wiebe, P. H. (1998). Low mitochondrial diversity and small effective population sizes of the copepods *Calanus finmarchicus* and *Nannocalanus minor*: possible impact of climatic variation during recent glaciation. *Journal of Heredity*, *89*(5), 383–392. https://doi.org/10.1093/jhered/89.5.383

Carvalho, G. R., & Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries*, *4*(3), 326–350. https://doi.org/10.1007/BF00042908

Castillo, J., Barbieri, M. A., & Gonzalez, A. (1996). Relationships between sea surface temperature, salinity, and pelagic fish distribution off northern Chile. *ICES Journal of Marine Science*, *53*(2), 139–146. https://doi.org/10.1006/jmsc.1996.0014

Coelho-Souza, S. A., López, M. S., Guimarães, J. R. D., Coutinho, R., & Candella, R. N. (2012). Biophysical interactions in the Cabo Frio upwelling system, southeastern Brazil. *Brazilian Journal of Oceanography*, *60*(3), 353–365. https://doi.org/10.1590/S1679-87592012000300008

Cowen, R. K., & Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science*, *1*(1), 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

da Silva, R., Veneza, I., Sampaio, I., Araripe, J., Schneider, H., & Gomes, G. (2015). High Levels of Genetic Connectivity among Populations of Yellowtail Snapper, *Ocyurus chrysurus* (Lutjanidae – Perciformes), in the Western South Atlantic Revealed through Multilocus Analysis. *PLOS ONE*, *10*(3), e0122173. https://doi.org/10.1371/journal.pone.0122173

Domingues, R. R., Hilsdorf, A. W. S., Shivji, M. M., Hazin, F. V. H., & Gadig, O. B. F. (2018). Effects of the Pleistocene on the mitochondrial population genetic structure and demographic history of the silky shark (*Carcharhinus falciformis*) in the western Atlantic Ocean. *Reviews in Fish Biology and Fisheries*, *28*(1), 213–227. https://doi.org/10.1007/s11160-017-9504-z

Drinan, D. P., Gruenthal, K. M., Canino, M. F., Lowry, D., Fisher, M. C., & Hauser, L. (2018). Population assignment and local adaptation along an isolation-by-distance gradient in Pacific cod (*Gadus macrocephalus*). *Evolutionary Applications*, *11*(8), 1448–1464. https://doi.org/10.1111/eva.12639

Drummond, A. J. (2005). Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. *Molecular Biology and Evolution*, *22*(5), 1185–1192. https://doi.org/10.1093/molbev/msi103

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Eytan, R. I., & Hellberg, M. E. (2010). Nuclear and mitochondrial sequence data reveal and conceal different demographic histories and population genetic processes in Caribbean reef fishes. *Evolution*, *64*(12), 3380–3397. https://doi.org/10.1111/j.1558-5646.2010.01071.x

FAO. (2016). The State of World Fisheries and Aquaculture. In *Contributing to food security and nutrition for all*. https://doi.org/10.5860/CHOICE.50-5350

Ford, M. J. (2002). Applications of selective neutrality tests to molecular ecology. *Molecular Ecology*, *11*(8), 1245–1262. https://doi.org/10.1046/j.1365-294X.2002.01536.x

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: a review. *Genetical Research*, *66*(02), 95. https://doi.org/10.1017/S0016672300034455

Froese, R., & Pauly, D. (2019). Fish Base. World Wide Web electronic publication.

Fu, Y. (1997). Statistical Tests of Neutrality of Mutations Against Population Growth, Hitchhiking and Background Selection. *Genetics*, *147*, 915–925. https://doi.org/genetics.org//147/2/915

Gaggiotti, O. E., Bekkevold, D., Jørgensen, H. B. H., Foll, M., Carvalho, G. R., Andre, C., & Ruzzante, D. E. (2009). Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: Atlantic herring as a case study. *Evolution*, *63*(11), 2939–2951. https://doi.org/10.1080/00049182.2018.1449710

Gomes, G., Sampaio, I., & Schneider, H. (2012). Population Structure of *Lutjanus purpureus* (Lutjanidae - Perciformes) on the Brazilian coast: further existence evidence of a single species of red snapper in the western Atlantic. *Anais Da Academia Brasileira de Ciências*, *84*(4), 979–999. https://doi.org/10.1590/S0001-37652012000400013

Grant, W. S. (2015). Problems and Cautions With Sequence Mismatch Analysis and Bayesian Skyline Plots to Infer Historical Demography. *Journal of Heredity*, *106*(4), 333–346. https://doi.org/10.1093/jhered/esv020

Hauser, L., Adcock, G. J., Smith, P. J., Bernal Ramirez, J. H., & Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New

Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences*, *99*(18), 11742–11747. https://doi.org/10.1073/pnas.172242899

Hauser, Lorenz, & Carvalho, G. R. (2008). Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish and Fisheries*, *9*, 333–362. https://doi.org/10.1164/ajrccm.160.5.9903088

Hoareau, T. B., Boissin, E., Paulay, G., & Bruggemann, J. H. (2013). The Southwestern Indian Ocean as a potential marine evolutionary hotspot: perspectives from comparative phylogeography of reef brittle-stars. *Journal of Biogeography*, *40*(11), 2167–2179. https://doi.org/10.1111/jbi.12155

Jenkins, T. L., Castilho, R., & Stevens, J. R. (2018). Meta-analysis of northeast Atlantic marine taxa shows contrasting phylogeographic patterns following post-LGM expansions. *PeerJ*, *6*, e5684. https://doi.org/10.7717/peerj.5684

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Knutsen, H., Jorde, P. E., Andre, C., & Stenseth, N. C. (2003). Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. *Molecular Ecology*, *12*(2), 385–394. https://doi.org/10.1046/j.1365-294X.2003.01750.x

Lehnert, S. J., DiBacco, C., Van Wyngaarden, M., Jeffery, N. W., Ben Lowen, J., Sylvester, E. V. A., … Bradbury, I. R. (2019). Fine-scale temperature-associated genetic structure between inshore and offshore populations of sea scallop (*Placopecten magellanicus*). *Heredity*, *122*(1), 69–80. https://doi.org/10.1038/s41437-018-0087-9

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*(11), 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Liu, J.-X., Gao, T.-X., Wu, S.-F., & Zhang, Y.-P. (2006). Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck & Schlegel, 1845). *Molecular Ecology*, *16*(2), 275–288. https://doi.org/10.1111/j.1365-294X.2006.03140.x

Ludt, W. B., & Rocha, L. A. (2015). Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. *Journal of Biogeography*, *42*(1), 25–38. https://doi.org/10.1111/jbi.12416

Luiz, O. J., Madin, J. S., Robertson, D. R., Rocha, L. A., Wirtz, P., & Floeter, S. R. (2012). Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1730), 1033–1040. https://doi.org/10.1098/rspb.2011.1525

Lukoschek, V., Riginos, C., & van Oppen, M. J. H. (2016). Congruent patterns of connectivity can inform management for broadcast spawning corals on the Great Barrier Reef. *Molecular Ecology*, *25*(13), 3065–3080. https://doi.org/10.1111/mec.13649

Mariani, S., Hutchinson, W. F., Hatfield, E. M. C., Ruzzante, D. E., Simmonds, E. J., Dahlgren, T. G., … Carvalho, G. R. (2005). North Sea herring population structure revealed by microsatellite analysis. *Marine Ecology Progress Series*, *303*, 245–257. https://doi.org/10.3354/meps303245

Momigliano, P., Harcourt, R., Robbins, W. D., Jaiteh, V., Mahardika, G. N., Sembiring, A., & Stow, A. (2017). Genetic structure and signatures of selection in grey reef sharks (*Carcharhinus amblyrhynchos*). *Heredity*, *119*(3), 142–153. https://doi.org/10.1038/hdy.2017.21

Nielsen, E. E., Nielsen, P. H., Meldrup, D., & Hansen, M. M. (2004). Genetic population structure of turbot (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. *Molecular Ecology*, *13*(3), 585–595. https://doi.org/10.1046/j.1365-294X.2004.02097.x

O'Brien, S. M., Gallucci, V. F., & Hauser, L. (2013). Effects of species biology on the historical demography of sharks and their implications for likely consequences of contemporary climate change. *Conservation Genetics*, *14*(1), 125–144. https://doi.org/10.1007/s10592-012-0437-8

O'Connor, M. I., Bruno, J. F., Gaines, S. D., Halpern, B. S., Lester, S. E., Kinlan, B. P., & Weiss, J. M. (2007). Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proceedings of the National Academy of Sciences*, *104*(4), 1266–1271. https://doi.org/10.1073/pnas.0603422104

Okello, J. B. A., Wittemyer, G., Rasmussen, H. B., Arctander, P., Nyakaana, S., Douglas-Hamilton, I., & Siegismund, H. R. (2008). Effective population size dynamics reveal impacts of historic climatic events and recent anthropogenic pressure in African elephants. *Molecular Ecology*, *17*(17), 3788–3799. https://doi.org/10.1111/j.1365-294X.2008.03871.x

Ovenden, J. R., Berry, O., Welch, D. J., Buckworth, R. C., & Dichmont, C. M. (2013). Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. *Fish and Fisheries*, *16*(1), 125–159. https://doi.org/10.1111/faf.12052

Ovenden, J. R., Peel, D., Street, R., Courtney, A. J., Hoyle, S. D., Peel, S. L., & Podlich, H. (2006). The genetic effective and adult census size of an Australian population of tiger prawns (*Penaeus esculentus*). *Molecular Ecology*, *16*(1), 127–138. https://doi.org/10.1111/j.1365-294X.2006.03132.x

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, *25*, 547–572.

Palumbi, S. R., Grabowsky, G., Duda, T., Geyer, L., & Tachino, N. (1997). Speciation and population genetic structure in tropical pacific sea urchins. *Evolution*, *51*(5), 1506–1517. https://doi.org/10.1111/j.1558-5646.1997.tb01474.x

Pinheiro, H. T., Rocha, L. A., Macieira, R. M., Carvalho-Filho, A., Anderson, A. B., Bender, M. G., … Floeter, S. R. (2018). South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. *Diversity and Distributions*, *24*(7), 951–965. https://doi.org/10.1111/ddi.12729

R Core Team (2019). *R: A language and environment for statistical computing*. Retrieved from https://www.r-project.org/

Ramos-Onsins, S. E., & Rozas, J. (2002). Statistical Properties of New Neutrality Tests Against Population Growth. *Molecular Biology and Evolution*, *19*(12), 2092–2100. https://doi.org/10.1093/oxfordjournals.molbev.a004034

Reed, D. H., Briscoe, D. A., Frankham, R., Ballou, J., O'Grady, J., Kalinowski, S., … Lowe, E. (2002). Inbreeding and extinction: The effect of environmental stress and lineage. *Conservation Genetics*, *3*(3), 301–307. https://doi.org/10.1023/A:1019948130263

Riginos, C., & Nachman, M. W. (2001). Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology*, *10*(6), 1439–1453. https://doi.org/10.1046/j.1365-294X.2001.01294.x

Rocha, L. A. (2003). Patterns of distribution and processes of speciation in Brazilian reef fishes. *Journal of Biogeography*, *30*(8), 1161–1171. https://doi.org/10.1046/j.1365-2699.2003.00900.x

Rocha, L. A., Robertson, D. R., Roman, J., & Bowen, B. W. (2005). Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1563), 573–579.

https://doi.org/10.1098/2004.3005

Silva-Oliveira, G. C., Rego, P., Schneider, H., Sampaio, I., & Vallinoto, M. (2008). Genetic characterization of populations of the critically endangered Goliath grouper (*Epinephelus itajara*, Serranidae) from the Northern Brazilian coast through analyses of mtDNA. *Genetics and Molecular Biology*, *31*(4).

Sjöqvist, C., Godhe, A., Jonsson, P. R., Sundqvist, L., & Kremp, A. (2015). Local adaptation and oceanographic connectivity patterns explain genetic differentiation of a marine diatom across the North Sea-Baltic Sea salinity gradient. *Molecular Ecology*, *24*(11), 2871–2885. https://doi.org/10.1111/mec.13208

Souza, A. S. de, Dias Júnior, E. A., Galetti Jr, P. M., Machado, E. G., Pichorim, M., & Molina, W. F. (2015). Wide-range genetic connectivity of Coney, *Cephalopholis fulva* (Epinephelidae), through oceanic islands and continental Brazilian coast. *Anais Da Academia Brasileira de Ciências*, *87*(1), 121–136. https://doi.org/10.1590/0001-3765201520130411

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdana, Z. A., Finlayson, M., … Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*, *57*(7), 573. https://doi.org/10.1641/B570707

Stuart-Smith, R. D., Edgar, G. J., & Bates, A. E. (2017). Thermal limits to the geographic distributions of shallow-water marine species. *Nature Ecology & Evolution*, *1*(12), 1846–1852. https://doi.org/10.1038/s41559-017-0353-x

Tajima, F. (1989). Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics*, *123*, 585–595.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, *24*(8), 1596–1599. https://doi.org/10.1093/molbev/msm092

Waples, R. S., Punt, A. E., & Cope, J. M. (2008). Integrating genetic data into management of marine resources: How can we do it better? *Fish and Fisheries*, *9*(4), 423–449. https://doi.org/10.1111/j.1467-2979.2008.00303.x

White, C., Selkoe, K. A., Watson, J., Siegel, D. A., Zacherl, D. C., & Toonen, R. J. (2010). Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1688), 1685–1694. https://doi.org/10.1098/rspb.2009.2214

WWF-Brasil. (2016). *Situação atual e tendências da pesca marinha no brasil e o papel dos subsídios*. Sao Paulo.

Xue, D. X., Wang, H. Y., Zhang, T., & Liu, J. X. (2014). Population genetic structure and demographic history of *Atrina pectinata* based on mitochondrial DNA and microsatellite markers. *PLoS ONE*, *9*(5). https://doi.org/10.1371/journal.pone.0095436

7. SUPPLEMENTARY MATERIAL

Table S1. List of analysed species, biological information (Fish Base), markers, number of sequences for each marker (N) and number of sites, represented by Brazilian coastal states (N sites). Some species had two markers analysed, so are repeated in the list.

| Species	Family	Order	IUCN	Habitat	Distribution	Depth range (m)	Brackish related	Maximum size	Trophic level	Marker	N	N sites
Auxis thazard	Scombridae	Perciformes	LC	pelagic	tropical	50 - ?	No	65	4.4	CytB	12	4
Cephalopholis fulva	Serranidae	Perciformes	LC	reef	subtropical	1 - 150	No	41	4.1	CytB	18	4
Chaetodipterus faber	Ephippidae	Perciformes	LC	reef	subtropical	3 a 35	Yes	91	4.5	COI	9	9
Chaetodipterus faber	Ephippidae	Perciformes	LC	reef	subtropical	3 a 35	Yes	91	4.5	CytB	98	2
Conodon nobilis	Haemulidae	Perciformes	LC	demersal	subtropical	1 - 100	No	33.6	3.6	COI	13	3
Conodon nobilis	Haemulidae	Perciformes	LC	demersal	subtropical	1 - 100	No	33.6	3.6	CytB	9	2
Cynoscion jamaicensis	Sciaenidae	Perciformes	LC	demersal	subtropical	1 a 70	Yes	50	3.8	COI	21	3
Cynoscion jamaicensis	Sciaenidae	Perciformes	LC	demersal	subtropical	1 a 70	Yes	50	3.8	CytB	13	5
Lopholatilus villarii	Malacanthidae	Perciformes	NA	demersal	subtropical	NA	No	107	3.8	CytB	20	4
Lutjanus analis	Lutjanidae	Perciformes	NT	reef	tropical	25 - 95	Yes	94	3.9	CytB	12	4
Lutjanus jocu	Lutjanidae	Perciformes	DD	reef	subtropical	2 a 40	Yes	128	4.4	CytB	10	2
Lutjanus purpureus	Lutjanidae	Perciformes	NA	demersal	tropical	26 a 340	No	100	3.6	CytB	14	2
Lutjanus synagris	Lutjanidae	Perciformes	NT	reef	subtropical	10 - 400	No	60	3.8	CytB	17	4
Menticirrhus americanus	Sciaenidae	Perciformes	LC	demersal	subtropical	? – 40	Yes	50	3.5	COI	23	3
Ocyurus chrysurus	Lutjanidae	Perciformes	DD	reef	subtropical	0 - 180	No	86.3	4	COI	30	4
Ocyurus chrysurus	Lutjanidae	Perciformes	DD	reef	subtropical	0 - 180	No	86.3	4	CytB	16	7
Opisthonema oglinum	Clupeidae	Clupeiformes	LC	reef	tropical	1 a 50	No	38	4.5	COI	13	3
Pomatomus saltatrix	Pomatomidae	Perciformes	VU	pelagic	subtropical	0 a 200	Yes	130	4.5	CytB	68	5
Scomber japonicus	Scombridae	Perciformes	LC	pelagic	temperate	NA	Yes	65	3.9	CytB	20	3
Scomberomorus brasiliensis	Scombridae	Perciformes	LC	reef	tropical	NA	No	125	3.3	COI	22	3
Stellifer rastrifer	Sciaenidae	Perciformes	LC	demersal	tropical	0 a 40	Yes	32.1	3.4	COI	16	5
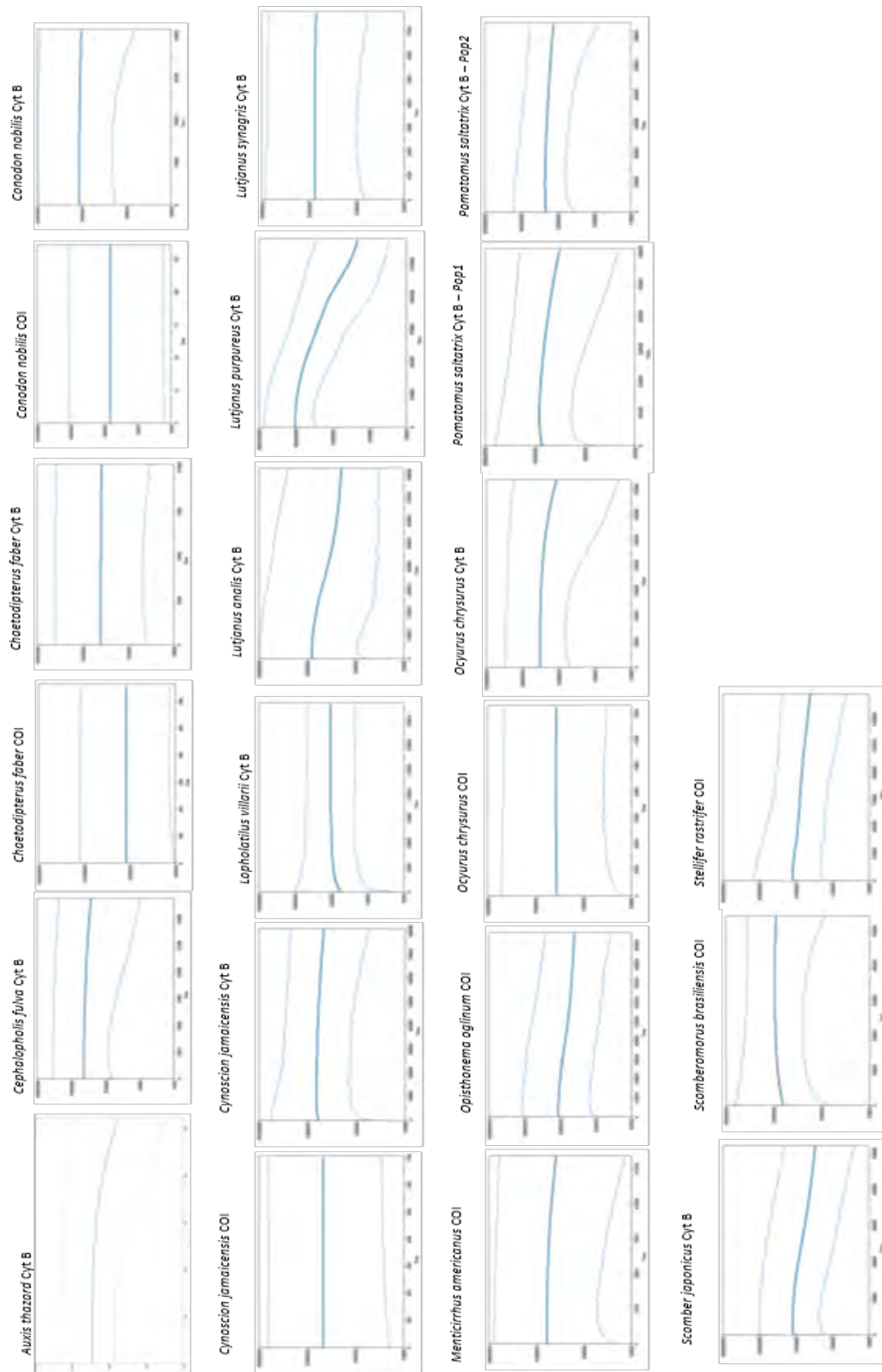
Fig. S2. Bayesian Skyline Plot for each Brazilian commercially exploited marine fish species or population and marker. Axis x is time and y is effective population size.

PAST NICHE AVAILABILITY AND EFFECTIVE POPULATION SIZE OF TWO TROPICAL REEF FISH

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ABSTRACT

The relationship between temporal variation in effective population size and environmental changes can be explored to better predict the consequences of anthropogenic impacts. Here we characterize the past effective population sizes for two commercially exploited reef fish species - *Lutjanus jocu* and *Sparisoma axillare* - in the Southwest Atlantic and test whether past climate variability correlates with changes in population size. Using thousands of SNP markers and Bayesian species distribution modelling we found that both species underwent population expansions in the past, although during different periods of time. The population size of *S. axillare* increased until 5,000 ybp, and has been stable ever since. This expansion coincides with the increased probability of having suitable habitat, as indicated by the increase in suitable areas in the niche modeling results. For *L. jocu*, the results suggest that, after a period of population expansion, its population size has remained stable over the last 1 million years. Habitat suitability for *L. jocu* is currently more restricted than in the past, but this was not reflected by simultaneous changes in estimated effective population size.

KEYWORDS: Bayesian analysis, Effective Population Size, Species Distribution Models.

1. INTRODUCTION

Knowledge of species distributions and abundance is critical to effective conservation management. Both are a consequence of habitat suitability, which in turn, is influenced by climate. In the marine environment, past climatic change is known to have affected sea level and water temperature. In periods of low sea level (e.g. glacial periods) the reconfiguration of the coastline and the shortening of the continental shelf may isolate previously connected parts of an area occupied by a species, resulting in genetic divergence. This has been described for a shallow Indo-Pacific region and also for the region between the Caribbean and the South Atlantic (Ludt & Rocha, 2015). Low sea level may also increase the importance of permeable barriers, such as river outflow (Rocha, 2003), affecting the dispersal of species sensitive to low salinities, but facilitating the dispersal of brackish dependent species. Temperature variability can also have an important effect on species distribution if the change is higher than the thermal niche of the species. Temperature changes can change the population dynamics of a species by forcing individuals to move to more suitable areas (Parmesan, 2006). During these periods of climate change, species may contract to areas of suitable habitat known as refugia (Keppel et al., 2012).

Climate change in the coming centuries will be characterised by increasing inter-annual, decadal and multi-decadal fluctuations in conjunction with other anthropogenic impacts (IPCC, 2014; Peterson et al., 2002). The impact of climate change will be influenced by the environmental sensitivity of each species and its adaptive capacities (Jezkova et al., 2011). In the oceans, climate change will mainly affect sea level, temperature, salinity and acidity, factors known to affect species distribution.

Species Distribution Models (SDMs) link spatial abundance or occurrence information with environmental variables to predict where (and how much) a species distribution is likely to be present in unsampled locations or other time periods (Guisan & Thuiller, 2005). It uses environmental information from known occurrence points to model and predict where species have

high or low probability of occurrence according to the differences in the environment. SDMs can be used to predict the occurrence and abundance of species in the present, in a future climate change context and also in the past, due to species niche conservatism potential (Peterson, 1999). Applying SDMs using past climatic events can contribute to understanding how events that changed the climate have had lasting impacts to the present day (Gür, 2013). Clearly, these methods have some caveats because it is impossible to include all factors that affect the distribution of a species (for example, biotic interactions), but they are still useful.

Analysis of whether and how these past processes affect species distributions may reveal fundamental phenomena that would likely go unrecognized in the observational record (e.g., novel and disappearing climates). In this sense, the integration of traditional SDMs with other methodologies and past data will benefit our understanding of ecological and biogeographic processes underlying species distribution and abundance.

Genetic data can be used to estimate current and past effective population size (N_e). In the terrestrial environment, most wild populations have a much smaller N_e than that provided by a census count, averaging one tenth the size of the census (Frankham, 1995). For marine species, which often have larger populations, large numbers of offspring and high mortality among juveniles, the effective size can be proportionately even smaller (Waples et al., 2016). Estimates of N_e can also be applied to understand demographic fluctuations over time (Hare et al., 2011). The integration of N_e fluctuation information and past niche modelling can help understand how species population size was affected by past climatic events and predict how it will respond to future event (e.g. Zhu et al., 2016; Khanal et al., 2018). Evidence of population expansion during periods of high temperature, for example, may suggest that species will benefit from future temperature increases. In addition, population size stability during periods of poor habitat suitability may indicate high persistence capacity for a species or high niche plasticity (Jezkova et al., 2011).

Here, using genetic data and current occurrence data for two tropical marine reef fish species, commonly targeted by fishers, we aimed to: i) calculate the past variation in their N_e, and ii) model their past and current distribution probability to identify correlations between habitat suitability and N_e. For that, we used a two-step approach, first using genomic data (SNPs markers) to estimate N_e and then SDMs to infer niche variability.

2. METHODS

2.1 Species

We focused on two fish species that are relatively abundant and exploited along the Brazilian coast and its oceanic islands: the dog snapper *Lutjanus jocu* and the grey parrotfish *Sparisoma axillare*. Both species share some characteristics, such as being reef dependence, having a pelagic larval phase and preferring relatively shallow waters (up to 40m deep) (Froese & Pauly, 2019). Both species are considered data deficient by the International Union for Conservation of Nature (IUCN). *Sparisoma axillare*, according to the Brazilian Red List of Aquatic Endangered Animals, is currently classified as Vulnerable (MMA, 2014). Both species are regularly exploited by the Brazilian fisheries, especially artisanal fisheries. *Lutjanus jocu* is widely exploited by artisanal fisheries in Brazil, being present in almost 40% of landings (Frédou et al., 2006). The exploitation of *Sparisoma axillare* is more heterogeneous, although increases in the last decades have resulted in some signs that the species is under heavy exploitation pressure (Floeter et al., 2006).

On the other hand, the two species also have contrasting biological features, being *L. jocu* a large snapper that grows up to 130 cm of length and lives up to 25 years; its occurrence ranges from Southeastern Brazil to the Caribbean Sea (Cervigón, 1993; Rezende & Ferreira, 2004; Floeter et al., 2003). Like many other snappers, *L. jocu* is carnivorous and uses different habitats according to its ontogeny: juveniles are frequently in estuaries and inner-shelf reefs, while adults are mainly

present in mid-shelf reefs (Moura et al., 2011). Conversely, *S. axillare* is an herbivorous parrotfish endemic to Brazil that grows up to 43 cm of length and can live up to 12 years (Froese & Pauly, 2019; Gaspar, 2006). In addition to being depth limited, its distribution is probably limited by the Amazon outflow (Araújo et al., 2019; Robertson et al., 2006).

2.2 Study area and sampling

Tissue samples were collected from fish caught along the Brazilian coast and around oceanic islands (Fig. 1, Table 1). For *L. jocu*, the northern limit of sampling was the Brazilian state of Maranhão, the southern limit was the state of Espírito Santo, and the sampled island was Fernando de Noronha. For *S. axillare*, the northern sampling limit was the state of Rio Grande do Norte, the southern limit the state of Rio de Janeiro, and the sampled islands included Fernando de Noronha, Abrolhos and Trindade. Because *S. axillare* coming from Trindade has significant genetic divergence from fish from other regions (Chapter 4 of this thesis), this site was excluded from the analyses.

For the distribution modelling analysis, geo-referenced presence records of both species were extracted from the Global Biodiversity Information Facility (GBIF) and the SpeciesLink online databases. Before running the analyses, presence data were checked for duplicate removal.
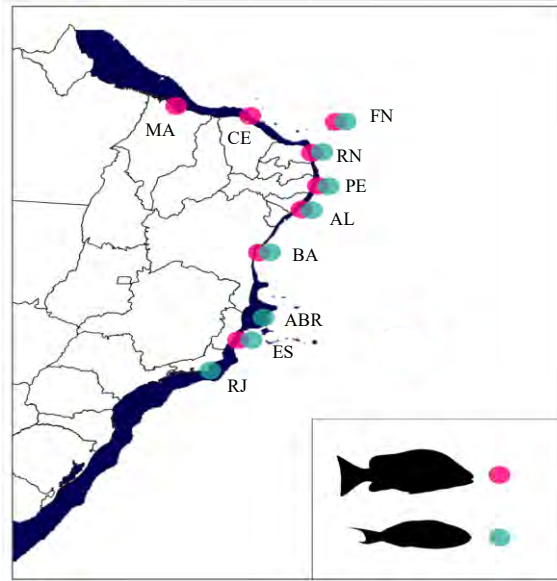
Fig. 1: Location of sampling sites for *Lutjanus jocu* (pink) and *Sparisoma axillare* (green). MA – Maranhão, CE – Ceará, RN – Rio Grande do Norte, FN – Fernando de Noronha, PE – Pernambuco, AL – Alagoas, BA – Bahia, ABR – Abrolhos, ES – Espírito Santo, RJ – Rio de Janeiro.

Table 1: Sample number for each locality, for each species.

Species	Maranhão	Ceará	Rio Grande do Norte	Pernambuco	Alagoas	Bahia	Espírito Santo	Rio de Janeiro	Fernando de Noronha	Abrolhos	Total
Lutjanus jocu	12	9	11	10	3	10	2	0	14	0	**71**
Sparisoma axillare	0	0	8	12	12	12	4	10	10	12	**80**

2.3 Estimating Effective Population Size (N_e)

2.3.1 DNA extraction, sequencing and SNPs filtering

DNA extraction, SNP discovery and genotyping were performed by Diversity Arrays Technology Pty. Ltd using their basic protocol described by Jaccoud et al. (2001). SNPs were selected based on their call rate, i.e. the minimum samples that the SNP was called (97% for *S. axillare* and 90% for *L. jocu*), reproducibility, i.e. the consistency in technical replicates (100%

for *S. axillare* and 97% for *L. jocu*), and Minimum Allele Frequency, to avoid SNPs resulting from sequencing errors (threshold of 2% for both species). We excluded SNPs in the same fragment to avoid physical linkage, and SNPs that significantly deviated from Hardy-Weinberg Equilibrium using the dartR package applying Bonferroni correction and alpha value of 0.05 (Gruber & Georges, 2019) in R (R Core Team, 2019). We excluded individuals with more than 20% of missing data (*S. axillare*, N = 1; *L. jocu* N = 11). After these filtering steps we had data on 5,093 SNPs for 80 individual of *S. axillare,* whereas for *L. jocu* our dataset included 3,955 SNPs for 71 individuals.

2.3.2 Genetic Population Structure and Isolation by Distance

We tested for population structure and isolation by distance before continuing the N_e, calculation because genetic differentiation may bias N_e estimates. The genetic structure was described using the admixture analysis in the LEA package in R software (Pritchard et al., 2000; Frichot & François, 2015). This multilocus approach provides a probability of K (number of populations or clusters) that will be characterised by a determined allele frequency. To test isolation by distance, we used the most usual test, the Mantel tests using the mantel.randtest function in R. We used linear geographic distance between sites and pairwise Fst, calculated using Arlequin 3.5 (Excoffier & Lischer, 2010).

2.3.3 Historical Effective Population Size (Coalescent method)

We identified changes in effective population sizes over time by constructing a Bayesian Skyline Plot using Beast v1 and Beauti (Drummond et al., 2005). This coalescent method reconstructs demographic history based on contemporary sampled gene sequences and provides more detailed information on effective population size history than previous methods (Drummond et al., 2005). The parameters included in Beauti to build the input file for Beast were clock rate =

6.8 x 10^{-8}, based on the substitution rate for SNPs described for other fish species (Roesti et al., 2015). The chain length used was 10,000,000, but this number was increased when the effective sample sizes of any parameter was smaller than 200. The Bayesian Skyline Reconstruction analysis was performed in Beast and graphics were visualized using Tracer v1.7.1.

2.4 Environmental variables

The potential explanatory variables used here, which are those known to affect the distribution of marine organisms were: bathymetry (m), maximum sea surface temperature (ºC), range of sea surface temperature (ºC), and mean sea surface salinity (PSS). All variables were extracted with a spatial resolution of 0.01 x 0.01 degrees, from the MARSPEC databases (http://www.marspec.org; Sbrocco & Barber, 2013) for present-day, mid-Holocene (6 kya) and Last Glacial Maximum (21 kya) scenarios.

In particular, bathymetry was selected as a possible predictor of both species distributions as many faunal changes seem to follow a depth gradient (e.g. Costa et al., 2017; Dell'Apa et al., 2016; Roos et al., 2015). Temperature and salinity were considered in the analyses, as they can be used to locate thermal fronts and productivity hotspots and therefore determine the influence of these characteristics on species distribution (Pennino et al., 2013).

Following the protocol established by Zuur et al. (2009), these variables were explored for correlation, co-linearity, outliers, and missing data before their use in the analyses and modelling. A Spearman's correlation test was used to test for correlation among variables using the 'corrplot' function in R software. Collinearity was tested by computing the generalized variance-inflation factors (GVIF), which are the corrected VIF values by the number of degrees of freedom of a predictor variable. GVIF was assessed using the 'corvif' function (Fox & Weisberg, 2018) in R.

Finally, after an exploratory analysis, to better interpret the direction (positive or negative) and magnitudes (effect sizes) of parameter estimates relative to the others, the explanatory

variables were standardized (difference from the mean divided by the corresponding standard deviation) (Gelman, 2008).

2.3.2 Bayesian Distribution Modelling

We used a hierarchical Bayesian point-reference spatial model (H-BSM) to estimate the specific habitat requirements, and this was implemented for populations of each species identified by the genetic structure analysis. Habitat availability was estimated for the present-day, mid-Holocene and Last Glacial Maximum conditions. H-BSMs can understood as a spatial extension of Generalized Linear Models (GLMs) because the modelling process describes variability in the response variable as a function of the explanatory variables, but with the addition of a stochastic spatial effect to model the residual spatial autocorrelation (Muñoz et al., 2013).

For the response variable (presence/absence of the species) a binomial distribution was used with a logarithmic link function. Because only species presence data were available, pseudo-absences were randomly generated for the entire area, using the 'srswor' function of the sampling package (Tillé & Matei, 2016) in R. In each case, the number of pseudo-absences was the same as the number of real presences (Barbet-Massin et al., 2012). Pseudo-absences were then combined with real presences into a single presence-absence dataset to be used for the binomial model. We opted for a binomial distribution with a H-BSM, instead of a less accurate model that allows the use of presence-only data (e.g. BIOCLIM, MAXENT). This enabled us to include a spatial effect to deal with spatial autocorrelation, and also to explicitly quantify uncertainties (Costa et al., 2017; Pennino et al., 2018; Roos et al., 2015).

For all H-BSMs, Bayesian parameter estimates and predictions were obtained throughout the Integrated Nested Laplace Approximations (INLA) approach (Rue et al., 2009) and R-package (http:\\www.r-inla.org). INLA uses Stochastic Partial Differential Equations (SPDE) approach

(Lindgren et al., 2011) for the spatially structured random effect (see Martínez-Minaya et al., 2018 for more details about the spatial effects).

Vague zero-mean Gaussian prior distributions with a variance of 100 were assigned for all fixed effect parameters, which are approximations of non-informative priors and are designed to have little influence on the posterior distributions. As recommended by Lindgren & Rue (2015), multivariate Gaussian distributions with a mean of zero and a spatially-structured covariance matrix were assumed for the spatial component.

The environmental variable selection with all possible interaction terms was mainly based on the Watanabe-Akaike information criterion (WAIC) (Watanabe et al., 2015) and the mean logarithmic of the approximated conditional predictive ordinate (LCPO) (Gneiting & Raftery, 2007). While WAIC values indicate the goodness of fit of the models, the LCPO evaluates the predictive capacity. Lower values for both WAIC and LCPO represent the best compromise between fit and parsimony.

To predict the suitable habitats for *L. jocu* and *S. axillare* populations, we used a Bayesian kriging approach. This approach treats parameters as random variables to consider parameter uncertainty. We created a Delaunay triangulation around the presence-absence points using the INLA SPDE module (Lindgren & Rue, 2015). Then, using linear interpolations we finally obtained maps of the probability of current occurrence (habitat suitability) of each species (present-day model). Past period scenarios were predicted based on the present-day model estimation.

3. RESULTS

3.1 Estimating Effective Population Sizes

For both species there was a weak genetic structure (most probable number of K = 1) and no evidence of isolation by distance (IBD) (*S. axillare*, Mantel test p-value: 0.23; *L. jocu*, Mantel

test p-value: 0.14; Fig. 2). Thus, for both species, samples from all locations were considered to be from the same population.
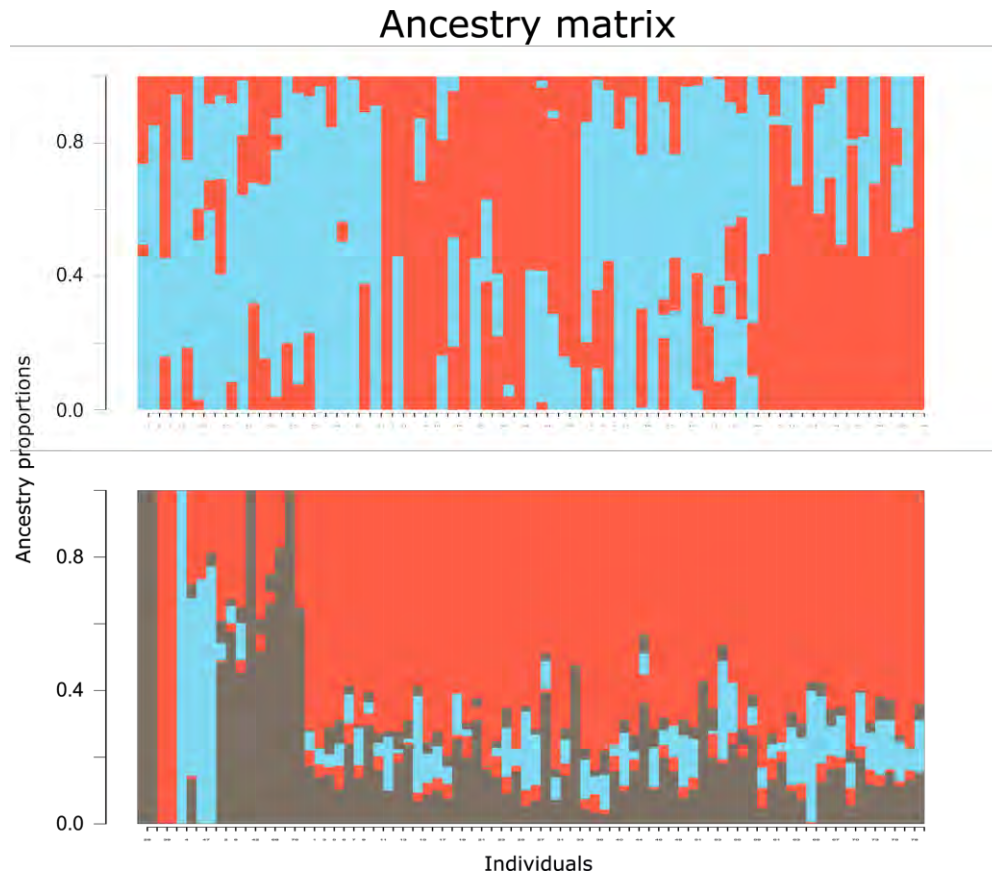

Fig. 2: Admixture results for a) *Lutjanus jocu* and b) *Sparisoma axillare* indicating lack of structure and geographical distance effect on genetic distribution.

3.1.1 Historical Population Effective Size (Coalescent estimate)

The population size of *S. axillare* increased until 5,000 ybp, after which the population remained stable (Fig. 3). The increase in suitable habitats during the expansion period is consistent with the increase in population size. The population of *L. jocu* has been stable for the last 1 million years (Fig. 3). Due to differences in datasets and genetic diversity between the two species, the automatic output of the analysis provided different time spans (Fig. 3).
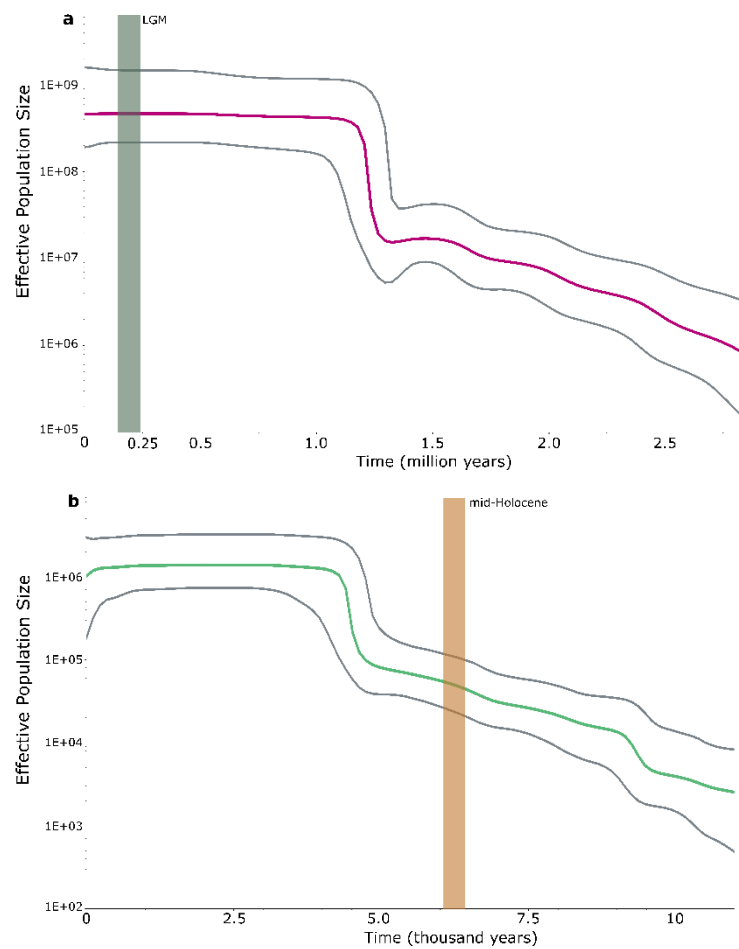
Fig. 3: Bayesian Skyline Plot indicating Effective Population Size variability from present (zero) to past for a) *Lutjanus jocu* and b) *Sparisoma axillare*.

3.2 Bayesian Distribution Modelling

For both species, the maximum sea surface temperature was highly correlated with the sea surface temperature (r>0.80) with these variables having a Generalized Variance Inflation Factors of (GVIF)>3. Thus, separate runs of H-BSM were performed and each run included only one of the highly correlated variables at a time to determine which would account for most of the species variance.

For *L. jocu*, the variables that showed the most predictive power (based on the lowest WAIC and LCPO values, Supplementary Material Table S1) were the maximum sea surface

temperature and bathymetry. The findings showed a positive relationship between the occurrence of *L. jocu* and the maximum sea surface temperature (posterior mean = 0.835; 95% CI = [-0.976, 2.839]) and bathymetry (posterior mean = 1.932; 95% CI = [1.307, 1.872]). Probability maps of the presence of *L. jocu* revealed that the maximum extension of its distribution occurred in the mid-Holocene period (Fig. 4b), while the Last Glacial Maximum scenario presented more suitable habitats in the southern coast of Brazil, especially in deeper waters. Currently, the most suitable habitats for *L. jocu* are restricted to the northern coast of Brazil (Fig. 4).
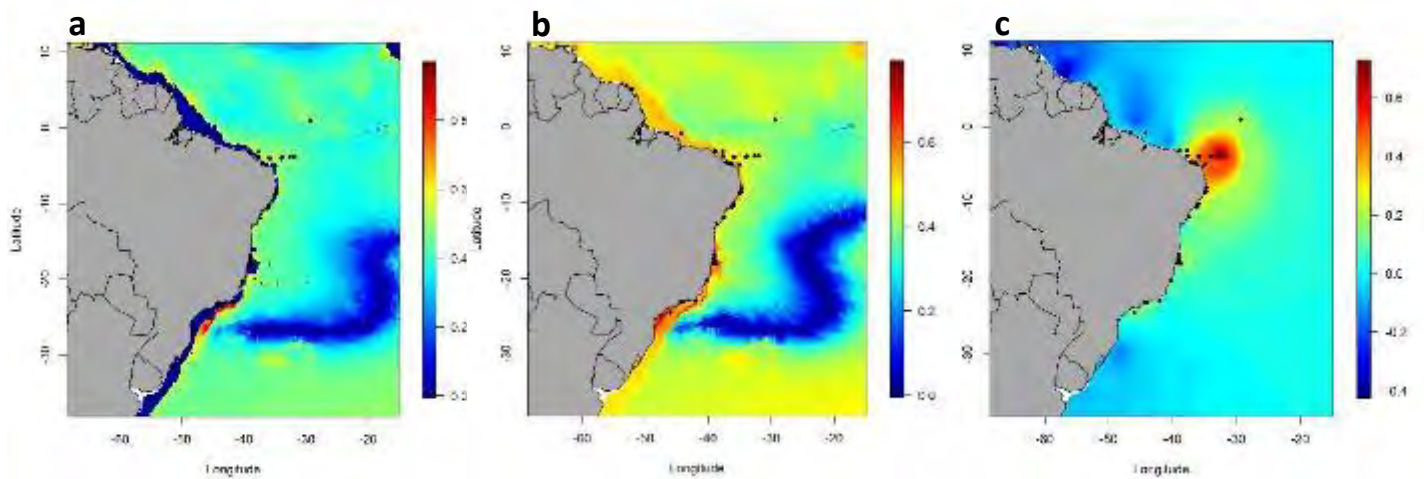

Fig 4. Predicted distribution of *Lutjanus jocu*, for a) Last Glacial Maximum (21kya), b) mid-Holocene (6kya), and c) present.
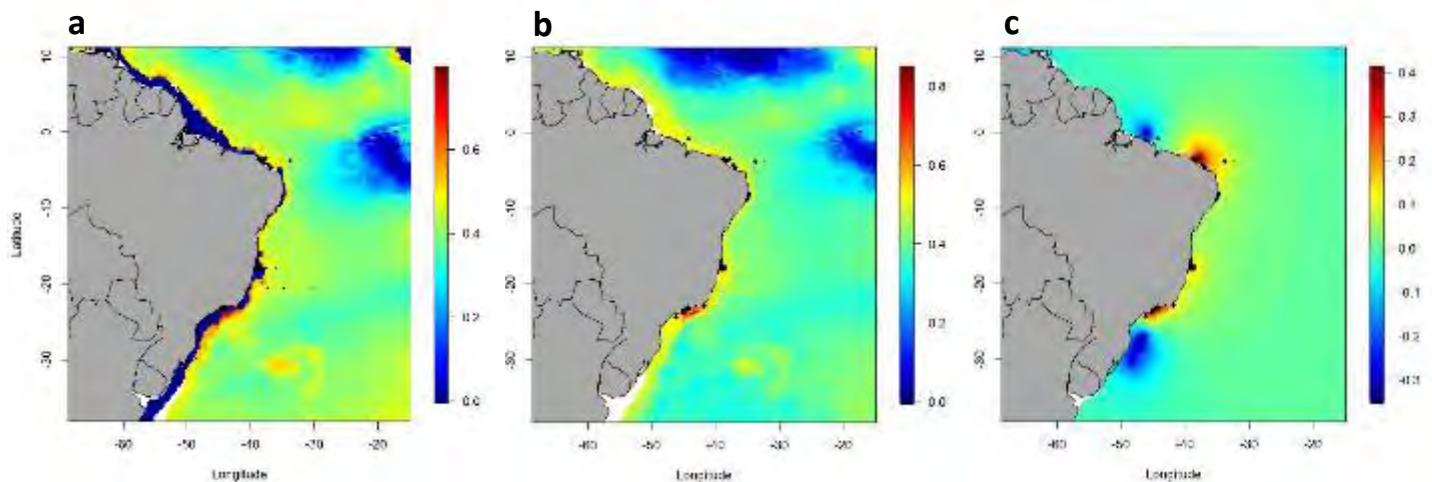

Fig 5. Predicted distribution of *Sparisoma axillare*, for a) Last Glacial Maximum (21kya), b) mid-Holocene (6kya), and c) present. The warmer the color, the more likely species occurrence.

For *S. axillare*, the most important environmental variables to predict its distribution were mean sea surface salinity, sea surface temperature range and bathymetry. The model selected was the third best one regarding the values of WAIC and LCPO (Supplementary Material Tables S2). We opted for the third best model because the WAIC and LCPO values were not significantly different than the first and second ones, whereas the third model included more variables, which could provide more information on how these variables changed through time. The distribution of *Sparisoma axillare* was negatively related to sea surface temperature range (posterior mean = -0.1365; 95% CI = [-1.1318, 0.8539]) and positively related to both sea surface salinity (posterior mean = 3.5897; 95% CI = [1.4402, 5.7318]) and bathymetry (posterior mean = 4.3433; 95% CI = [1.2926, 7.3931]). In the three periods, higher suitability for this species was found in the southeast coast of Brazil, near the state of Rio de Janeiro. The north-northeast region was also identified as suitable under current conditions (Fig. 5).

5. DISCUSSION

We found limited genetic structure, large effective population sizes (N_e) and some evidence of population stability after a period of sharp expansion for both the dog snapper *Lutjanus jocu* and the grey parrotfish *Sparisoma axillare*. We suggest that the expansion was caused by different reasons for each species, since each expansion period ended at a different time. For *S. axillare*, the expansion sharply followed the Holocene, which may be consistent with the small increase in niche availability seen in the results of distribution models or as a result of a recent bottleneck. The population of *L. jocu* has remained stable for over a million years, prior to the large increase in habitat suitability that occurred between the LGM and the mid-Holocene periods. Despite the

sympatric occurrence in most of the distribution of both species, differences in their biology and, mainly, in their demographic history could help explain these results.

Sparisoma axillare is endemic to Brazil and appears to be more dependent on shallow waters (1-35 m, Moura et al., 2001), which could help explain the population size increases after the Holocene periods. Indeed, with sea-level rise after glacial melt, a greater extent of shallow habitats became available on the continental shelf, as can be observed when comparing the distribution prediction maps for the LGM and mid-Holocene. The endemism of *S. axillare* and its low connectivity attributed to isolation by depth indicates that this species has a limited dispersal capacity. This feature can cause variability in population size due to habitat availability and may have resulted in a recent bottleneck that could explain its short reconstruction span in comparison with *L. jocu*. Glacial cycles could affect the abundance and distribution of other coastal non-pelagic species (Liu et al., 2006; Liu et al., 2011). Population size variability might also be a result of speciation, which for *S. axillare* happened much earlier than the population expansion detected here. The separation between *Sparisoma rubripinne* from East-Atlantic and *S. rubripinne* and *S. axillare* (West-Atlantic) occurred almost 3 million years ago, while the separation between the Caribbean *S. rubripinne* and *S. axillare* was around 2.2 million years ago (Floeter et al., 2007). Therefore, the population size variability in *S. axillare* is most likely a response to environmental changes.

In contrast, *L. jocu* has a larger distribution, from the southern Brazil to the Caribbean Sea (Cervigón, 1993; Floeter et al., 2003), probably because the species seems less limited by depth and other environmental factors, such as rivers outflows. Thus, for *L. jocu*, historical factors, including speciation, might have shaped patterns with N_e more so than habitat suitability. Population expansions during the Pleistocene, such as those observed with *L. jocu,* also occurred in other Atlantic fish (Larmuseau et al., 2009). It is known that some marine species may not be so sensitive to glacial cycles, due to their capacity to move to more suitable areas and to their large

population sizes (Francisco et al., 2011). For these species, even if climate change decreased their population size in some regions, this reduction may not be sufficient to impact its overall population size (Francisco et al., 2011). During the Pleistocene some reef habitats were maintained, regardless of sea level fluctuations, and this may have supported some reef species during glacial cycles (Ludt & Rocha, 2015). It has been hypothesized that the genus *Lutjanus* can be resistant to climatic fluctuations and could have avoided the extinctions of the Oligocene, which have affected other reef species, due to its ability to move and occupy refugia areas (Cowman & Bellwood, 2011; Frédérich & Santini, 2017). Thus, the demographic history of *L. jocu* and the expansion of its population in Brazil could be more related to the divergence of the populations than to climate oscillation in the south-western Atlantic. The species *L. jocu* and *Lutjanus argentiventris* probably diverged about 2 million years ago, and this could explain the earlier expansion of *L. jocu* population (Frédérich & Santini, 2017).

Detecting relationships between past environmental variability and genetic diversity or N_e can help predict how species will respond to future changes in climate. Climate change can affect species in three main ways: i) species can adapt to the new climate, keeping their population stable, and this will depend on the species' adaptation capacity (Williams et al., 2008); ii) species can move to more suitable areas, which will depend on their dispersal capacity and habitat availability (Peterson et al., 2002), or iii) species can be affected negatively or positively by changes, and their population sizes may decrease or expand, depending on whether the changes reduce (e.g. Moller et al., 2008) or increase habitat suitability, respectively. Identifying the capacity for taxa to adapt or not is needed to refine the predictions of climate change effect on biodiversity (Jezkova et al., 2011). Recent increases in temperature are known to be forcing species to move towards the pole (Parmesan & Yohe, 2003).

Our results showed that the N_e of *S. axillare* is sensitive to environmental changes, probably because the dispersal of this species is limited by the environment. These observations

suggest that *S. axillare* may be relatively more vulnerable to future climate change and habitat loss. The current threatened status of *S. axillare* is mainly due to overfishing. Reduction in its population size and the concomitant reduction in genetic variation can further reduce its adaptive capacity (Frankham et al., 2002). In addition, the fact that its preferred habitat is reefs puts further pressure on this endemic species, as reefs are also endangered due to both climate change and overfishing (Hoegh-Guldberg et al., 2007; Hughes, 2003).

However, even species that seem more resilient to past climate variability, such as *L. jocu*, can still be affected by future climate change. The current rate of change is much higher than past natural changes, such as those that took place during the LGM or the Holocene (Karl & Trenberth, 2003). The last century has seen the greatest increase in temperature in the millennium, and predictions are that the pace of change will pick up speed (IPCC, 2014; Jones et al., 2001). Then, even if a species has adaptive or dispersal capacity to deal with changes or move to more suitable areas, it is possible that it simply does not have time for that (Jump & Penuelas, 2005).

Ultimately, further examination of this topic will increase our understanding of how organisms adapt and respond to environmental variation. These studies are likely to be valuable for planning conservation management for threatened and endangered species, which require detailed information on factors affecting demographic parameters such as N_e. The results described here can be especially valuable for species where quantitative N_e models are needed to prioritize certain areas and populations for protection.

5. ACKNOWLEDGMENTS

the paper. JTV collected the data, analysed and wrote the manuscript. RS analysed data. BPF collected data. PFML, SMQL and AS reviewed the manuscript.

6. REFERENCES

Araújo, R. G., Silva, R. D. F., Sampaio, I., & Guimarães-Costa, A. (2019). Does DNA barcoding offer meaningful insights into the diversity of the parrotfish of the genus *Sparisoma* (Scaridae)? *Journal of Applied Ichthyology*, jai.13932. https://doi.org/10.1111/jai.13932

Barbet-Massin, M., Jiguet, F., Albert, C. H., & Thuiller, W. (2012). Selecting pseudo-absences for species distribution models: how, where and how many? *Methods in Ecology and Evolution*, *3*(2), 327–338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Cervigón, F. (1993). *Los peces marinos de Venezuela Volumen II* (F. C. L. Roques, Ed.). Caracas.

Costa, T. L. A., Pennino, M. G., & Mendes, L. F. (2017). Identifying ecological barriers in marine environment: The case study of *Dasyatis marianae*. *Marine Environmental Research*, *125*, 1–9. https://doi.org/10.1016/j.marenvres.2016.12.005

Cowman, P. F., & Bellwood, D. R. (2011). Coral reefs as drivers of cladogenesis: expanding coral reefs, cryptic extinction events, and the development of biodiversity hotspots. *Journal of Evolutionary Biology*, *24*(12), 2543–2562. https://doi.org/10.1111/j.1420-101.2011.02391.x

Dell'Apa, A., Pennino, M. G., & Bonzek, C. (2016). Modeling the habitat distribution of spiny dogfish (*Squalus acanthias*), by sex, in coastal waters of the northeastern United States. *Fishery Bulletin*, *115*(1), 89–100. https://doi.org/10.7755/FB.115.1.8

Drummond, A. J. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, *22*(5), 1185–1192. https://doi.org/10.1093/molbev/msi103

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver3.5: a new series of programs to

perform population genetics analyses under Linux and Windows. *Molecular ecology resources*, *10*(3), 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Floeter, S. R., Halpern, B. S., & Ferreira, C. E. L. (2006). Effects of fishing and protection on Brazilian reef fishes. *Biological Conservation*, *128*(3), 391-402. https://doi.org/10.1016/j.biocon.2005.10.005

Floeter, S. R., Rocha, L. A., Robertson, D. R., Joyeux, J. C., Smith-Vaniz, W. F., Wirtz, P., … Bernardi, G. (2007). Atlantic reef fish biogeography and evolution. *Journal of Biogeography*, 071009214220004 https://doi.org/10.1111/j.1365-2699.2007.01790.x

Floeter, S. R, Gasparini, J. L., Rocha, L. A., Ferreira, C. E. L., Rangel, C. A., & Feitoza, B. M. (2003). *Brazilian reef fish fauna: checklist and remarks*. 1–22.

Fox, J., & Weisberg, S. (2018). *An R companion to applied regression*. Sage Publications.

Francisco, S. M., Faria, C., Lengkeek, W., Vieira, M. N., Velasco, E. M., & Almada, V. C. (2011). Phylogeography of the shanny *Lipophrys pholis* (Pisces: Blenniidae) in the NE Atlantic records signs of major expansion event older than the last glaciation. *Journal of Experimental Marine Biology and Ecology*, *403*(1–2), 14–20. https://doi.org/10.1016/j.jembe.2011.03.020

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: a review. *Genetical Research*, *66*(02), 95. https://doi.org/10.1017/S0016672300034455

Frankham, R., Briscoe, D. A., & Ballou, J. D. (2002). *Introduction to Conservation Genetics*. Cambridge University Press.

Frédérich, B., & Santini, F. (2017). Macroevolutionary analysis of the tempo of diversification in snappers and fusiliers (Percomorpha: Lutjanidae). *Belgian Journal of Zoology*, *147*(1). https://doi.org/10.26496/bjz.2017.2

Frédou, T., Ferreira, B. P., & Letourneur, Y. (2006). A univariate and multivariate study of reef fisheries off northeastern Brazil. *ICES Journal of Marine Science*, *63*(5), 883-896.

https://doi.org/10.1016/j.icesjms.2005.11.019

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*(8), 925–929. https://doi.org/10.1111/2041-210X.12382

Froese, R., & Pauly, D. (2019). Fish Base.

Gaspar, A. L. B. (2006). *Idade, crescimento e padrões de recrutamento do Bobó,* Sparisoma axillare*, na APA Costa dos Corais* (Master's thesis, Universidade Federal de Pernambuco).

Gelman, A. (2008). Scaling regression inputs by dividing by two standard deviations. *Statistics in Medicine*, *27*(15), 2865–2873. https://doi.org/10.1002/sim.3107

Gneiting, T., & Raftery, A. E. (2007). Strictly proper scoring rules, prediction, and estimation. *Journal of the American Statistical Association*, *102*(477), 359–378. https://doi.org/10.1198/016214506000001437

Gruber, B., & Georges, A. (2019). dartR: importing and analysing SNP and silicodart data generated by genome-wide restriction fragment analysis. *R Package Version v.2*. Retrieved from https://cran.r-project.org/package=dartR

Guisan, A., & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, *8*(9), 993–1009. https://doi.org/10.1111/j.1461-0248.2005.00792.x

Gür, H. (2013). The effects of the Late Quaternary glacial-interglacial cycles on Anatolian ground squirrels: range expansion during the glacial periods? *Biological Journal of the Linnean Society*, *109*(1), 19–32. https://doi.org/10.1111/bij.12026

Hare, M. P., Nunney, L., Schwartz, M. K., Ruzzante, D. E., Burford, M., Waples, R. S., … Palstra, F. (2011). Understanding and estimating effective population size for practical application in marine species management. *Conservation Biology*, *25*(3), 438–449. https://doi.org/10.1111/j.1523-1739.2010.01637.x

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., …

> Hatziolos, M. E. (2007). Coral reefs under rapid climate change and ocean acidification. *Science*, *318*(5857), 1737–1742. https://doi.org/10.1126/science.1152509

Hughes, T. P. (2003). Climate change, human impacts, and the resilience of coral reefs. *Science*, *301*(5635), 929–933. https://doi.org/10.1126/science.1085046

IPCC. (2014). *Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernamental Panel on Climate Change.* (R. K. P. and L. A. M. Core Writing Team, Ed.). Geneva.

Jaccoud, D., Peng, K., Feinstein, D., & Kilian, A. (2001). Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, *29*(4), e25. https://doi.org/10.1093/nar/29.4.e25

Jezkova, T., Olah-Hemmings, V., & Riddle, B. R. (2011). Niche shifting in response to warming climate after the last glacial maximum: inference from genetic data and niche assessments in the chisel-toothed kangaroo rat (*Dipodomys microps*). *Global Change Biology*, *17*(11), 3486–3502. https://doi.org/10.1111/j.1365-2486.2011.02508.x

Jones, P. D., Osborn, T. J., & Briffa, K. R. (2001). The evolution of climate over the last millennium. *Science*, *292*(5517), 662–667. https://doi.org/10.1126/science.1059126

Jump, A. S., & Penuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters*, *8*(9), 1010–1020. https://doi.org/10.1111/j.1461-0248.2005.00796.x

Karl, T. R., & Trenberth, K. E. (2003). Modern global climate change. *Science*, *302*(5651), 1719–1723. https://doi.org/10.1126/science.1090228

Keppel, G., Van Niel, K. P., Wardell-Johnson, G. W., Yates, C. J., Byrne, M., Mucina, L., … Franklin, S. E. (2012). Refugia: identifying and understanding safe havens for biodiversity under climate change. *Global Ecology and Biogeography*, *21*(4), 393–404.

https://doi.org/10.1111/j.1466-8238.2011.00686.x

Khanal, L., Chalise, M. K., He, K., Acharya, B. K., Kawamoto, Y., & Jiang, X. (2018). Mitochondrial DNA analyses and ecological niche modeling reveal post-LGM expansion of the Assam macaque (*Macaca assamensis*) in the foothills of Nepal Himalaya. *American journal of primatology*, *80*(3), e22748. https://doi.org/10.1002/ajp.22748

Larmuseau, M. H. D., Van Houdt, J. K. J., Guelinckx, J., Hellemans, B., & Volckaert, F. A. M. (2009). Distributional and demographic consequences of Pleistocene climate fluctuations for a marine demersal fish in the north-eastern Atlantic. *Journal of Biogeography*, *36*(6), 1138–1151. https://doi.org/10.1111/j.1365-2699.2008.02072.x

Lindgren, F., & Rue, H. (2015). Bayesian spatial modelling with R-INLA. *Journal of Statistical Software*, *63*(19).

Lindgren, F., Rue, H., & Lindström, J. (2011). An explicit link between Gaussian fields and Gaussian Markov random fields: the stochastic partial differential equation approach. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*(4), 423–498. https://doi.org/10.1111/j.1467-9868.2011.00777.x

Liu, J.-X., Gao, T.-X., Wu, S.-F., & Zhang, Y.-P. (2006). Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck & Schlegel, 1845). *Molecular Ecology*, *16*(2), 275–288. https://doi.org/10.1111/j.1365-294X.2006.03140.x

Liu, J.-X., Tatarenkov, A., Beacham, T. D., Gorbachev, V., Wildes, S., & Avise, J. C. (2011). Effects of Pleistocene climatic fluctuations on the phylogeographic and demographic histories of Pacific herring (*Clupea pallasii*). *Molecular Ecology*, *20*(18), 3879–3893. https://doi.org/10.1111/j.1365-294X.2011.05213.x

Ludt, W. B., & Rocha, L. A. (2015). Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. *Journal of Biogeography*, *42*(1), 25–

38. https://doi.org/10.1111/jbi.12416

Luikart, G., Ryman, N., Tallmon, D. A., Schwartz, M. K., & Allendorf, F. W. (2010). Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics*, *11*(2), 355–373. https://doi.org/10.1007/s10592-010-0050-7

Martínez-Minaya, J., Cameletti, M., Conesa, D., & Pennino, M. G. (2018). Species distribution modeling: a statistical review with focus in spatio-temporal issues. *Stochastic Environmental Research and Risk Assessment*, *32*(11), 3227–3244. https://doi.org/10.1007/s00477-018-1548-7

MMA (2014) *Portaria MMA Nº 445, de 17 de dezembro de 2014*.

Moller, A. P., Rubolini, D., & Lehikoinen, E. (2008). Populations of migratory bird species that did not show a phenological response to climate change are declining. *Proceedings of the National Academy of Sciences*, *105*(42), 16195–16200. https://doi.org/10.1073/pnas.0803825105

Moura, R., Figueiredo, J., & Sazima, I. (2001). A new parrotfish (Scaridae) from Brazil, and revalidation of *Sparisoma amplum* (Ranzani, 1842), *Sparisoma frondosum* (Agassiz, 1831), *Sparisoma axillare* (Steindachner, 1878) and *Scarus trispinosus* Valenciennes, 1840. *Bulletin of Marine Science*, *68*(3), 505–524.

Moura, R. L., Francini-Filho, R. B., Chaves, E. M., Minte-Vera, C. V., & Lindeman, K. C. (2011). Use of riverine through reef habitat systems by dog snapper (*Lutjanus jocu*) in eastern Brazil. *Estuarine, Coastal and Shelf Science*, *95*(1), 274–278. https://doi.org/10.1016/j.ecss.2011.08.010

Muñoz, F., Pennino, M. G., Conesa, D., López-Quílez, A., & Bellido, J. M. (2013). Estimation and prediction of the spatial occurrence of fish species using Bayesian latent Gaussian models. *Stochastic Environmental Research and Risk Assessment*, *27*(5), 1171–1180.

https://doi.org/10.1007/s00477-012-0652-3

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37–42. https://doi.org/10.1038/nature01286

Pennino, M. G., Muñoz, F., Conesa, D., López-Quílez, A., & Bellido, J. M. (2013). Modeling sensitive elasmobranch habitats. *Journal of Sea Research*, *83*, 209–218. https://doi.org/10.1016/j.seares.2013.03.005

Pennino, M. G., Rufener, M.-C., Thomé-Souza, M. J. F., Carvalho, A. R., Lopes, P. F. M., & Sumaila, U. R. (2018). Searching for a compromise between biological and economic demands to protect vulnerable habitats. *Scientific Reports*, *8*(1), 7791. https://doi.org/10.1038/s41598-018-26130-z

Peterson, A. T., Soberón, J., & Sánchez-Cordero, V. (1999). Conservatism of ecological niches in evolutionary time. *Science*, *285*(5431), 1265-1267. https://doi.org/10.1126/science.285.5431.1265

Peterson, A. T., Ortega-Huerta, M. A., Bartley, J., Sánchez-Cordero, V., Soberón, J., Buddemeier, R. H., & Stockwell, D. R. B. (2002). Future projections for Mexican faunas under global climate change scenarios. *Nature*, *416*(6881), 626–629. https://doi.org/10.1038/416626a

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

R Core Team (2019). *R: A language and environment for statistical computing*.

Rezende, S. D. M., & Ferreira, B. P. (2004). Age, growth and mortality of dog snapper *Lutjanus jocu* (Bloch & Schneider, 1801) in the northeast coast of Brazil. *Brazilian Journal of*

Oceanography, *52*(2), 107-121. http://dx.doi.org/10.1590/S1679-87592004000200003

Robertson, D., Karg, F., Leao de Moura, R., Victor, B. C., & Bernardi, G. (2006). Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes. *Molecular Phylogenetics and Evolution*, *40*(3), 795–807. https://doi.org/10.1016/j.ympev.2006.04.011

Rocha, L. A. (2003). Patterns of distribution and processes of speciation in Brazilian reef fishes. *Journal of Biogeography*, *30*(8), 1161–1171. https://doi.org/10.1046/j.1365-2699.2003.00900.x

Roesti, M., Kueng, B., Moser, D., & Berner, D. (2015). The genomics of ecological vicariance in threespine stickleback fish. *Nature Communications*, *6*(1), 8767. https://doi.org/10.1038/ncomms9767

Roos, N. C., Carvalho, A. R., Lopes, P. F. M., & Pennino, M. G. (2015). Modeling sensitive parrotfish (Labridae: Scarini) habitats along the Brazilian coast. *Marine Environmental Research*, *110*, 92–100. https://doi.org/10.1016/j.marenvres.2015.08.005

Sbrocco, E. J., & Barber, P. H. (2013). MARSPEC: ocean climate layers for marine spatial ecology. *Ecology*, *94*(4), 979–979. https://doi.org/10.1890/12-1358.1

Tillé, Y., & Matei, A. (2016). *sampling: Survey Sampling. R package version 2.8.*

Waples, R. K., Larson, W. A., & Waples, R. S. (2016). Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, *117*(4), 233–240. https://doi.org/10.1038/hdy.2016.60

Watanabe, D., Nomura, M., Watanabe, K., Sakitani, N., Iwasawa, H., Takano, S., & Moriyama, H. (2015). Efficacy of cane and crutch in middle aged and older individuals: a systematic review and meta-analysis. *Physiotherapy*, *101*(April), e1606–e1607. https://doi.org/10.1016/j.physio.2015.03.1619

Williams, S. E., Shoo, L. P., Isaac, J. L., Hoffmann, A. A., & Langham, G. (2008). Towards an Integrated Framework for Assessing the Vulnerability of Species to Climate Change. *PLoS*

Biology, *6*(12), e325. https://doi.org/10.1371/journal.pbio.0060325

Zhu, G. P., Ye, Z., Du, J., Zhang, D. L., Zhen, Y. H., Zheng, C. G., ... & Bu, W. J. (2016). Range wide molecular data and niche modeling revealed the Pleistocene history of a global invader (*Halyomorpha halys*). *Scientific reports*, *6*, 23192. https://doi.org/10.1038/srep23192

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2009). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

7. SUPPLEMENTARY MATERIAL

Table S1. Models ordered by lower WAIC for *Lutjanus jocu*. sstmax = maximum sea surface temperature, bathy = bathymetry, sssmean = mean sea surface salinity, sstrange = sea surface temperature range. The selected model is marked with *

Models	Dic	Waic	LCPO
resp ~ -1 + beta0 + sstmax + bathy + f(spatial, model=spde)*	Inf	89.02447	0.437493
resp ~ -1 + beta0 + sssmean + sstmax + bathy + f(spatial, model=spde)	Inf	89.08444	0.510428
resp ~ -1 + beta0 + sssmean + bathy + f(spatial, model=spde)	Inf	92.44269	0.227434
resp ~ -1 + beta0 + bathy + f(spatial, model=spde)	Inf	92.78004	0.218704
resp ~ -1 + beta0 + sstrange + bathy + f(spatial, model=spde)	Inf	94.51518	0.2212
resp ~ -1 + beta0 + sstmax + sstrange + bathy + f(spatial, model=spde)	Inf	95.08167	0.421693
resp ~ -1 + beta0 + sssmean + sstrange + bathy + f(spatial, model=spde)	Inf	95.3008	0.227752
resp ~ -1 + beta0 + sssmean + sstmax + sstrange + bathy + f(spatial, model=spde)	Inf	96.18184	0.488195
resp ~ -1 + beta0 + sssmean + sstrange + bathy	98.66464	98.43446	0.223942
resp ~ -1 + beta0 + sstrange + bathy	98.68248	98.53212	0.223983
resp ~ -1 + beta0 + sstmax + sstrange + bathy	100.6286	100.5456	0.228752
resp ~ -1 + beta0 + sssmean + sstmax + sstrange + bathy	100.7644	100.6135	0.229141
resp ~ -1 + beta0 + bathy	104.2152	104.1162	0.236624
resp ~ -1 + beta0 + sstmax + bathy	104.7529	104.6711	0.237918
resp ~ -1 + beta0 + sssmean + bathy	105.0383	104.8411	0.238424

resp ~ -1 + beta0 + sssmean + sstmax + bathy	105.1525	105.0276	0.238893
resp ~ -1 + beta0 + sssmean + f(spatial, model=spde)	Inf	114.9229	0.363445
resp ~ -1 + beta0 + f(spatial, model=spde)	Inf	115.6833	0.444767
resp ~ -1 + beta0 + sstmax + f(spatial, model=spde)	Inf	116.3402	0.574597
resp ~ -1 + beta0 + sssmean + sstmax + f(spatial, model=spde)	Inf	116.5312	0.490733

Table S2. Models ordered by lower WAIC for *Sparisoma axillare*. Sstmax = maximum sea surface temperature, bathy = bathymetry, sssmean = mean sea surface salinity, sstrange = sea surface temperature range. The selected model is marked with *

Modelos	Dic	Waic	LCPO
resp ~ -1 + beta0 + sssmean + bathy + f(spatial, model=spde)	Inf	44.8949	0.565113
resp ~ -1 + beta0 + sssmean + sstmax + bathy + f(spatial, model=spde)	Inf	45.70809	2.589931
resp ~ -1 + beta0 + sssmean + sstrange + bathy + f(spatial, model=spde)*	Inf	46.56693	0.84463
resp ~ -1 + beta0 + sstmax + bathy + f(spatial, model=spde)	Inf	47.11027	2.877336
resp ~ -1 + beta0 + sssmean + sstmax + bathy	Inf	47.29084	2.616275
resp ~ -1 + beta0 + sssmean + sstmax + sstrange + bathy + f(spatial, model=spde)	Inf	47.76833	2.38396
resp ~ -1 + beta0 + sssmean + sstmax + sstrange + bathy	Inf	49.31936	2.361024
resp ~ -1 + beta0 + sssmean + bathy	Inf	50.68291	0.546576
resp ~ -1 + beta0 + bathy + f(spatial, model=spde)	Inf	51.58093	2.306042
resp ~ -1 + beta0 + sstmax + sstrange + bathy + f(spatial, model=spde)	Inf	52.488	3.49401
resp ~ -1 + beta0 + sssmean + sstrange + bathy	Inf	53.28182	0.806161

resp ~ -1 + beta0 + sstrange + bathy + f(spatial, model=spde)	Inf	59.01868	3.195748
resp ~ -1 + beta0 + sstmax + sstrange + f(spatial, model=spde)	Inf	76.29799	0.833684
resp ~ -1 + beta0 + f(spatial, model=spde)	Inf	76.34317	0.648771
resp ~ -1 + beta0 + sssmean + sstrange + f(spatial, model=spde)	Inf	76.43231	0.727529
resp ~ -1 + beta0 + sstrange + f(spatial, model=spde)	Inf	76.45967	0.612584
resp ~ -1 + beta0 + sstrange + bathy	Inf	76.78031	0.286919
resp ~ -1 + beta0 + sssmean + sstmax + sstrange + f(spatial, model=spde)	Inf	76.80654	0.963357
resp ~ -1 + beta0 + sssmean + f(spatial, model=spde)	Inf	76.9846	0.734833
resp ~ -1 + beta0 + sstmax + f(spatial, model=spde)	Inf	77.32697	0.870972

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COASTAL-ISLAND POPULATION GENETIC CONNECTIVITY OF THE REEF FISH

SPARISOMA AXILLARE

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ABSTRACT

If a species is distributed in isolated patches, over time the isolated populations can become genetically different through mutation, random genetic drift and local adaptation. Knowing how environmental features shape genetic connectivity can help predict the consequences of human activities on populations. Here, we evaluated the seascape characteristics that could explain the patterns of genetic connectivity and isolation of *Sparisoma axillare* off the coast of Brazil. We genotyped and analysed thousands of SNPs markers from individuals from six coastal sites and three islands (Fernando de Noronha, Abrolhos and Trindade) located at different distances from the coast. The population from Trindade is the only one to form a genetically distinct group. Seascape factors, such as bathymetry and oceanic currents, have more influence on genetic variation than geographic distance, while specific habitat requirements in Trindade could be driving genetic divergence.

1. INTRODUCTION

Reefs comprise the most diverse aquatic ecosystems in the world and provide many goods and services for human well-being. However, due to severe human-induced impacts, such as overfishing, pollution, and climate change, reefs are also the most endangered ecosystems worldwide (Hoegh-Guldberg et al., 2007; Hughes, 2003). Without proper management and mitigation strategies, reefs and their resources will perish within a few decades (Pandolfi, 2003), including full species or populations they host.

The effectiveness of managing populations depends on assuring that species will keep self-recruiting and connecting their populations (Momigliano et al., 2015). Connectivity may not be very important in the marine environment due to the large dispersal capacity of organisms (Costello & Connor, 2019), yet dispersal capacity doesn't necessarily equate to high connectivity. Effective connectivity is influenced by habitat area, quality and spatial arrangement, as well as by the dispersal ability of each individual and species. Species that can reach further distances due to their dispersal capacity tend to present more connected populations (Cushman et al., 2006). However, reaching further sites does not necessarily translate into the establishment of new populations, as even highly connected areas in terms of individuals may not have the habitat requirements for them to establish themselves (Adriaens et al., 2009; Burgess et al., 2014). Being able to disperse and to establish itself determines an effective connectivity.

Available and suitable habitats, such as reefs, are generally not evenly distributed across the landscape (Hanski, 1999). Reaching these suitable habitats is not necessarily easy depending on the environmental differences between two sites, for instance, in the space between coastal and isolated reefs. For example, many reef fishes will not be able to cross deep tracts of the ocean, making depth an effective barrier (Quimpo et al., 2018). Currents also influence the transportation of pelagic larvae and can either increase or decrease population connectivity (Gilg & Hilbish,

2003; Johnson & Black, 2006; Weersing & Toonen, 2009), depending on the oceanographic front where sites are located. Specifically, currents can carry larvae towards another population increasing connectivity, or carry larvae to the opposite site, decreasing it (Treml et al., 2008). The lack of connectivity between populations due to geographic distance, presence of barriers or low quality matrix can limit individual dispersal and gene flow and, then, lead to genetic differentiation (e.g. Riginos & Nachman, 2001; Dixo et al., 2009).

Seascape genetic tools are being developed to better understand the correlation between the marine environment and connectivity or gene flow. These spatially explicit analyses can contribute to clarify how the environment affects the genetic structure of a population, even at low levels of structure (Selkoe et al., 2016). Through seascape genetic tools we learned, for example, that depth contributed to explain the subtle genetic structure of anemonefishes (Saenz-Agudelo et al., 2015). For the common cockle, genetic differentiation in neutral and outlier loci was explained by oceanic currents and temperature, respectively, in addition to geographical distance (Coscia et al., 2019). The use of seascape genetics methods can also contribute to investigate gene flow direction, and indicate vulnerable areas for conservation (Selkoe et al., 2016).

The existence of oceanic barriers could help explain the high endemism and low genetic variability observed on islands, which would have low connectivity with continental areas (Floeter & Gasparini, 2000; Frankham, 1997; Pinheiro et al., 2018). Still, some species are common both on the coast and off islands, including species of the genus *Sparisoma* (Labridae), although they tend to be more common in oceanic islands than other parrotfishes from the same family (Mazzei et al., 2019). The genus *Sparisoma* is formed by herbivorous parrotfishes dependent on reef habitats (Froese & Pauly, 2019). It comprises 15 species, five of which endemic to Brazil and one of those occurring only in the reefs of the archipelago of Trindade, located more than a 1000 km offshore.

Historically, the diversification of *Sparisoma* occurred mainly through dispersal events (across the Atlantic, to islands) followed by divergence in allopatry, suggesting that environmental barriers probably played an important role (Robertson et al., 2006). For example, the great oceanic depth between West and East Atlantic is considered an effective barrier important for divergence, and was probably responsible for the separation of some sister lineages (Araújo et al., 2019). A complex of *S. rubripinne* from the Caribbean, plus *S. axillare* from southwest Atlantic, are separated from the Eastern Atlantic lineages of *S. rubripinne* (between 2.2 and 5.6 million of years ago, Robertson et al., 2006). River mouths seem to be effective barriers for *Sparisoma*, and are more important than the distance between the coast and offshore reefs and islands (Robertson et al., 2006). The Amazonas river, for example, is also likely to have affected the differentiation in this group by separating *Sparisoma axillare* and *S. rubripinne* from their Caribbean lineages between 0.5 and 5.6 million of years ago (Robertson et al., 2006).

Sparisoma axillare is a parrotfish relatively abundant in coastal habitats and on island reefs. The species is endemic to Brazil, occurring mainly in shallow waters (Froese & Pauly, 2019). Its larval pelagic phase is supposed to be long, similar to other species of *Sparisoma* (between 57 and 60 days on average, Robertson et al., 2006), allowing them to eventually reach and settle in further regions. The species occupies different environments along its ontogenetic development. Juveniles less than 5cm in length are mostly present in macroalgal beds and flat reefs, while juveniles larger than 5cm are more frequent in the back or fore reefs (Feitosa & Ferreira, 2015). *Sparisoma axillare* has a relatively large body size (40 cm), and is widely fished in some regions (Roos et al., 2015). For this reason, the species already shows signs of population decrease and is classified as Vulnerable in the Brazilian List of Endangered Aquatic Animals (MMA, 2014).

Because of its threatened status, it is important to understand how protected areas are helping sustain *Sparisoma axillare*. Models of larval connectivity showed extremely low connectivity between Brazilian Marine Protected Areas (MPAs): the recruitment that takes place

in other regions in Brazil is crucially dependent on the existence of a single large MPA of sustainable use at the Brazilian northeast region (Costa dos Corais) (Endo et al., 2019). If populations within MPAs are the only sustainable ones, then *Sparisoma axillare* is in a highly risky condition (Endo et al., 2019). However, larval models present some caveats, and the adding of genetic information could be important to identify seascape factors affecting population connectivity (Cowen & Sponaugle, 2009; Levin, 2006). Here, we aimed to identify the population genetic structure and environmental variables that affect genetic connectivity and the local requirements of *Sparisoma axillare* populations, on the Brazilian coast and islands. To do that, we: i) first tested if the populations of *S. axillare* presented some genetic structure using SNPs markers; ii) then we identified the main environmental and geographic variables that affect genetic diversity among sites and inferred the relative importance of adult and larval dispersal in connectivity. The results of this study can be applied to understand population dynamics in other similar reef species that occur in the same region and in others, and improve the understanding of the effectiveness of MPAs.

2. METHODOLOGY

2.1 Study area and sampling

Samples were collected along more than 4,000km of the Brazilian coast and included three islands complexes, Abrolhos Bank, Fernando de Noronha Archipelago and Trindade island (Fig. 1). The extensive >7,000 km of coastline in Brazil, together with its continental shelf and Exclusive Economic Zone (EEZ), encompass a wide diversity of habitats, including coastal and oceanic islands, with different origins, features and distances from the coast, and that are differently affected by oceanic currents. Coastal waters are mainly turbid, especially due to the presence of

large rivers, while the islands present clearer waters. This diversity of features can act as possible barriers for population connectivity.

Abrolhos is a large coastal archipelago, formed by five small islands, located 70 km off the coast of Bahia, a northeastern state. Abrolhos is close to the branching point of the Brazilian current in the northward and southward directions, and is formed by channels, reefs, sandbanks and volcanic islands. This region has some unique aspects in comparison with other Brazilian regions, including its large coral bank, which can be responsible for local adaptations and populations divergence (Leão & Kikuchi, 2001). Fernando de Noronha is a volcanic oceanic archipelago located about 400km from the northeast coast. Fernando de Noronha shares multiple features with the coast, including most of its fish fauna (Floeter & Gasparini, 2000). A strong oceanic current, heading from Fernando de Noronha towards the coast, can affect connectivity between Fernando de Noronha and the coast (mostly with Rio Grande do Norte and Pernambuco states). Trindade, located 1,100km from the northernmost point of the south eastern coast, connects to the coast through a large chain of 30 volcanic seamounts, called Vitoria-Trindade Chain (Fig. 1). These seamounts can act as stepping-stones to connect coastal and island populations. However, the Brazilian Current, flowing south along the Brazilian shelf, may reinforce the barrier between island – seamounts – and the coast (Pinheiro et al., 2015). The region was strongly affected by past changes in sea level, with the number of emerged islands varying according to the Glacial period (Pinheiro et al., 2017). Currently, two islands are present on the east limit of the chain.

In terms of marine species occurrence and endemism, Brazil is divided in six subprovinces (Pinheiro et al., 2018). Abrolhos is in the same subprovince as the coast, Fernando de Noronha (together with Atol das Rocas) consists of a separated subprovince, and Trindade with Vitoria-Trindade Chain form a third one (Pinheiro et al., 2018). There is some support, based on species similarity, that the three subprovinces are all relatively well connected (Pinheiro et al., 2018). Connectivity can be enabled by habitat characteristics between islands, i.e. organisms will

establish in sites with similar characteristics (Pinheiro et al., 2018). Speciation in islands in general are important for Brazilian marine diversification (Pinheiro et al., 2017), and recent changes in sea level had a large impact on island biodiversity patterns in the region, due to differences in connectivity levels (Pinheiro et al., 2017).

Eighty-nine tissue samples of *Sparisoma axillare* were collected between 2017 and 2018 at nine sites, through SCUBA diving (SISBIO authorization number 48112-7), in fish landings, and fish markets (when origin was known). Samples were stored in 95% ethanol. The number of samples per site was between eight and 12 (except for samples from Espírito Santo, which consisted of only four). The Pernambuco site consists of samples collected from two close localities: Tamandaré and Itamaracá. *Sparisoma axillare* geo-referenced presence data for distribution modelling analysis was extracted from online databases (Species Link and GBIF) and from information provided by experts.
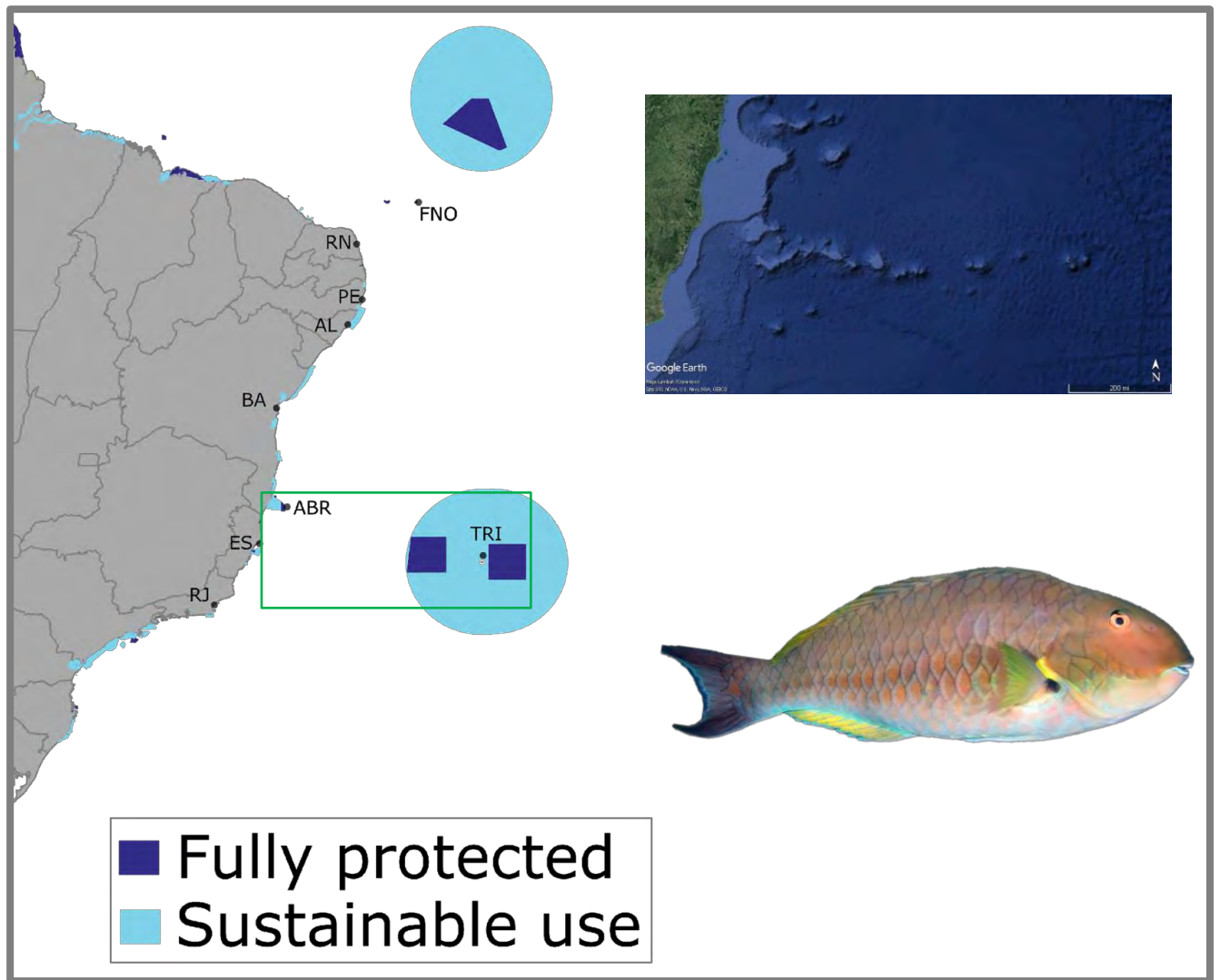
Fig. 1. Left side, the study area with sampling sites. Coastal sites (black dots): RN – Rio Grande do Norte, PE – Pernambuco, AL – Alagoas, BA – Bahia, ES – Espírito Santo, RJ – Rio de Janeiro. Islands sites are: FNO – Fernando de Noronha, ABR – Abrolhos, and TRI – Trindade. In detail on the right side, Google Earth image of Vitória-Trindade Chain and an image of an adult individual of *Sparisoma axillare*.

2.2 Environmental variables

The following environmental variables were used to test possible selection, isolation by resistance and distribution modeling. Those variables are known to affect marine organisms' fitness, dispersal and distribution.

2.2.1 Latent Factor Mixed Models (LFMM)

To identify correlation between outlier SNPs and the environment, the following uncorrelated (Pearson correlation, $r<0.8$) variables were extracted from bio-oracle (Assis et al., 2018; Tyberghein et al., 2012) and tested: mean chlorophyll (mg.m^{-3}), pH, phosphate (mol.m^{-3}), salinity (PSS) and minimum sea surface temperature (ºC), all with resolution of 0.01 x 0.01 degrees.

2.2.2 Isolation by Resistance

To test the environmental effect on connectivity, here called resistance, four environmental variables were used: bathymetry (m), mean velocity of currents (m^{-1}), mean curvature (º), and minimum sea surface temperature (ºC), using rasters provided by bio-oracle and re-categorized (Supplementary Material Table S1) in R (R Core Team, 2019).

2.2.3 Distribution modeling

The environmental variables used as explanatory variables of the species occurrence were extracted from bio-oracle and MARSPEC databases (http://www.marspec.org; Sbrocco & Barber, 2013), all with resolution of 0.01 x 0.01 degrees. The environmental variables tested were: bathymetry (m), and aspect, that is a measure of bottom complexity (both from MARSPEC), and mean chlorophyll (mg.m^{-3}), minimum sea surface temperature (ºC), range of sea surface temperature (ºC), phytoplankton (umol.m^{-3}) (from bio-oracle), and roughness. Roughness was derived from bathymetry using terrain function from the 'raster' package (Hijmans & van Etten, 2014). Roughness varies from 0 (no terrain variation, meaning less complexity) to 1 (high variation, consolidate substrates). These variables were selected because they could potentially affect *Sparisoma axillare* distribution. Bathymetry seems to be one of the main drivers to affect marine species distribution (e.g. Costa et al., 2017; Dell'Apa et al., 2016; Roos et al., 2015), and was selected because of the apparent preference for shallow waters by *S. axillare* (Froese & Pauly, 2019). Aspect and roughness were selected as a proxy of habitat complexity, which can be

important for reef species. Temperature range was included because latitudinal differences can be related to occurrence of different populations, and can affect local preferences. Chlorophyll and phytoplankton were selected as proxy of productivity, which can be important especially for an herbivorous species as *S. axillare*. The variables were tested for correlation (Pearson) and collinearity (Generalized Variance Inflation Index - VIF). As the variables were not highly correlated (r<0.8) and VIF was lower than 3, all were considered for the spatial distribution analysis after standardization (difference from the mean divided by the corresponding standard deviation, following (Gelman, 2008).

2.3 Genetic analysis

2.3.1 DNA sequencing and SNPs filtering analysis

SNPs were discovered and genotyped using the Diversity Arrays Technology Pty. Ltd. protocol (described by Jaccoud et al., 2001). For a high-quality dataset, the data was filtered using the following parameters: minimum call rate of 97%, 100% of reproducibility, and a Minimum Allele Frequency of 2%. These steps are important to reduce the chance of results from sequencing errors. When more than one SNP was found in the same fragment, only one was maintained for the analysis to avoid linked loci. Those deviating from Hardy-Weinberg Equilibrium were identified and excluded, using the 'dartR' package applying Bonferroni correction (Gruber et al., 2018) in R (R Core Team, 2019). One individual with more than 20% of missing data was excluded. Following these filtering steps, 5,093 SNPs and 88 individuals were kept for the subsequent analyses. This dataset is the same as the one used in Chapter 3.

2.3.2 F_{ST} outliers' detection and environment association

The identification of F_{ST} outliers was done using Outflank (Whitlock & Lotterhos, 2015). This approach was selected due to its low rate of false positives discovery and is based on F_{ST}

distributions for individual loci. Outflank analysis was run using the outflank function from the 'dartR' package in R (R Core Team, 2019). A trim value (left and right) of 0.05 was used, based on the estimate that 5% of any genome is affected by strong selection, as suggested by Whitlock & Lotterhos (2015), and considering nine populations (sites). To detect outlier SNPs correlated with environmental variables, a Latent Factor Mixed Models (LFMM) was used. The LFMM analysis tests the correlation between loci and environmental variables from samples sites, incorporating prior information on neutral genetic structure. The LFMM function from the 'LEA' package was used (Frichot & François, 2015). SNPs identified by LFMM analysis were searched for gene annotation using NCBI BLAST tool, using a minimum e-value of 0.0001 (Johnson et al., 2008). These SNPs were excluded from further analyses of neutral genetic population structure, although it can be an indicative of under selection genetic divergence.

2.3.3 Genetic population structure analysis

Two additional approaches to investigate genetic population structure were used. First, a pairwise F_{ST} analysis between sites was run using Arlequin software (Excoffier & Lischer, 2010), using the dataset without outlier SNPs. The parameters applied were a p-value lower than 0.05 for significance, and 100,000 permutations. To test for population structure without locality information we used the Structure Software v2.3.4 (Pritchard et al., 2000) testing from 1 to 9 possible clusters (10 iterations each). The Admixture ancestral model was used, considering the allele frequencies of each population as independent, with a run length of 100,000 (10,000 burn-in). The most probable number of K (number of clusters) was inferred based on ΔK (Evanno et al., 2005). The resulting genetic population structure was used to define the populations for the distribution modelling.

2.3.4 Demography

To test migration rate and direction between sites, we used the function divMigrate of the 'diveRsity' package (Keenan et al., 2013) in R, using Jost's D as metric for genetic differentiation (Jost, 2008). This method is based on the allele frequency of the pool of migrants in each pairwise comparison between sites, and the migration rate varies between 0 and 1.

2.3.5 Seascape genetic analysis - Isolation by Distance and Resistance

The geographic distance between sampling sites was measured in Google Earth. Four different rasters were used, one for each variable (bathymetry, currents, curvature and minimum sea surface temperature), using the nine sites as focal points. For bathymetry, curvature and minimum sea surface temperature, the calculation was made based on resistance (higher values meaning lower connectivity). For currents, we used "conductance", meaning that higher values correspond to higher connectivity. To define the resistance distance between sites, a matrix of resistance (distance) between sites was calculated using the Circuitscape software (McRae & Beier, 2007) and 'ResistanceGA' package (Peterman, 2018) in R for each variable. Circuitscape is based on the circuit theory approach to identify all possible pathways between sites (focal points). To test for correlations between geographic and resistance distances with genetic distances was used MLPE (maximum likelihood population effects, Peterman, 2018). We also ran these tests using geographic distance as a variable and using residuals from the geographic distance and genetic distance (to exclude the geographic distance effects and to identify seascape effects). The MLPE models were classified using AICc and the best models were the ones with lower AICc values and higher AIC weight.

2.4 Spatial Distribution Modelling

A hierarchical Bayesian point-reference spatial model (H-BSM) to estimate the specific habitat preferences was implemented for each population identified by genetic structure analysis. These models can also be considered to be a spatial extension of Generalized Linear Models (GLMs) because the modelling process describes the variability in the response variable as a function of the explanatory variables, with the addition of a stochastic spatial effect to model the residual spatial autocorrelation (Muñoz et al., 2013). For the response variable (presence/absence of the species) a binomial distribution was used with a logarithmic link function. As only species presence data was available, pseudo-absences were randomly generated for the entire area, using the 'srswor' function of the 'sampling' package (Tillé & Matei, 2016) in R. In each case, the number of generated pseudo-absences was the same as the number of real presences. Pseudo-absences were then combined with real presences into a single presence-absence dataset to be used for the binomial model. In particular, it is worth mentioning that a binomial distribution with a Bayesian spatial model was used instead of a less accurate model that allows the use of presence-only data (e.g. BIOCLIM, MAXENT) because it permits the inclusion of a spatial effect to deal with spatial autocorrelation and to explicitly quantify the uncertainties (Costa et al., 2017; Pennino et al., 2018; Roos et al., 2015). For all models, Bayesian parameter estimates and predictions were obtained throughout the Integrated Nested Laplace Approximations (INLA) approach (Rue et al., 2009) and package (http:\\www.r-inla.org) implemented in the R software. INLA uses Stochastic Partial Differential Equations (SPDE) approach (Lindgren et al., 2011) for the spatially structured random effect.

A vague zero-mean Gaussian prior distribution with a variance of 100 was assigned for all fixed effect parameters, while multivariate Gaussian distributions with mean zero and a spatially-structured covariance matrix were assumed for the spatial component. The environmental variable selection with all possible interaction terms was mainly based on the Watanabe-Akaike

information criterion (WAIC) (Watanabe et al., 2015), and the mean logarithmic of the approximated conditional predictive ordinate (LCPO) (Gneiting & Raftery, 2007). While WAIC values indicate the goodness of fit of the models, the LCPO evaluates the predictive capacity. Lower values for both WAIC and LCPO represent the best compromise between fit and parsimony.

To predict the probability of the species occurrence in the entire study area and define suitable habitats for *S. axillare* populations, a Bayesian kriging approach was used to calculate the posterior predictive distribution of the species. This approach treats parameters as random variables in order to consider parameters uncertainty and also uses additional functions that linearly interpolate the predicted values in non-sampled locations.

3. RESULTS

3.1.1 Genetic analysis

We found 66 outliers SNPs using Outflank (Supplementary Material Table S2) and 216 outliers correlated with environmental variables using the LFMM analysis. For three SNPs correlated with environmental variables, the search for gene annotation matched available genomes on Genbank (Table 1).

Table 1. List of SNPs identified as outliers or correlated with environmental variables.

Loci ID	Sequence	Blast	Cover	e-value	Environmental Variables
100025002	TGCAGTGTGCTGATCTCCTGTGTTCACATTAAAGTG TCCAGACTGGGCCTGAGCATGTCCGTGTTTTAT	cerebellar degeneration-related protein 2-like	94	5.00E-13	Phosphorus
38667479	TGCAGCTTCATCACGTCCACTTCCTCTACCGGCCCTGC CCAGACTTTCCTCCCAGCATGAGCGGTTCAG	CST complex subunit CTC1-like	85	1.00E-13	Chlorophyll and pH
38663128	TGCAGTCCTACTACGAGGCCAAAGCCCGCAGAGAGA G GAAGATCAAGAGCAAGAAGTACCACCGAGTCC	U3 small nucleolar RNA-associated protein 14 homolog A-like	97	5.00E-23	Salinity

3.1.2 Genetic population structure

Pairwise F_{ST} varied between 0.002039 (Abrolhos and Espírito Santo) and 0.15638 (Abrolhos and Trindade) (Table 2). Despite some significant differences, F_{ST} between all sites, except Trindade, was lower than 0.021. However, the F_{ST} between Trindade and all the other sites were always higher than 0.12. According to the Structure analysis, the most probable number of populations in our dataset is two (K=2; Fig 2), one cluster comprising all the coastal sites plus Abrolhos Bank and Fernando de Noronha Archipelago, and another cluster comprising only Trindade Island. As the divergence between Trindade and other sites was large, which could mask smaller divergences, we also ran Structure excluding the Trindade population. The result indicates K = 1.

Table 2: Pairwise F_{st} between sites (bold values indicate $p < 0.05$, * indicates Fst larger than 0.01, and ** larger than 0.1). Here we show the two sites from Pernambuco separately because the results were highly similar between them. FN – Fernando de Noronha archipelago, RN – Rio Grande do Norte, ITA-PE – Itacaré Pernambuco, TAM-PE – Tamandaré Pernambuco, AL – Alagoas, BA – Bahia, ABRO – Abrolhos, ES – Espírito Santo, RJ – Rio de Janeiro, TRIN – Trindade.

|	FN	RN	ITA-PE	TAM-PE	AL	BA	ABRO	ES	RJ	TRIN
FN	0									
RN	**0.0123***	0								
ITA-PE	0.00552	**0.01181***	0							
TAM-PE	0.00427	**0.01051***	0.00562	0						
AL	**0.01024***	**0.01282***	**0.00813**	**0.00675**	0					
BA	0.00214	**0.01005***	**0.00322**	0.00275	**0.00718**	0				
ABRO	**0.01152***	**0.02009***	0.01266	0.01186	**0.01419***	**0.00931**	0			
ES	**0.01252***	**0.01587***	0.01279	0.01321	**0.00943**	**0.00654**	0.02039	0		
RJ	**0.00739**	**0.01249***	**0.00695**	0.00525	**0.01263***	**0.00687**	**0.01544***	**0.00969**	0	
TRIN	**0.12743***	**0.13538***	**0.13936***	**0.13522***	**0.12683****	**0.12399***	**0.13422***	**0.15638***	**0.1326***	0
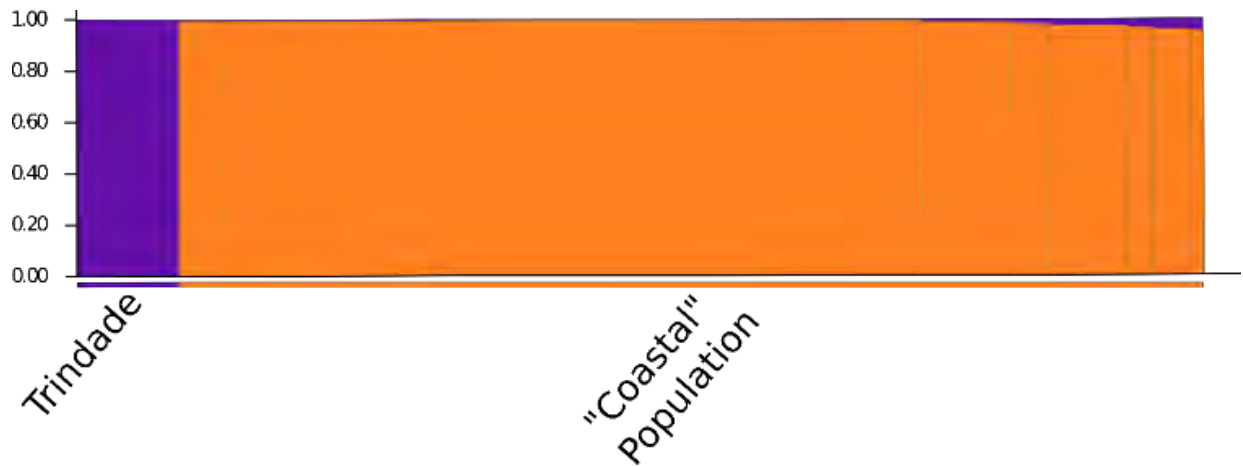
Fig. 2: Barplot of the Structure analysis showing the two clusters (K = 2): Trindade island population (in purple) and "coastal" population, which comprises all the coastal sites, plus Fernando de Noronha Archipelago and Abrolhos Bank.

3.1.3 Demography

The migration rate was high and in both directions between most of the sites, including all the coastal area and two islands, Abrolhos and Fernando de Noronha. The northern region, including Bahia, Alagoas, Pernambuco and Fernando de Noronha, was the most connected. Rio Grande do Norte presented a relatively small connectivity with other sites. Espírito Santo, located further south from most of other sampling places (with the exception of Rio de Janeiro), also presented lower connectivity, but this result could be due to a lower sampling number. Clearly, Trindade is the most isolated site, providing very few migrants to the other locations and receiving even fewer ones (Fig. 3, Table 3).
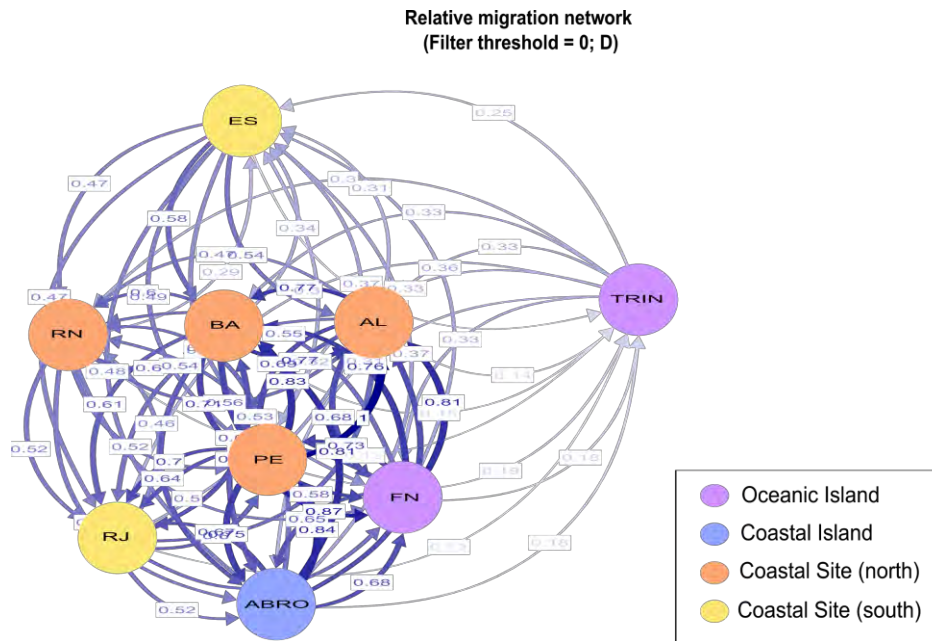
Fig. 3: Migration rate between sampling sites in oceanic islands (TRIN - Trindade Island and FN - Fernando de Noronha Archipelago), coastal island (ABRO - Abrolhos Bank), coastal sites in the northern region (RN - Rio Grande do Norte, PE - Pernambuco, AL - Alagoas and BA - Bahia) and coastal sites in the southern region (ES - Espírito Santo and RJ - Rio de Janeiro).

Table 3: Results of migration rate. Upper part of the matrix is the rate of migrants received by the site and left column is the rate of migrants leaving the site. FN – Fernando de Noronha Archipelago, BA – Bahia, ES – Espírito Santo, RJ – Rio de Janeiro, TRIN – Trindade Islands, ABRO – Abrolhos Bank, RN – Rio Grande do Norte, AL – Alagoas, PE – Pernambuco.

	FN	BA	ES	RJ	TRIN	ABRO	RN	AL	PE
		0.764383	0.358165	0.660179	0.179408	0.653852	0.551072	0.812282	0.728915
FN	NA								
	0.715115		0.336556	0.612645	0.151934	0.637237	0.595996	0.767748	
BA		NA							0.713022
	0.445093	0.575397		0.474794	0.151638	0.480988	0.474253	0.535208	0.488683
ES			NA						
	0.556676	0.667256	0.320466		0.125345	0.520215	0.458461	0.579369	0.600365
RJ				NA					
	0.330742	0.333373	0.251296	0.297055		0.307398		0.325227	0.325279
TRIN					NA		0.300648		

ABRO	0.675988	0.814687	0.367938	0.665491	0.183322	NA	0.531418	0.687572	0.843631
RN	0.496424	0.603772	0.291558	0.515889	0.129171	0.503382	NA	0.557759	0.51714
AL	0.683254	0.770836	0.311007	0.54025	0.138551	0.621399	0.473306	NA	0.692296
PE	0.867517	0.825268	0.368547	0.698167	0.185972	0.748388	0.558151	1	NA

3.1.4 Seascape genetic analysis - Isolation by distance and resistance

The best MLPE models had bathymetry resistance explaining the genetic distance (Table 4). Using the residuals from the correlation between genetic distance and geographic distance as a response variable, three models were equally good: bathymetry, currents, and curvature (Table 5). A visual inspection of the relationship between variables indicates that both bathymetry and current intensity resistance contribute to the isolation of the Trindade population (Fig 4).

Table 4: Model selection of variables explaining genetic distance ordered by AIC (MLPE analysis result).

	(Intrc)	Geo_dist	Currents	Bathymetry	Curvel	Temperature	df	logLik	AIC	delta
Bathymetry	-0.04068			-0.07664			4	130.485	-253	0
Currents	0.06785		0.005738				4	128.345	-248.7	4.28
currents+bathymetry	-0.09831		0.02522	-0.2029			5	128.253	-246.5	6.47
Curvel	0.05014				0.000355		4	126.439	-244.9	8.09
Temperature	0.0476					-0.00086	4	125.996	-244	8.98
curvel+bathymetry	-0.1291			-0.17	0.002949		5	126.09	-242.2	10.79
geo_dist	0.04781	-0.00012					4	124.908	-241.8	11.16
temperature+bathymetry	-0.00202			-0.04304		-0.00054	5	125.052	-240.1	12.87
geo_dist+bathymetry	-0.07468	0.00021		-0.1061			5	124.169	-238.3	14.63

Table 5: Model selection of variables explaining the residuals of genetic distance~geographic distance ordered by AIC (MLPE analysis result).

	(Intrc)	Geo_dist	Currents	Bathymetry	Curvel	Temperature	df	logLik	AIC	
Currents	1.37E-02		0.003918				4	157.819	-307.6	
bathymetry	-2.70E-04			-2.34E-04			4	157.45	-306.9	
curvel	5.07E-03				0.0007727		4	156.263	-304.5	
temperature	-1.60E-05					-6.54E-05	4	154.774	-301.5	
geo_dist	1.31E-09	4.07E-05					4	154.676	-301.4	
currents+bathymetry	1.46E-02		0.00403	4.17E-04			5	153.693	-297.4	
curvel+bathymetry	5.01E-03			-5.28E-05	0.0007718		5	152.116	-294.2	
temperature+bathymetry	-1.49E-04			-1.16E-04		-6.44E-05	5	150.636	-291.3	
geo_dist+bathymetry	-2.00E-04	3.97E-05		-1.73E-04			5	150.535	-291.1	
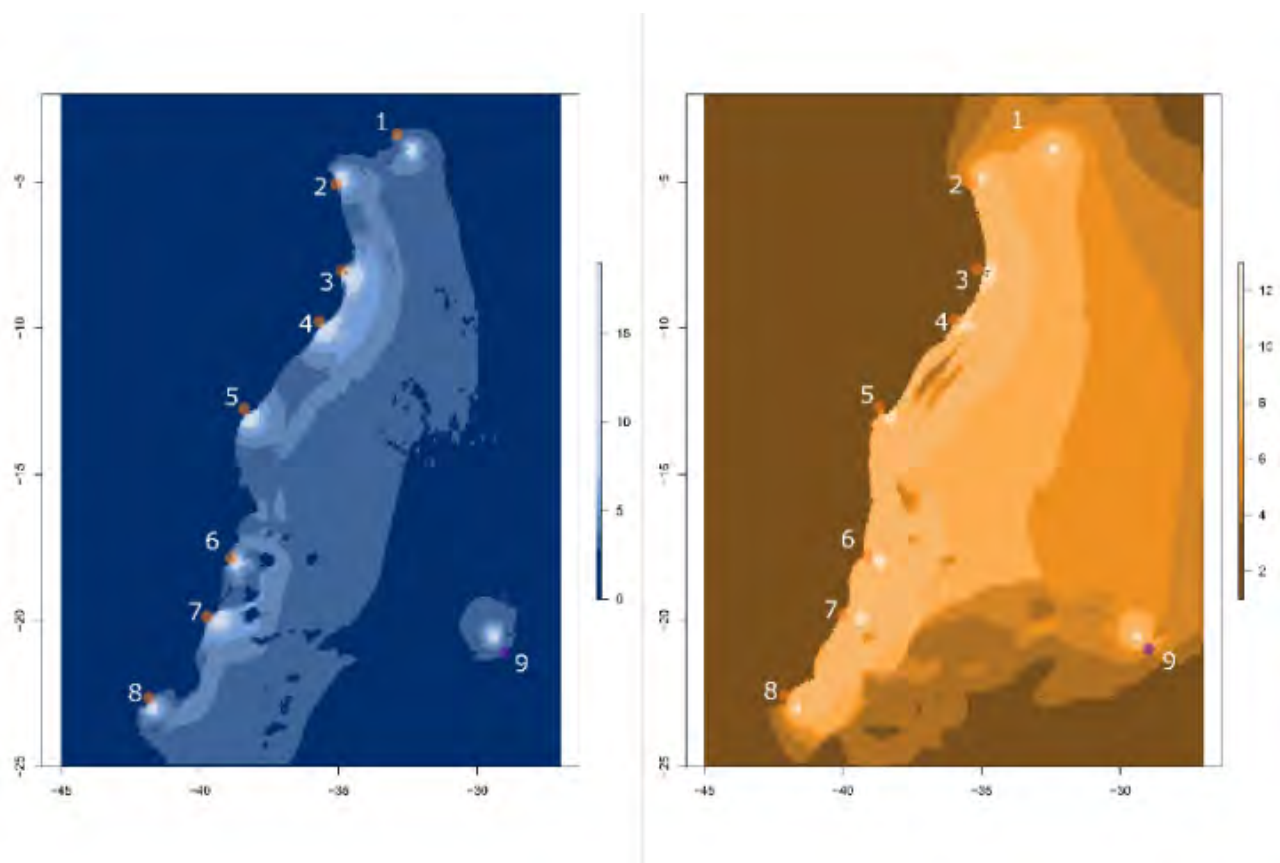
Fig 4. a) Bathymetry and b) oceanic currents intensity resistance maps. Higher values indicate lower resistance. 1 – Fernando de Noronha, 2 – Natal, 3 – Pernambuco, 4 – Alagoas, 5 – Bahia, 6 – Abrolhos, 7 – Espírito Santo, 8 – Rio de Janeiro, 9 - Trindade

3.2 Bayesian Distribution Modelling

As the genetic population structure analysis identified two clusters, two distribution models were run, and resulted in different requirements for each population, suggesting some difference in their niches. For the Coastal population, 122 occurrence sites and, for Trindade population, 25 were used for the spatial distribution modelling. For the Coastal population, the model with the lowest WAIC and LCPO showed a positive relationship with the Sea Surface Temperature Range and with Bathymetry, and also included the Spatial Effect. For this population there is a large area with high suitability along the coast and closer to islands (Fig 5a). Suitable areas were also present at the East Atlantic, near Africa. For the Trindade population, the model with best fit indicated a positive correlation with the Sea Surface Temperature Range and Aspect. The random spatial effect was also relevant. Suitable habitats are only available in close proximity to the island (Fig 5b).
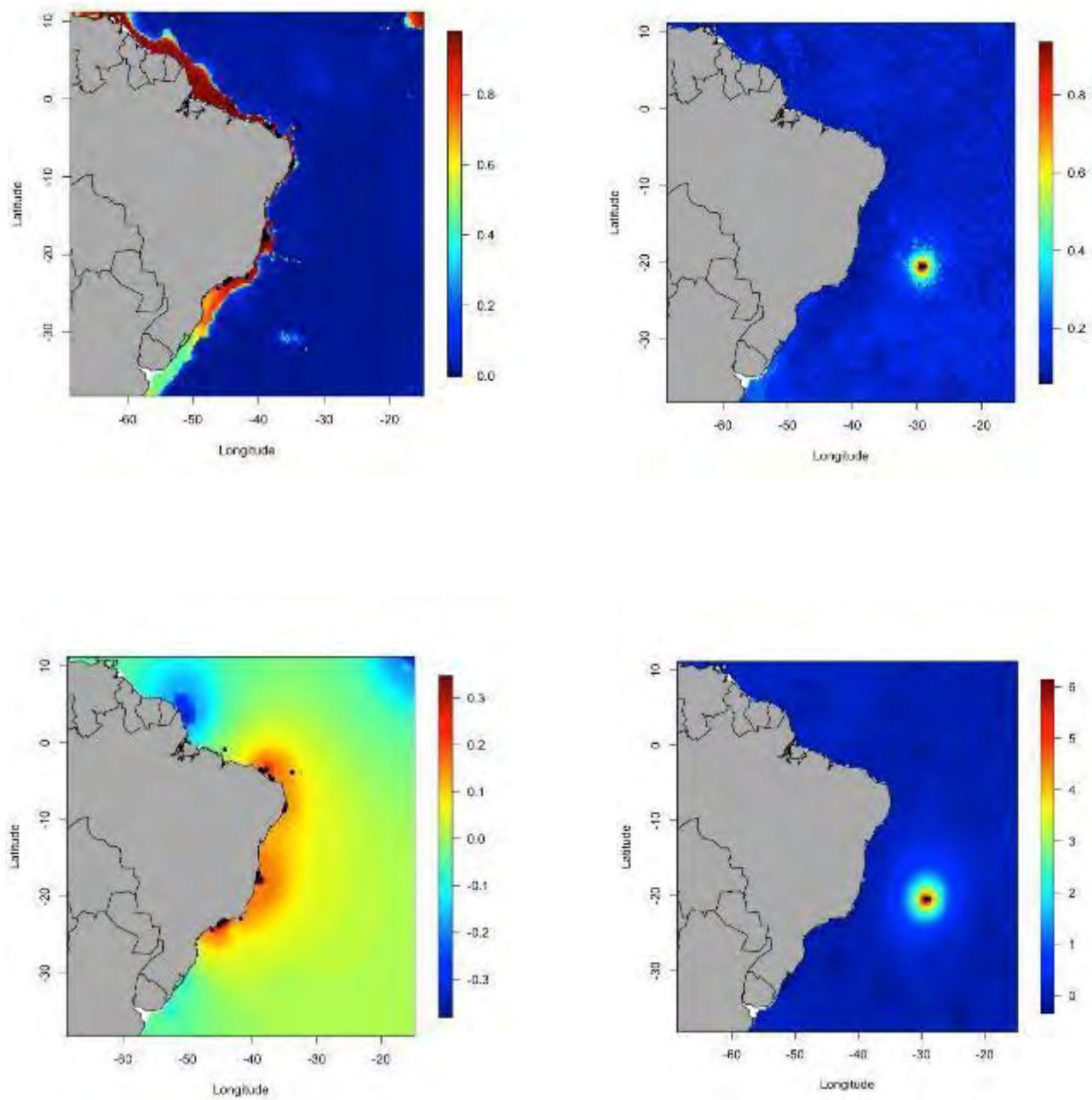
Fig. 5: Median of posterior probability (above) and spatial effect (below) for both populations, a) coastal, Abrolhos and Fernando de Noronha and b) Trindade.

4. DISCUSSION

This study showed that *S. axillare* has generally high levels of genetic connectivity in its distribution along the Brazilian coast. Only one population, from the furthest oceanic island (Trindade), showed limited genetic connectivity with the rest of the sampled sites (migration rate lower than 0.36). The observed genetic structure is explained by a combination of geographic distance and environmental resistance (bathymetry and oceanic currents intensity). There was also some evidence of local adaptation (specific habitat preference) for the Trindade population, which may further limit gene flow.

The migration rate between coastal sites is overall greater than 0.5, with the exception of Espírito Santo, which is located on the southeastern coast. However, the low migration rate to and from Espírito Santo could also be explained by the low sampling number (N = 4) from this site. The Fernando de Noronha Archipelago is highly connected to the coast, mainly Pernambuco, with a migration rate to the archipelago of 0.86, and with Alagoas, with a migration rate to the coast of 0.81. Some previous modelling have reported that larvae spawned in Fernando de Noronha can travel relatively long distances (mainly during the winter) due to the North Brazilian Current and the North Equatorial Counter Current, connecting the populations of Fernando de Noronha to the coastal populations (Endo et al., 2019). Also according to the larval modelling, a large MPA located in Pernambuco and Alagoas (Costa dos Corais) is important for the recruitment that occurs in other northern and southern MPAs (Endo et al., 2019). This result corroborates with our findings that Pernambuco has high genetic connectivity with Fernando de Noronha and other sampled sites. Despite its relevance to the viability of the distribution of *S. axillare*, this large MPA is categorized as low protection, equivalent to IUCN category IV (Dudley, 2008), and can be heavily impacted by fishing.

This MPA and the MPA at Trindade were the only ones where self-recruitment occurred, according to the larval modeling study (Endo et al., 2019). Important factors that affect self-recruitment are larval productivity, local habitat quality, and site isolation (Sponaugle et al., 2002). Our findings suggest that the self-recruitment in Trindade is probably due to the lack of connectivity of this region with others, and this increases the vulnerability of this population. Although the larval model suggests that most populations located in MPAs are not well connected (Endo et al., 2019), the level of genetic connectivity is consistent with this because very few migrants per generation are needed to homogenize allele frequencies. So, the inconsistency between larval and genetic results can be because of the different time scales these processes operate at.

The low levels of genetic structure found for *S. axillare* has been reported for other species in the region (da Silva et al., 2015; Silva-Oliveira et al., 2008). The level of genetic divergence was not completely explained by the geographic distance among sites, and the islands (Fernando de Noronha and Abrolhos) were no less connected than other coastal areas. Due to differences in the environment and environmental resistance between islands and coast, our results were contrary to what we expected. For instance, the area between the coast and Fernando de Noronha is characterised by deep areas, which we expected to restrict dispersal. As it did not, the Brazil Current could be playing an important role in connecting Fernando de Noronha with the coast when passing through Fernando de Noronha southward. However, the currents alone do not explain the connectivity, since the observed connectivity is apparently happening in both directions. The geographic proximity could explain the connectivity between Abrolhos and the points closest to the coast, especially with the state of Bahia. Migration between Abrolhos and Bahia is stronger towards the coast (0.81) than towards Abrolhos Bank (0.63), which reinforces the importance of this unique protected area for the maintenance of the coastal population of *S. axillare*.

The genetic divergence between Trindade and other localities suggests that the Vitoria-Trindade Chain (VTC) is not acting as a stepping-stone for *S. axillare*, as it does for other species (Joyeux et al., 2008; Simon et al., 2013). In fact, *S. axillare* has never been reported on the VTC seamounts, where other six species of the same genus have been registered (Pinheiro et al., 2015), suggesting that the dispersal of this species may be especially limited by depth (Lessios & Robertson, 2006). The colonization of Trindade could be due to sporadic events, which were probably more frequent during periods of low sea level around 20kya (Cowen, 1985). During the Pleistocene, all the seamounts were exposed, which probably led to increases in the biodiversity of Trindade (Macieira et al., 2015; Simon et al., 2013; Thomas et al., 2009). Despite the significant population structure between Trindade Islands and the coast, there is still some migration between them, especially towards the coast (migration rate towards the coast from 0.25 to 0.33; towards Trindade from 0.12 to 0.18).

Bathymetry and oceanic currents seemed to contribute to the lack of connectivity between the populations of *S. axillare* from the coast and Trindade. The Trindade Islands region experiences different oceanographic conditions than the Fernando de Noronha Archipelago and Abrolhos Bank regions (Rodrigues et al., 2007; Lumpkin & Garzoli, 2005; Rudorff et al., 2009). While in Fernando de Noronha, the currents are stronger and head towards the coast (northward and southward), in Trindade currents are weaker with no clear direction. The Brazilian Current, flowing south along the Brazilian shelf, may also reinforce the lack of connectivity between Trindade and the coast (Pinheiro et al., 2015).

Besides neutral genetic differences caused by low connectivity between sites, population adaptation to local conditions can also induce genetic divergence. So, even with some level of migration, genetic differentiation can be maintained. Here we found that possibly under selection SNPs were significantly associated with environmental variables (salinity, phosphorus,

chlorophyll and pH), indicative of localized adaptation. So, some degree of genetic differentiation in *Sparisoma axillare* populations can be explained by differences in local conditions.

The levels and patterns of genetic connectivity identified here can also help predict impacts of future climate change. Of the three factors found to affect the connectivity between the coast and Trindade populations, ocean currents can be strengthened by future climate change. The characteristics of the oceanic currents near Trindade already play an important role in reducing the connectivity, probably due to meandering processes and vortices (Lima, 2019). Future changes in currents in the region are predicted to decrease the larval connectivity further (Lima, 2019), whereas effects of temperature can hinder the local larval establishment (Lima, 2019).

Anthropogenic effects continue to threaten *S. axillare*, especially the Trindade population. Despite its vulnerable status, *S. axillare* is an important fishing resource in some Brazilian regions (Roos et al., 2015). Fisheries can have an especially negative impact on islands, and Trindade is no exception (Pinheiro et al., 2010). Despite the fact that Trindade is technically protected to some degree, the archipelago is still subject to some fishing by the visitors. Recently in Brazil, large offshore MPAs were established, increasing the protected areas from 1.5% to impressive 25%. However, there is some debate how the criteria used to select these areas, which are mostly in the open ocean and do not include reef areas, seamounts, or consider where the biodiversity is distributed (Magris & Pressey, 2018). More than that, these MPAs are loosely regulated and easily changed by political and economic pressures. Here we showed that an isolated population of *S. axillare* is restricted by very limited habitat availability in Trindade island, and that the area is not receiving migrants from the coast, increasing its vulnerability.

5. ACKNOWLEDGEMENTS

6. REFERENCES

Adriaens, D., Jacquemyn, H., Honnay, O., & Hermy, M. (2009). Conservation of remnant
populations of *Colchicum autumnale* – The relative importance of local habitat quality and
habitat fragmentation. *Acta Oecologica*, *35*(1), 69–82.
https://doi.org/10.1016/j.actao.2008.08.003

Araújo, R. G., Silva, R. D. F., Sampaio, I., & Guimarães-Costa, A. (2019). Does DNA barcoding
offer meaningful insights into the diversity of the parrotfish of the genus *Sparisoma* (Scaridae)?
Journal of Applied Ichthyology, jai.13932. https://doi.org/10.1111/jai.13932

Assis, J., Tyberghein, L., Bosch, S., Verbruggen, H., Serrão, E. A., & De Clerck, O. (2018). Bio-
ORACLE v2.0: Extending marine data layers for bioclimatic modelling. *Global Ecology and
Biogeography*, *27*(3), 277–284. https://doi.org/10.1111/geb.12693

Burgess, S. C., Nickols, K. J., Griesemer, C. D., Barnett, L. A. K., Dedrick, A. G., Satterthwaite,
E. V., … Botsford, L. W. (2014). Beyond connectivity: how empirical methods can quantify
population persistence to improve marine protected-area design. *Ecological Applications*, *24*(2),
257–270. https://doi.org/10.1890/13-0710.1

Coscia, I., Wilmes, S. B., Ironside, J. E., Goward-Brown, A., O'Dea, E., Malham, S. K., ... &
Robins, P. E. (2019). Fine-scale seascape genomics of an exploited marine species, the common
cockle *Cerastoderma edule*, using a multi-modelling approach. *bioRxiv*, 724062.
https://doi.org/10.1101/724062

Costa, T. L. A., Pennino, M. G., & Mendes, L. F. (2017). Identifying ecological barriers in
marine environment: The case study of *Dasyatis marianae*. *Marine Environmental Research*,
125, 1–9. https://doi.org/10.1016/j.marenvres.2016.12.005

Costello, M. J., & Connor, D. W. (2019). Connectivity is generally not important for marine
reserve planning. *Trends in Ecology & Evolution*, *34*(8), 686–688.
https://doi.org/10.1016/j.tree.2019.04.015

Cowen, R. K. (1985). Large scale pattern of recruitment by the labrid, *Semicossyphus pulcher*:
Causes and implications. *Journal of Marine Research*, *43*(3), 719–742.
https://doi.org/10.1357/002224085788440376

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity.
Annual Review of Marine Science, *1*(1), 443–466.
https://doi.org/10.1146/annurev.marine.010908.163757

Cushman, S. A., McKelvey, K. S., Hayden, J., & Schwartz, M. K. (2006). Gene flow in complex
landscapes: testing multiple hypotheses with causal modeling. *The American Naturalist*, *168*(4),
486–499. https://doi.org/10.1086/506976

da Silva, R., Veneza, I., Sampaio, I., Araripe, J., Schneider, H., & Gomes, G. (2015). High levels of genetic connectivity among populations of Yellowtail snapper, *Ocyurus chrysurus* (Lutjanidae – Perciformes), in the Western South Atlantic revealed through multilocus analysis. *PLOS ONE*, *10*(3), e0122173. https://doi.org/10.1371/journal.pone.0122173

Dell'Apa, A., Pennino, M. G., & Bonzek, C. (2016). Modeling the habitat distribution of spiny dogfish (*Squalus acanthias*), by sex, in coastal waters of the northeastern United States. *Fishery Bulletin*, *115*(1), 89–100. https://doi.org/10.7755/FB.115.1.8

Dixo, M., Metzger, J. P., Morgante, J. S., & Zamudio, K. R. (2009). Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biological Conservation*, *142*(8), 1560-1569. https://doi.org/10.1016/j.biocon.2008.11.016

Dudley, N. (2008). *Guidelines for Applying Protected Area Management Categories*. Gland.

Endo, C. A. K., Gherardi, D. F. M., Pezzi, L. P., & Lima, L. N. (2019). Low connectivity compromises the conservation of reef fishes by marine protected areas in the tropical South Atlantic. *Scientific Reports*, *9*(1), 8634. https://doi.org/10.1038/s41598-019-45042-0

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Feitosa, J. L. L., & Ferreira, B. P. (2015). Distribution and feeding patterns of juvenile parrotfish on algal-dominated coral reefs. *Marine Ecology*, *36*(3), 462–474. https://doi.org/10.1111/maec.12154

Floeter, S. R., & Gasparini, J. L. (2000). The southwestern Atlantic reef fish fauna: Composition and zoogeographic patterns. *Journal of Fish Biology*, *56*(5), 1099–1114. https://doi.org/10.1006/jfbi.2000.1231

Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity*, *78*(3), 311–327. https://doi.org/10.1038/hdy.1997.46

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*(8), 925–929. https://doi.org/10.1111/2041-210X.12382

Froese, R., & Pauly, D. (2019). Fish Base.

Gelman, A. (2008). Scaling regression inputs by dividing by two standard deviations. *Statistics in Medicine*, *27*(15), 2865–2873. https://doi.org/10.1002/sim.3107

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: coupling genetics with fine-scale physical oceanography. *Ecology*, *84*(11), 2989–2998. https://doi.org/10.1890/02-0498

Gneiting, T., & Raftery, A. E. (2007). Strictly proper scoring rules, prediction, and estimation. *Journal of the American Statistical Association*, *102*(477), 359–378. https://doi.org/10.1198/016214506000001437

Gruber, B., Unmack, P. J., Berry, O. F., & Georges, A. (2018). dartR package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources*, *18*(3), 691–699. https://doi.org/10.1111/1755-0998.12745

Hanski, I. (1999). *Metapopulation Ecology*. Oxford University Press.

Hijmans, R. J., & van Etten, J. (2014). raster: Geographical data analysis and modeling. *R Package Version v.2*.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., … Hatziolos, M. E. (2007). Coral reefs under rapid climate change and ocean acidification. *Science*, *318*(5857), 1737–1742. https://doi.org/10.1126/science.1152509

Hughes, T. P. (2003). Climate change, human impacts, and the resilience of coral reefs. *Science*, *301*(5635), 929–933. https://doi.org/10.1126/science.1085046

Jaccoud, D., Peng, K., Feinstein, D., & Kilian, A. (2001). Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, *29*(4), e25. https://doi.org/10.1093/nar/29.4.e25

Johnson, M., & Black, R. (2006). Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. *Evolution*, *60*(12), 2498–2506. https://doi.org/10.1111/j.0014-3820.2006.tb01885.x

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: a better web interface. *Nucleic acids research*, *36*(suppl_2), W5-W9.

Jost, L. O. U. (2008). G_{ST} and its relatives do not measure differentiation. *Molecular ecology*, *17*(18), 4015-4026. https://doi.org/10.1111/j.1365-294X.2008.03887.x

Joyeux, J.-C., Floeter, S. R., Ferreira, C. E. L., & Gasparini, J. L. (2008). Biogeography of tropical reef fishes: The South Atlantic puzzle. *Journal of Biogeography*, *28*(7), 831–841. https://doi.org/10.1046/j.1365-2699.2001.00602.x

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: an R package for the estimation of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* 4: 782–788.

Leão, Z. M. A. N., & Kikuchi, R. K. P. (2001). *The Abrolhos Reefs of Brazil*. https://doi.org/10.1007/978-3-662-04482-7_7

Lessios, H., & Robertson, D. (2006). Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1598), 2201–2208. https://doi.org/10.1098/rspb.2006.3543

Levin, L. A. (2006). Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, *46*(3), 282–297. https://doi.org/10.1093/icb/icj024

Lima, L. S. (2019). *Conectividade demográfica e aclimatação de peixes recifais em cenário de mudanças climáticas*. INPE.

Lindgren, F., Rue, H., & Lindström, J. (2011). An explicit link between Gaussian fields and Gaussian Markov random fields: the stochastic partial differential equation approach. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*(4), 423–498. https://doi.org/10.1111/j.1467-9868.2011.00777.x

Lumpkin, R., & Garzoli, S. L. (2005). Near-surface circulation in the tropical Atlantic Ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, *52*(3), 495-518. https://doi.org/10.1016/j.dsr.2004.09.001

Macieira, R. M., Simon, T., Pimentel, C. R., & Joyeux, J. C. (2015). Isolation and speciation of tidepool fishes as a consequence of Quaternary sea-level fluctuations. *Environmental Biology of Fishes*, *98*(1), 385–393. https://doi.org/10.1007/s10641-014-0269-0

Magris, R. A., & Pressey, R. L. (2018). Marine Protected Areas: Just for show? *Science*, *360*(6390), 723.2-724. https://doi.org/10.1126/science.aat6215

Mazzei, E. F., Pinheiro, H. T., Morais, R. A., Floeter, S. R., Veras, D. P., Queiroz, L. V., … Ferreira, C. E. L. (2019). Parrotfishes of the genus *Scarus* in southwestern Atlantic oceanic reef environments: occasional pulse or initial colonization? *Marine Biodiversity*, *49*(1), 555–561. https://doi.org/10.1007/s12526-017-0827-8

McRae, B. H., & Beier, P. (2007). Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences*, *104*(50), 19885–19890. https://doi.org/10.1073/pnas.0706568104

MMA (2014) *Portaria MMA Nº 445, de 17 de dezembro de 2014*

Momigliano, P., Harcourt, R., Robbins, W. D., & Stow, A. (2015). Connectivity in grey reef sharks (*Carcharhinus amblyrhynchos*) determined using empirical and simulated genetic data. *Scientific reports*, 5, 13229. https://doi.org/10.1038/srep13229

Muñoz, F., Pennino, M. G., Conesa, D., López-Quílez, A., & Bellido, J. M. (2013). Estimation and prediction of the spatial occurrence of fish species using Bayesian latent Gaussian models. *Stochastic Environmental Research and Risk Assessment*, *27*(5), 1171–1180. https://doi.org/10.1007/s00477-012-0652-3

Pandolfi, J. M. (2003). Global trajectories of the long-term decline of coral reef ecosystems. *Science*, *301*(5635), 955–958. https://doi.org/10.1126/science.1085706

Pennino, M. G., Rufener, M.-C., Thomé-Souza, M. J. F., Carvalho, A. R., Lopes, P. F. M., & Sumaila, U. R. (2018). Searching for a compromise between biological and economic demands to protect vulnerable habitats. *Scientific Reports*, *8*(1), 7791. https://doi.org/10.1038/s41598-018-26130-z

Peterman, W. E. (2018). ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms. *Methods in Ecology and Evolution*, *9*(6), 1638–1647. https://doi.org/10.1111/2041-210X.12984

Pinheiro, H. T., Bernardi, G., Simon, T., Joyeux, J. C., Macieira, R. M., Gasparini, J. L., … Rocha, L. A. (2017). Island biogeography of marine organisms. *Nature*, *549*(7670), 82–85. https://doi.org/10.1038/nature23680

Pinheiro, H. T., Mazzei, E., Moura, R. L., Amado-Filho, G. M., Carvalho-Filho, A., Braga, A. C., … Joyeux, J.-C. (2015). Fish Biodiversity of the Vitória-Trindade Seamount Chain, Southwestern Atlantic: An Updated Database. *PLOS ONE*, *10*(3), e0118180. https://doi.org/10.1371/journal.pone.0118180

Pinheiro, H. T., Rocha, L. A., Macieira, R. M., Carvalho-Filho, A., Anderson, A. B., Bender, M. G., … Floeter, S. R. (2018). South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. *Diversity and Distributions*, *24*(7), 951–965. https://doi.org/10.1111/ddi.12729

Pinheiro, H. T., Martins, A. S., & Gasparini, J. L. (2010). Impact of commercial fishing on Trindade Island and Martin Vaz Archipelago, Brazil: characteristics, conservation status of the species involved and prospects for preservation. *Brazilian Archives of Biology and Technology*, *53*(6), 1417–1423. https://doi.org/10.1590/S1516-89132010000600018

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Quimpo, T. J. R., Cabaitan, P. C., Olavides, R. D. D., Dumalagan Jr., E. E., Munar, J., & Siringan, F. P. (2018). Spatial variability in reef-fish assemblages in shallow and upper mesophotic coral ecosystems in the Philippines. *Journal of Fish Biology*, jfb.13848. https://doi.org/10.1111/jfb.13848

R Core Team (2019). *R: A language and environment for statistical computing*.

Riginos, C., & Nachman, M. W. (2001). Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology*, *10*(6), 1439–1453. https://doi.org/10.1046/j.1365-294X.2001.01294.x

Robertson, D., Karg, F., Leão de Moura, R., Victor, B. C., & Bernardi, G. (2006). Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes. *Molecular Phylogenetics and Evolution*, *40*(3), 795–807. https://doi.org/10.1016/j.ympev.2006.04.011

Rodrigues, R. R., Rothstein, L. M., & Wimbush, M. (2007). Seasonal Variability of the South Equatorial Current Bifurcation in the Atlantic Ocean: A Numerical Study. *Journal of Physical Oceanography*, *37*(1), 16–30. https://doi.org/10.1175/JPO2983.1

Roos, N. C., Carvalho, A. R., Lopes, P. F. M., & Pennino, M. G. (2015). Modeling sensitive parrotfish (Labridae: Scarini) habitats along the Brazilian coast. *Marine Environmental Research*, *110*, 92–100. https://doi.org/10.1016/j.marenvres.2015.08.005

Rudorff, C. A. G., Lorenzzetti, J. A., Gherardi, D. F., & Lins-Oliveira, J. E. (2009). Application of remote sensing to the study of the pelagic spiny lobster larval transport in the tropical Atlantic. *Brazilian Journal of Oceanography*, *57*(1), 7-16. http://dx.doi.org/10.1590/S1679-87592009000100002

Rue, H., Martino, S., & Chopin, N. (2009). Approximate Bayesian inference for latent Gaussian models by using integrated nested Laplace approximations. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *71*(2), 319–392. https://doi.org/10.1111/j.1467-9868.2008.00700.x

Saenz-Agudelo, P., Dibattista, J. D., Piatek, M. J., Gaither, M. R., Harrison, H. B., Nanninga, G. B., & Berumen, M. L. (2015). Seascape genetics along environmental gradients in the Arabian Peninsula: insights from ddRAD sequencing of anemonefishes. Molecular ecology, 24(24), 6241-6255. https://doi.org/10.1111/mec.13471

Sbrocco, E. J., & Barber, P. H. (2013). MARSPEC: ocean climate layers for marine spatial ecology. *Ecology*, *94*(4), 979–979. https://doi.org/10.1890/12-1358.1

Selkoe, K. A., Aloia, C. C., Crandall, E. D., Iacchei, M., Liggins, L., Puritz, J. B., ... & Toonen, R. J. (2016). A decade of seascape genetics: contributions to basic and applied marine connectivity. *Marine Ecology Progress Series*, *554*, 1-19. https://doi.org/10.3354/meps11792

Silva-Oliveira, G. C., Rego, P., Schneider, H., Sampaio, I., & Vallinoto, M. (2008). Genetic characterisation of populations of the critically endangered Goliath grouper (*Epinephelus itajara*, Serranidae) from the Northern Brazilian coast through analyses of mtDNA. *Genetics and Molecular Biology*, *31*(4). http://dx.doi.org/10.1590/S1415-47572008005000016

Simon, T., Macieira, R. M., & Joyeux, J.-C. (2013). The shore fishes of the Trindade-Martin Vaz insular complex: an update. *Journal of Fish Biology*, *82*(6), 2113–2127. https://doi.org/10.1111/jfb.12126

Sponaugle, S., Cowen, R., Shanks, A., Morgan, S. G., Leis, J. M., Pineda, J.… Munro, J. L. (2002). Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. *Bulletin of Marine Science*, *70*(1), 341–375.

Thomas, A. L., Henderson, G. M., Deschamps, P., Yokoyama, Y., Mason, A. J., Bard, E., … Camoin, G. (2009). Penultimate deglacial sea-level timing from Uranium/Thorium dating of Tahitian corals. *Science*, *324*(5931), 1186–1189. https://doi.org/10.1126/science.1168754

Tillé, Y., & Matei, A. (2016). *sampling: Survey Sampling. R package version 2.8.*

Treml, E. A., Halpin, P. N., Urban, D. L., & Pratson, L. F. (2008). Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. *Landscape Ecology*, *23*(S1), 19–36. https://doi.org/10.1007/s10980-007-9138-y

Tyberghein, L., Verbruggen, H., Pauly, K., Troupin, C., Mineur, F., & De Clerck, O. (2012). Bio-ORACLE: a global environmental dataset for marine species distribution modelling. *Global Ecology and Biogeography*, *21*(2), 272–281. https://doi.org/10.1111/j.1466-8238.2011.00656.x

Watanabe, D., Nomura, M., Watanabe, K., Sakitani, N., Iwasawa, H., Takano, S., & Moriyama, H. (2015). Efficacy of cane and crutch in middle aged and older individuals: a systematic review and meta-analysis. *Physiotherapy*, *101*(April), e1606–e1607. https://doi.org/10.1016/j.physio.2015.03.1619

Weersing, K., & Toonen, R. (2009). Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*, *393*, 1–12. https://doi.org/10.3354/meps08287

Whitlock, M. C., & Lotterhos, K. E. (2015). Reliable detection of loci responsible for local adaptation: inference of a null model through trimming the distribution of Fst. *The American Naturalist*, *186*(S1), S24–S36. https://doi.org/10.1086/682949

7. SUPPLEMENTARY MATERIAL

Table S1. Reclassified values of rasters for Circuitscape analysis.

Bathymetry			Currents Velocity			Curvature		
From	To	Value	From	To	Value	From	To	Value
-6124	-5000	100	0.002732	0.04	1	0.002732	0.01	10
-5000	-4000	85	0.04	0.05	2	0.01	0.015	9
-4000	-950	60	0.05	0.06	3	0.015	0.03	8
-950	-900	55	0.06	0.1	8	0.03	0.05	7
-900	-850	50	0.1	0.4	9	0.05	0.08	6
-850	-800	45	0.4	0.5	10	0.08	0.1	5
-800	-750	40	0.5	0.6	11	0.1	0.3	4
-750	-700	35	0.6	0.9	12	0.3	0.5	3
-700	-650	30	0.9	1.196877	13	0.5	0.8	2
-650	-600	29				0.8	1.196877	1
-600	-550	28						
-550	-500	27						
-500	-450	26						
-450	-400	25						
-400	-350	24						
-350	-300	23						
-300	-250	22						
-250	-200	20						
-200	-150	19						
-150	-100	10						
-100	-50	9						
-50	-45	8						
-45	-40	7						
-40	-35	6						
-35	-30	5						
-30	-25	5						
-25	-20	4						
-20	-15	3						
-15	-10	2						
-10	-5	1						
-5	-1	0						

Table S2. Description of SNPs outliers identified by Outflank analysis.

Locus ID	He	FST	p-values	Analysis
38647324.38.G.A	0.471526	0.241035	0.001203	Outflank
38638701.15.A.G	0.35124	0.342985	4.22E-05	Outflank
38657762.22.G.T	0.344977	0.3164	0.000106	Outflank
38633868.67.G.A	0.297521	0.333804	4.82E-05	Outflank
38645610.17.G.A	0.275245	0.452833	4.82E-07	Outflank
38660991.60.G.A	0.21875	0.245738	0.001207	Outflank
38640598.24.A.G	0.192601	0.354591	1.58E-05	Outflank
38651072.6.G.A	0.192601	0.309762	8.12E-05	Outflank
38653692.9.T.C	0.192601	0.277949	0.00033	Outflank
38634945.65.G.T	0.192601	0.23375	0.002551	Outflank
38658223.36.G.A	0.174522	0.599814	1.09E-09	Outflank
38648768.52.T.A	0.183626	0.241403	0.001889	Outflank
38634194.27.T.C	0.174522	0.26877	0.000703	Outflank
38659355.24.C.T	0.165289	0.276509	0.000511	Outflank
38669619.28.T.C	0.155927	0.403429	2.43E-06	Outflank
38771743.12.C.G	0.136816	0.43749	5.38E-07	Outflank
38651003.20.G.C	0.136816	0.466452	2.62E-07	Outflank
38657615.47.C.T	0.136816	0.248073	0.000812	Outflank
38641964.24.C.A	0.127066	0.288849	0.000174	Outflank
38651783.14.C.T	0.127066	0.291469	0.000159	Outflank
38638581.65.G.A	0.127066	0.293866	0.000217	Outflank
38663644.32.A.T	0.117188	0.42114	1.18E-06	Outflank
38660376.5.G.T	0.10718	0.368133	1.21E-05	Outflank

38658152.54.A.G	0.465055	0.213364	0.002666	Outflank
38650951.48.G.C	0.425362	0.255703	0.001004	Outflank
38670252.21.A.G	0.420907	0.23447	0.002521	Outflank
38639071.37.G.A	0.366231	0.27939	0.000336	Outflank
38653058.23.T.A	0.325413	0.220239	0.002337	Outflank
38660996.19.C.T	0.297521	0.231256	0.001312	Outflank
38650467.27.C.T	0.267562	0.540792	1.25E-08	Outflank
38659398.45.T.A	0.2828	0.249262	0.00126	Outflank
38659807.15.G.A	0.275245	0.294072	0.000251	Outflank
38633314.45.C.A	0.251808	0.384899	7.93E-06	Outflank
38646715.59.A.T	0.21875	0.230175	0.002568	Outflank
38657927.58.T.C	0.192601	0.296261	0.000223	Outflank
38642768.35.A.G	0.174522	0.536232	1.32E-08	Outflank
38637152.56.G.A	0.147972	0.23957	0.001158	Outflank
38658319.35.T.C	0.147972	0.415768	1.95E-06	Outflank
38652576.20.G.A	0.138261	0.44852	5.13E-07	Outflank
38659265.52.C.T	0.127066	0.743307	2.83E-12	Outflank
38636125.50.T.C	0.128419	0.239209	0.001441	Outflank
38633786.52.T.C	0.117188	0.430344	8.30E-07	Outflank
38646260.39.C.T	0.118444	0.226141	0.002079	Outflank
38659748.5.T.A	0.117188	0.341354	5.57E-05	Outflank
38638549.54.G.A	0.108337	0.473108	1.38E-07	Outflank
38650532.64.G.C	0.10718	0.364228	8.64E-06	Outflank
38663202.48.G.A	0.377857	0.306004	0.000163	Outflank
38664655.8.T.C	0.328181	0.229034	0.001979	Outflank
38648774.54.C.T	0.128419	0.286436	0.000278	Outflank
38635719.9.G.A	0.108337	0.374596	1.53E-05	Outflank
38655259.35.T.A	0.417185	0.288261	0.000244	Outflank
38650276.58.T.C	0.397174	0.407301	2.58E-06	Outflank
38660577.17.G.A	0.40238	0.236828	0.002415	Outflank
38666681.10.G.A	0.337683	0.276749	0.000243	Outflank

38662822.29.C.A	0.187399	0.525121	1.60E-08	Outflank
38652077.61.C.T	0.1298	0.376287	6.88E-06	Outflank
38648155.11.A.G	0.282976	0.37357	7.47E-06	Outflank
38635427.43.C.A	0.250865	0.223615	0.002267	Outflank
38656552.54.A.G	0.18	0.598512	8.05E-10	Outflank
38649522.28.T.A	0.170519	0.286315	0.000251	Outflank
38654996.20.A.C	0.151142	0.741783	1.80E-12	Outflank
38649819.19.G.A	0.151142	0.406007	1.28E-06	Outflank
38655469.60.C.T	0.151142	0.247611	0.000415	Outflank
38651233.38.G.T	0.151142	0.526581	2.75E-08	Outflank
38771879.10.G.A	0.141246	0.24945	0.000723	Outflank
38650394.41.T.A	0.131211	0.304555	0.000212	Outflank

GENERAL CONCLUSION

Fishery management aims to ensure the long-term sustainability of fish stocks and to conserve vulnerable species and habitats. In this thesis, I integrated genetic methods, climate modeling and catch-based analysis to provide novel insight to the biology and conservation status of vulnerable species in Brazil. Some approaches applied here are new for the tropical marine environment, and have successfully filled gaps of knowledge that exist due to lack of data and investments in this region.

I analysed 137 species that are commercially fished in Brazil in order to better understand the sustainability of existing management. My results indicated that for some groups of species there will be negative effects from climate change. Vulnerability is associated with enhanced sensitivity to warming following a period of overexploitation. In addition, I show how environmental changes alter distributions, and to this end I evaluate the risk of isolation.

I identified multiple factors that increase the chance of stock collapse. In Chapter 1, using annual catch data for 132 species, climate models and data from across a 61-year period, I examined the external factors (fishery and climate) and intrinsic factors (biological traits) that influence vulnerability. First, based on historical catch data, the exploitation status for each species was placed into four categories: collapsed, overexploited, fully exploited, early stages of exploitation. Results indicate that species with small body sizes are more vulnerable to stock collapse. Another surprising result was the relationship between species that already have had large population declines and sea temperature. These species are also negatively affected by warming, while less vulnerable groups (overexploited, fully exploited and in development) were found to be positively affected by increases in temperature. Despite the need for a more detailed study of this effect, I suggest that the mechanism of increased vulnerability due to climatic variability is a consequence of reduced population size.

The goal of Chapter 2 was to identify a general pattern of genetic structure along the Brazilian coast, using sequence data from mitochondrial DNA for 17 commercially exploited species. The main conclusion was that for 15 species there was no indication of genetic structure, suggesting that these species

can be managed as single stocks. However, for two species, *Pomatomus saltatrix* and *Cynoscion jamaicensis*, I found some degree of genetic structure. For *P. saltatrix*, an environmental barrier could be the difference in temperature between north and south of Rio de Janeiro. For this species, it should be considered that there are at least two fish stocks in Brazil that should be managed independently. The results for *C. jamaicensis* suggest the presence of isolation by geographic distance.

To better understand how the past climate affects the current characteristics of marine fish populations in Brazil, more sensitive markers (SNPs) and Bayesian niche modeling were applied in Chapter 3. Due to the expense, only two species were analysed in this chapter: the dog snapper *Lutjanus jocu* and the grey parrotfish *Sparisoma axillare*. In this chapter, I also found a lack of genetic structure along the coast, despite the occurrence of one single isolated population of *S. axillare* at Trindade Island (Chapter 4). The main goal of this chapter was to identify the relationship between effective population size variability and past climate variability (Last Glacial Maximum – 21 thousand years ago – and Mid-Holocene – 6 thousand years ago). I concluded that both species had population expansions followed by stability, but during different periods. The analysis showed that *L. jocu* population was stable for hundreds of years, despite climate changes. *S. axillare* was stable for around 4 thousand years, and the population expansion can be related to increases in preferential areas after mid-Holocene. The results indicate that *S. axillare* can be more sensitive to changes in the environment, and this could be explained by its lower dispersal capacity. These results reinforce that *S. axillare* needs management strategies and protection, especially given overexploitation and the signal of a declining population leading to its classification as vulnerable by the Brazilian Environmental Ministry.

In the Chapter 4, the objective was to more deeply understand the connectivity between coastal and island reefs, using the parrotfish *Sparisoma axillare* as a model. The results indicate a high connectivity between almost all sites, including two islands (Fernando de Noronha and Abrolhos), with the exception of Trindade. Trindade Island is located more than 1 thousand kilometers from the coast of Espírito Santo, but it is often considered connected with the coast due to the presence of a submerged chain of seamounts, the Vitória-Trindade Chain. Other studies indicated a stepping-stone pattern of connection in this area (Joyeux et al., 2008; Simon et al., 2013). However, our results indicate that, for *S. axillare*, the chain is not acting

as a corridor. Besides the geographical distance, environmental characteristics – mainly depth and oceanic currents – have a role in limiting the connectivity between Trindade and coast. Also, results from distribution modeling suggest that it is possible that the isolated population of Trindade is going through a process of local adaptation.

This thesis highlights the complexity of the marine environment and contributes towards disentangling the many contemporary and historical factors that have shaped fish stock structure. In turn, this knowledge can be applied towards more effective measures of protection and management. This is especially needed in tropical waters, because factors relating to the sustainability of fisheries have received less attention than in temperate regions. Here, I suggest that smaller species, negatively affected by warmer temperatures should be a conservation focus in tropical regions. Also, special attention should be given to the parrotfish *Sparisoma axillare* due to its sensitivity to environmental change. The Trindade Island population of *S. axillare* should also be a conservation focus due to its isolation, and this is likely to be the reality for other species inhabiting the Trindade Island region.

REFERENCES

Begg, G. A., & Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fisheries Research*, *43*(1–3), 35–44. https://doi.org/10.1016/S0165-7836(99)00065-X

Joyeux, J.-C., Floeter, S. R., Ferreira, C. E. L., & Gasparini, J. L. (2008). Biogeography of tropical reef fishes: the South Atlantic puzzle. *Journal of Biogeography*, *28*(7), 831–841. https://doi.org/10.1046/j.1365-2699.2001.00602.x

Simon, T., Macieira, R. M., & Joyeux, J.-C. (2013). The shore fishes of the Trindade-Martin Vaz insular complex: an update. *Journal of Fish Biology*, *82*(6), 2113–2127. https://doi.org/10.1111/jfb.12126

ANNEX I

Other publications

Published scientific papers not directly related to this thesis

1. **Verba, J. T.**, Lima de Oliveira Borges, M., Ferreira da Silva, M. N., Costa Pinto, L., & Rabello Neto, J. G. (2018). Mice on menu: opportunistic feeding behaviour of the Amazonian silver arowana *Osteoglossum bicirrhosum*. *Journal of Fish Biology*, 93(1), 132-133. https://doi.org/10.1111/jfb.13665

2. Lopes, P. F., **Verba, J. T.**, Begossi, A., & Pennino, M. G. (2019). Predicting species distribution from fishers' local ecological knowledge: a new alternative for data-poor management. *Canadian Journal of Fisheries and Aquatic Sciences*, *76*(8), 1423-1431. https://doi.org/10.1139/cjfas-2018-0148

3. Farias, I. P., Willis, S., Leao, A., **Verba, J. T.**, Crossa, M., Foresti, F., Porto-Foresti, F, Sampaio, I., & Hrbek, T. (2019). The largest fish in the world's biggest river: Genetic connectivity and conservation of *Arapaima gigas* in the Amazon and Araguaia-Tocantins drainages. *PloS One*, *14*(8). https://doi.org/10.1371/journal.pone.0220882

JOURNAL OF **FISH** BIOLOGY

Mice on menu: opportunistic feeding behaviour of the Amazonian silver arowana *Osteoglossum bicirrhosum*

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Two silver arowana, *Osteoglossum bicirrhosum*, one male (49.5 cm standard length, L_S) and one female (52.5 cm L_S) that had fed on small-eared colilargo or small-eared pygmy rice rat *Oligoryzomys microtis*, were sampled during the rising water period in the Brazilian Amazon. The identified prey were 6 and 8 cm long and occupied the entire stomachs of both individuals. This opportunistic feeding behaviour appears to be a strategy for this fish to acquire nutrients during times when its usual aquatic prey is dispersed throughout the flooded forest.

KEYWORDS

allochthonous feeding, *bicirrhosum*, freshwater food web, *Oligoryzomys*, *Osteoglossum bicirrhosum*

The Amazon floodplains consist of an extensive area of flooded forests that are regulated by the flood pulse (Junk *et al.*, 1989). The water column can increase to > 20 m in some areas and remain that way for several months. This temporary flooding causes significant changes to local ecological processes as a result of aquatic environment expansion, thus creating new habitats to be explored by faunal communities (Winemiller & Jepsen, 1998). The flooded forests benefit fish for example, by providing a wide diversity of habitat options and decreasing the density of possible predators because of the expansion of suitable occupancy areas (Lowe-McConnell, 1999; Winemiller & Jepsen, 1998). With habitat expansion, not only does the density of predators decrease, but also the density of available prey. Hence, feeding can be more difficult for piscivorous animals. To compensate for this drawback, some carnivorous fish explore a high diversity of allochthonous food resources in flooded forest areas (Winemiller & Jepsen, 1998). The silver arowana *Osteoglossum bicirrhosum* (Cuvier 1829) is a good example of this. This species is an opportunistic generalist carnivorous predator that inhabits lakes and flooded forests in

the Amazon Basin (Castillo *et al.*, 2012; Saint-Paul *et al.*, 2000). *Osteoglossum bicirrhosum* hunts along lake borders and within flooded forests and it supplements its food consumption during the high-water period by leaping from the water to seek allochthonous prey, such as terrestrial invertebrates (Aragão, 1984; Goulding, 1989). This species can reach more than 1 m in length and weigh up to 3 kg.

The occasional consumption of non-volant small mammals by *O. bicirrhosum* was observed during sampling for a project on *O. bicirrhosum* mating systems and parentage (Verba *et al.*, 2014). Forty-five fish specimens were collected using a traditional harpoon during the rising water season between February and March 2011 in a lake and the surrounding flooded forest in the lower Purus River, Amazonas State, Brazil (04° 11′ 12.0″ S; 61° 57′ 56.4″ W). Among the sampled fish were one male (49.5 cm standard length, L_S) and one female (52.5 cm L_S) that presented abnormally large stomachs that caught the collectors' attention. As the objective of the sampling was not feeding analysis, only the stomachs of these two individuals were removed for content analysis. The consumption of two rodents by each individual was found.
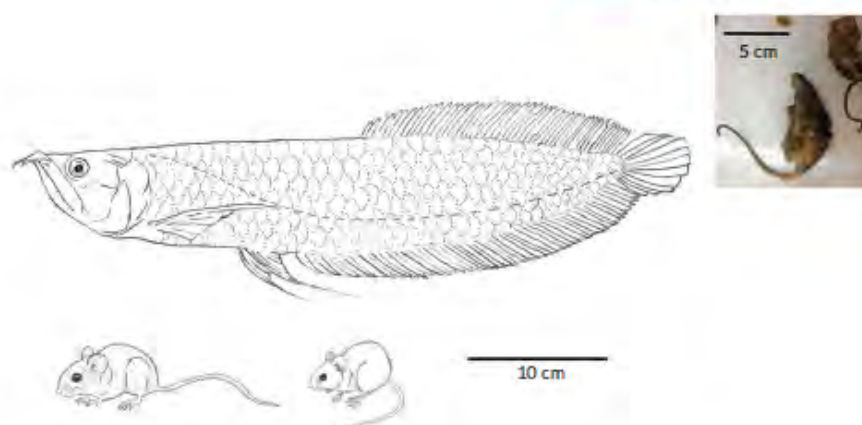
166

FIGURE 1 *Osteoglossum bicirrhosum* and two *Oligoryzomys microtis* found in an *O. bicirrhosum* stomach

The rodents found in the female individual stomach were 6 and 8 cm long (Figure 1). The rodents found in the male *O. bicirrhosum* stomach were in an advanced stage of decomposition and not measured. In both stomachs, the rodents occupied almost the entire space. Two of the four rodents that were in an early stage of decomposition were identified to species and deposited in the Instituto Nacional de Pesquisas da Amazônia, Manaus, mammal collection (INPA 7257 field#19/2011-1; INPA7258 field#19/2013-2). On the basis of external morphological characteristics and known geographical distribution, these rodents were identified as small-eared colilargo or small-eared pygmy rice rat *Oligoryzomys microtis*, a scansorial species (Paglia *et al.*, 2012) that climbs trees to explore the vegetation above the waterline in flooded forests (Emmons & Feer, 1997). It is abundant in dense grasses along river margins and on floating grass mats (Patton *et al.*, 2000).

Osteoglossum bicirrhosum is an important resource for artisanal fisheries in the Amazon. Some reproductive characteristics, such as its intense parental care and aggregations during care, may contribute to the species persistence despite intense exploitation. Additionally, their generalist feeding behaviour and capacity to exploit allochthonous food items during the high-water season may contribute to this adaptability (Lowry *et al.*, 2005). Future research to quantify the contribution of allochthonous food sources in the overall diet of this fish might provide valuable insights.

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REFERENCES

Aragão, L. P. (1984). Contribuição ao estudo da biologia do aruanã, *Osteoglossum bicirrhosum* Vandelli 1829, do lago Janauacá - estado do Amazonas, Brasil. I – Desenvolvimento e alimentação larval (Osteichtys – Osteoglossiformes). *Ciência Agronômica, 15*, 7–17.

Castillo, B. T. D., Marín, C. P., & Koo, F. C. (2012). Hábitos alimentícios de la Arahuana Osteoglossum bicirrhosum (Cuvier, 1829) en la Cuenca Medio del río Putumayio – el estrecho, Loreto, Perú. *Ciencia Amazónica (Iquitos), 2*, 17–26.

Emmons, L. H., & Feer, F. (1997). *Neotropical rainforest mammals: A field guide* (2nd ed.). Chicago, IL: University of Chicago Press.

Goulding, M. (1989). *The fishes and the forest: Explorations in Amazonian natural history*. Berkeley, CA: University of California Press.

Junk, W. J., Bayley, P. B., & Sparks, R. E. (1989). The flood pulse concept in river-floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences, 106*, 110–127.

Lowe-McConnell, R. H. (1999). *Estudos Ecológicos de Comunidades de Peixes Tropicais*. São Paulo, Brazil: Editora da Universidade de São Paulo (EDUSP).

Lowry, D., Wintzer, A. P., Matott, M. P., Whitenack, L. B., Huber, D. R., Dean, M., & Motta, P. J. (2005). Aerial and aquatic feeding in the silver arawana, Osteoglossum bicirrhosum. *Environmental Biology of Fishes, 73*, 453–462. https://doi.org/10.1007/s10641-005-3214-4

Paglia, A. P., da Fonseca, G. A., Rylands, A. B., Herrmann, G., Aguiar, L. M., Chiarello, A. G., ... Mendes, S. L. (2012). Lista anotada dos mamíferos do Brasil 2ª Edição annotated checklist of Brazilian mammals. *Occasional Papers in Conservation Biology, 6*, 76.

Patton, J. L., Da Silva, M. N. F., & Malcolm, J. R. (2000). Mammals of the Rio Juruá and the evolutionary and ecological diversification of Amazonia. *Bulletin of the American Museum of Natural History, 1*, 306. https://doi.org/10.1206/0003-0090(2000)244<0001:MOTRJA>2.0.CO

Saint-Paul, U., Zuanon, J., Correa, M. A. V., García, M., Fabré, N. N., Berger, U., & Junk, W. J. (2000). Fish communities in central Amazonian white-and Blackwater floodplains. *Environmental Biology of Fishes, 57*, 235–250. https://doi.org/10.1023/A:1007699130333

Verba, J. T., Neto, R., Gurgel, J., Zuanon, J., & Farias, I. (2014). Evidence of multiple paternity and cooperative parental care in the so called monogamous silver arowana Osteoglossum bicirrhosum (Osteoglossiformes: Osteoglossidae). *Neotropical Ichthyology, 12*, 145–151. https://doi.org/10.1590/S1679-62252014000100015

Winemiller, K. O., & Jepsen, D. B. (1998). Effects of seasonality and fish movement on tropical river food webs. *Journal of Fish Biology, 53*, 267–296. https://doi.org/10.1111/j.1095-8649.1998.tb01032.x

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Predicting species distribution from fishers' local ecological knowledge: an alternative for data-poor management

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19

Abstract

Many developing countries lack information to manage their endangered species, urging
the need for affordable and reliable information. We used Bayesian hierarchical spatial
models, with oceanographic variables, to predict the distribution range of *Epinephelus
marginatus*, the dusky grouper, for the entire Southwest Atlantic. We ran a model using
scientific information gathered from the literature, and another using information gathered
from fishers on species presence or absence. In both models, temperature was an important
determinant of species occurrence. The predicted occurrence of the dusky grouper
overlapped widely (Schoener's D= 0.71; Warren's I = 0.91) between the models, despite
small differences on the southern and northern extremes of the distribution. These results
suggest that basic information provided by fishers on species occurrence in their area can
be reliable enough to predict species occurrence over large scales and can be potentially
useful for marine spatial planning. Fishers' knowledge may be an even more viable
alternative to data collection than what was previously thought, for countries that both
struggle with financial limitations and have urgent conservation needs.

Introduction

One of the main obstacles that developing countries face to manage fisheries or assign a conservation status to target species is a lack of basic and reliable information on species, habitats and ecosystems (Kaufman et al. 2004; Mora et al. 2009; Houk et al. 2012; Worm and Branch 2012; Pauly and Zeller 2016). Without this information, species management is just a guessing game subject to failure (Hilborn 2007; Cinti et al. 2010; Finkbeiner and Basurto 2015). Lack of reliable information also opens the possibility for criticism, with resource users, for instance, opposing measures because they are not convinced that they are necessary or legitimate (Jentoft 2000; Lopes et al. 2013b; Di Dario et al. 2015). Precautionary approaches, which are usually advisable in such cases (Johannes 1998; Kaufman et al. 2004), are rarely implemented because of the high immediate political, social and economic costs associated with management (Marshall and Marshall 2007; Gaines et al. 2010). While the "burden of proof" remains unjustly on science rather than on users (Dayton 1998), managers and researchers need basic supporting data to justify the need for immediate management (Houk et al. 2012; Roos et al. 2015, 2016).

However, acquiring species, ecosystem or fisheries data in developing countries is proportionally costly, not only because funding for science and research is limited (science usually ranks low in government priorities) (Chao et al. 2015; Pinheiro et al. 2015), but also because such countries are burdened with bureaucracy, fear of biopiracy, lack of specific tax incentives and access to technologies, and neglect for resource use statistics (Barber et al. 2014; Pauly and Zeller 2016). In such a context, developing country researchers face the constant need to seek innovative affordable solutions to data acquisition and effective management implementation (Berkes 2003).

64   In fact, globally, two of the most successful management systems seem to be well-

65   established practices in the developing tropics: co-management and customary management

66   (Berkes 2003; Mora et al. 2009; Lopes et al. 2011; Cinner and Huchery 2014). These

67   solutions include users in both the decision-making and surveillance processes (Castello et

68   al. 2009) and rely on very little data (Castello, 2004).

69   Researchers have proposed that such knowledge and data gaps be filled with

70   information provided by fishers, based on their knowledge and experience, which is known

71   in the literature as fishers' local ecological knowledge - FEK or LEK (Johannes 1981;

72   Moller et al. 2004; Silvano and Valbo-Jorgensen 2008; Silvano and Begossi 2010). Since

73   the beginning, these studies have shown that fishers retain an amazing amount of accurate

74   information on fish biology and ecology, such as diet, reproduction, habitat preference, and

75   schooling habit of their target species (Neis et al. 1999; Silvano and Begossi 2010). More

76   recently, studies using LEK have demonstrated the potential to tap into fishers' knowledge

77   to reconstruct information on fish stocks and to detect stock declines unknown to science,

78   given the lack of past scientific data. With regards to the latter, older fishers provide

79   evidence for stock decline by reporting on their past catches (Tesfamichael et al. 2014;

80   Bender et al. 2014; Damasio et al. 2015).

81   Clearly, fishers' LEK is not taken for granted, as it indeed should not be (Davis and

82   Wagner 2003). Fishers' information, similar to any other information provided by humans,

83   can be distorted by their cultural and socioeconomic background and by their perception

84   and interpretation of reality (Jones et al. 2008; Ruddle and Davis 2011). For instance,

85   fishers may attribute a strong predatory role to a given species if they think it competes

86   with them for valuable prey (Davis et al. 2004). They can also overemphasize the role of

87   certain food items on fish diets, if the food item is used as bait (Begossi et al. 2016).

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88 Moreover, fishers may recollect their most positive memories (largest catch or largest fish)

89 much better than their average day-to-day fishing success (Damasio et al. 2015). The role

90 of research is to identify the information that is most applicable to management (Silvano

91 and Valbo-Jorgensen 2008).

92   Taking this a step further, recent studies have started to test the usefulness of LEK

93 data in ecosystems modeling (Anadón et al. 2010; Eddy et al. 2010). Although less detailed

94 than traditional scientific modeling, LEK-based models can provide some of the required

95 information for proper ecosystem management (Bevilacqua et al. 2016). It is known that

96 fishers can identify areas in their own region where target species are, however, it remains

97 to be tested whether fishers' LEK can be used to predict species occurrence (niche

98 distribution). (Begossi et al. 2013). Although basic, the prediction of species occurrence

99 may represent non-existent information for certain areas and for certain species (Roos et al.

100 2015). Knowing species–environment relationships and their suitable habitats could

101 contribute to the implementation of marine spatial planning and, if necessary, the protection

102 of relevant habitats for the conservation of some species (Agardy et al. 2011; Pennino et al.

103 2016). Habitat and species mapping are especially relevant for conservation programs for

104 threatened or endangered species because they provide clear pictures of their distribution,

105 and thus facilitate management of the marine environment (Roos et al. 2015).

106   Within this context, we modeled the distribution of *Epinephelus maginatus* (Lowe

107 1834), the dusky grouper, along the Southern Atlantic coast (South American coast). This

108 species is economically relevant, has been widely exploited in Europe, Africa and South

109 America, has been extensively studied in the Mediterranean (e.g., Bouchereau et al. 1999;

110 Gilles et al. 2000; Andrello et al. 2013), and is considered endangered by the IUCN Red

111 List. In the South Atlantic the species is much less known (Machado et al. 2003, 2008;

112   Andrade et al. 2003; Irigoyen et al. 2005). However, some studies have already suggested

113   that in the Southern Atlantic *E. marginatus* has a smaller effective population size

114   compared to its Mediterranean counterpart (Priolli et al. 2016), and that fishers have

115   noticed significant declines in their catches over time (Lopes et al. 2013a). We also

116   compared the distribution prediction generated from traditional scientific data to the one

117   generated from information provided by fishers. Our intention was to provide alternative

118   basic, reliable and cheap information for spatial planning and management of fish species

119   that can be applied worldwide, particularly in areas where there is no other source of

120   information available.

121

122   **2. Material and methods**

123   *Scientific data*

124   The scientific data used here to establish where *E. marginatus* was present or absent

125   along the south Atlantic coast came from three different sources: 1) our own data collected

126   through multiple projects, 2) bibliographic sources, and 3) online databases that provide

127   data on species presence and absence (all sources are in Supplement S1). With respect to

128   our own data, we used information present in our databases for the period 1988-2016,

129   combined with geographical location of fishing grounds, which are published elsewhere

130   (Begossi et al. 2013). With the exception of the period 2013-2016, when our research was

131   specifically designed to sample and study *E. marginatus,* the remaining studies concerned

132   small-scale fisheries in general, and we simply registered the presence of the dusky grouper

133   whenever available in the landings (sometimes in numbers, sometimes in kilograms,

134   sometimes just whether or not it was present). This means that the occurrence of *E.*

135   *marginatus* may have been registered in a given site (fishing grounds) multiple times, as

136 our data is fishing data and not scientific sampling data. As most of our research to date has

137 covered a specific part of the extensive Brazilian coast, we also carried out an exhaustive

138 bibliographic review of previous research to account for existing information about geo-

139 referenced occurrences or absences of *E. marginatus* in all South American waters

140 (Supplement S1). The studies we identified were mostly scientific samplings that registered

141 the presence of the species in a given site, using methods such as transects; where the

142 researcher samples multiples areas near one another. For those cases, we randomly took

143 one of the sampled locations and assigned the species presence (or absence) to it. To make

144 sure we included all available information, we extracted presence data from online

145 databases (Kaschner et al. 2013) and excluded duplicate observations. We then combined

146 the three data sources pertaining to the presence or absence of *E. marginatus* into a single

147 presence-absence dataset to perform a Bayesian spatial model with a binomial distribution.

148   Overall, we registered 826 suitable observations to run in the model (Figure 1a):

149 578 presences (356 from our own data, six presences from the AQUAMAPS database, and

150 216 presences from the bibliographic sources) and 26 absences (all from the bibliographic

151 sources). Of the presence data, 43 were unique presences (registered just once).

152 *Ethnobiological data*

153   Following the same procedure used for the scientific datasets, an exhaustive

154 literature review of previous studies was performed to collect occurrence information for all

155 South American waters that were provided by fishers (Supplement S1). This information

156 was usually obtained through interviews when fishers were asked if *E. marginatus* was

157 present in their region. As these studies relied on interviews, no geo-referenced information

158 was available. To account for an approximate location, we estimated the presence or

159 absence of the species in a coastal point close to the village where the interview took place

160 (average of 120 meters away from the shore).

161   We recorded a total of 60 observations, of which 37 referred to presences and 23 to

162 absences of *E. marginatus* (Figure 1b).

163 *Environmental data*

164   Overall, six environmental variables were considered to be potential predictors of *E.*

165 *marginatus* distribution, both for the scientific and for the ethnobiological data: sea surface

166 temperature (SST in °C), sea surface salinity (SSS in PSU), net primary productivity (NPP

167 in mg C m^{-2} d^{-1}), depth (in meters), slope (% grade) and concavity of the seabed. The latter

168 variable is not a physical unit, but the second derivative of the bathymetry layer (or the

169 slope of the slope) and represents whether a raster cell is on a hill (negative values) or in a

170 valley (positive values). These variables were chosen as they had previously been

171 associated with the presence of either the same species or a related species (Harmelin and

172 Harmelin-Vivien 1999; Andrello et al. 2013).

173   SST and SSS were both extracted with a spatial resolution of 1° x 1° (~ 100 km)

174 from NODS_WOA09 as long-term monthly climatological means provided by the

175 NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their web site at

176 http://www.esrl.noaa.gov/psd/.

177   Net primary productivity (NPP) was retrieved from a 920 x 1680 global grid of NPP

178 and calculated as a function of chlorophyll, available light, and photosynthetic efficiency

179 using the entire SeaWIFS chlorophyll record (1998-2016) on the Ocean Productivity

180 website with a spatial resolution of 1° x 1°

181 (http://www.science.oregonstate.edu/ocean.productivity/index.php).

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182   Bathymetric   features   were   derived   from   the   MARSPEC   database,

183   (http://www.marspec.org), which is a world ocean dataset with a spatial resolution of 1km x

184   1 km (30 arc-second) developed for marine spatial ecology (Sbrocco and Barber 2013).

185   To ensure the same spatial resolution, all environmental data were aggregated to the

186   lowest spatial resolution common to them ($1° \times 1°$), using the "raster" package in R

187   (Hijmans et al. 2016) to avoid downscaling. This means that more detailed information,

188   when available (e.g., slope), was retained. All environmental variables were standardized in

189   order to enable relative weight comparisons between variables (Kinas and Andrade 2014).

190   Multicollinearity was checked using Pearson's correlation index. Other typical data

191   exploration procedures were routinely done according to Zuur et al. (2010).

192   High correlations were found between SSS and SST ($r = 0.79$), NNP and SSS ($r = $ -

193   0.91) and NPP and SST ($r = 0.69$). Consequently, these variables were introduced

194   separately in the different models.

195   *Sampling uncertainties*

196   As data were collected from different sources, a potential additional origin of

197   variation on the species occurrence could be due to the observer effect, especially for the

198   observations collected from the literature, from AQUAMAPS, and from fishers for whom

199   we do not have any additional sampling information. The behavior of individual observers

200   caused by random aspects, and/or unobserved sampling characteristics could have caused

201   some of the variation in the data. Ignoring such non-independence of the data can lead to an

202   invalid statistical inference (Roos et al. 2015; Costa et al. 2017). To remove any bias caused

203   by observer-specific differences in the sampling process, an observer effect was added to

204   the models (both for the scientific and ethnobiological datasets) as a random effect because

205   there was no interest in knowing the specific nature of the observers.

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206   *Statistical models*

207   The probability of *E. marginatus* occurrence (for both the scientific and

208   ethnobiological data) was modeled using Bayesian Gaussian iCAR (intrinsic conditional

209   autoregressive model) to take into account spatial autocorrelation (Latimer et al. 2006) and

210   different sources of uncertainties. Bayesian species distribution models are especially

211   suitable for data limited situations (Costa et al. 2017). We used a Gaussian iCAR (Besag

212   1974) to account for any eventual spatial autocorrelation between observations, assuming

213   that the probability of species presence at one site depends on the probability of species

214   presence in neighboring sites. Assuming the random variable Y_i follows a Bernoulli

215   distribution, it can take on a value of 1 or 0 depending on whether the habitat is suitable (Y_i

216   $= 1$) or not ($Y_i = 0$), then:

217 $$Y_i \sim \text{Bernoulli}(\pi_i)$$

218 $$\text{logit}(\pi_i) = X_i\beta + Z_i + \rho_{j(i)}$$

219   where β represents the vector of the regression coefficients, X is the matrix of covariates, Z_i

220   is the observer random effect, and ρ represents the spatial random effect for any given cell

221   j. The relation between π_i, the covariates of interest and spatial effect is the usual logit link.

222   We used uninformative priors centered at zero with a fixed large variance of 100 for

223   all parameters involved in the model and adopted a uniform distribution for the variance of

224   the spatial effect.

225   We fit these models using the "hSDM" package (Vieilledent et al. 2014) of the R

226   statistical environment (R Development Team 2017).

227   We fit the resulting models obtained by combining the mentioned variables and the

228   respective interactions and compared them using both backwards and forwards approaches

229　using the standard Deviance Information Criterion (DIC) (Spiegelhalter et al. 2002). Lower

230　values of DIC represent better compromises between fit and parsimony.

231　　　　Finally, in order to plot the functional response between the selected environmental

232　variables and the predicted values, we used the "ggplot" package of the R software to apply

233　a smoothing function to attempt to capture the general patterns in the spatial trend of the

234　species, while also reducing the noise. This technique uses locally weighted scatterplot

235　smoothing (lowess), which is an outlier-resistant method that estimates a polynomial

236　regression curve by using local and bootstrap techniques with the percentile method in

237　order to account for the variability in the original lowess fit. With this methodology, each

238　functional response shows a 95% confidence interval for the original lowess.

239　　　　*Model validation for occurrence*

240　　　　We validated the best selected model by using an internal 10-fold cross validation

241　based on randomly selected training and test datasets (created by a random selection of

242　75% and 25% of the data respectively) (Fielding and Bell 1997), with the

243　"*PresenceAbsence*" package in R (Freeman and Moisen 2008). We assessed the model's

244　performance by using the area under the receiver-operating characteristic curve (AUC)

245　(Fielding and Bell 1997) and the "True Skill Statistic" (TSS) (Allouche et al. 2006).

246　*Comparison of model predictions*

247　　　　We compared the predictions of *E. marginatus* distribution from the scientific and

248　ethno datasets using the similarity statistics Shoener's D and Warren's I (Warren et al.

249　2008). These statistics range from 0 (no overlap between areas) to 1 (distributions are

250　identical) (Pennino et al. 2016; 2017). We carried out these analyses using the

251　*nicheOverlap* function of the *dismo* package (Hijmans et al. 2011) in R software.

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253   **Results**

254   *Scientific data*

255   The occurrence of *E. marginatus* seems to be primarily determined by the depth,

256   slope, and SST of the habitat, in addition to the spatial component that accounted for the

257   residual spatial autocorrelation and the observer random effect (Table 1). The species is

258   more likely to be present in shallow ($\cong$300-400 m, posterior mean = 3.71; 95% CI = [1.99,

259   5.64]) and cooler waters (11-25° C max, posterior mean = -4.63; 95% CI = [-8.10, -1.51])

260   (Figure 2a - c). Although the species seems to be present in areas ranging from sandy (low

261   slope values) to rocky bottoms (high slope values), it showed a marked preference for a

262   structured (rocky in most cases of the study area) seabed (slope value preference = 75-90;

263   posterior mean = 0.18; 95% CI = [0.10, 0.99]), demonstrated by the highest occurrence

264   probability at higher slope values (Figure 2b).

265   The best conditions for species occurrence in South America were met in shallow

266   and structured grounds, with slightly cooler waters than those found in the north (observed

267   by plotting the median posterior occurrence probability), specifically between the

268   southeastern coast of Brazil (state of Espírito Santo) and Rio de Plata in Uruguay (Figure

269   3). The expected occurrence of *E. marginatus* in the north of Argentinian Patagonia was

270   driven by three reports of its presence in the literature, but there are few observations here

271   and the presences could be rare events.

272   *Ethnobiological data*

273   Based on the ethnobiological dataset, the main predictors of *E. marginatus* habitats

274   were depth and SST, along with the spatial component that accounted for the residual

275   spatial autocorrelation and the observer random effect (Table 2). In this specific case, slope

276   was not relevant. This was expected given that the occurrence locations were assigned to

277   the ocean grid point closest to the reporting village, therefore disregarding the local type of

278   seabed.

279   According to the presence data credited to the fishers, *E. marginatus* was also

280   expected to occur in shallow (posterior mean = 3.49; 95% CI = [2.35, 7.10]) and cooler

281   waters (posterior mean = -5.24; 95% CI = [-7.48, -0.22]). However, given that species

282   presence or absence was assigned to the ocean grid point closest to the reporting village in

283   our study, i.e., areas which are usually shallow, the actual most relevant information is the

284   effect of water temperature (Figure 4). Higher occurrence probabilities were found at a

285   depth range of ≅250-450 m (Figure 4a). Interestingly, the species occurrence probability

286   was shown to be most likely at a temperature range of between 11° C and 25° C, which is

287   the exact range predicted from the scientific dataset (Figure 4 b).

288   The expected geographical distribution of *E. marginatus* in South America suggests

289   a wider distribution pattern along the Brazilian coast than the one we observed in the

290   scientific database (based on its median posterior probability). For instance, based on the

291   ethnobiological data, the species is expected to occur further up north, especially between

292   the northeastern Brazilian state of Sergipe and the southern state of Santa Catarina (Figure

293   5).

294   *Model performance and comparisons*

295   For both datasets, all the best models achieved AUC values of >0.65, which

296   indicates a good degree of discrimination between the locations where a species is present

297   and where it is absent. For the scientific dataset the AUC value was 0.83, whereas this

298   value was slightly lower for the ethnobiological dataset (0.73). Similarly, a higher TSS

299   value was achieved using the scientific dataset (0.74 versus 0.67). However, again both

300   datasets achieved values higher than 0.60, indicating a good ability for the models to

301   predict real absence and presence records for *E. marginatus*.

302   Interestingly, the predictions generated from both models showed an excellent

303   degree of overlap, suggesting high similarity between them (Schoener's D= 0.71; Warren's

304   I = 0.91).

305

306   Discussion

307   This study highlights two major points of relevance: (i) it is the first reliable

308   occurrence prediction of the dusky grouper *E. marginatus* carried out using a state-of-the-

309   art species distribution model (Costa et al. 2017), (ii) and it provides evidence that such

310   models can be built from data-poor situations, such as when the only information available

311   is that provided by fishers.

312   Similar to what has been shown in the Mediterranean, where *E. marginatus* has

313   been widely studied, in our study we also expected that the species would have a strong

314   preference for sub-tropical shallower waters, and for habitats with complex topography

315   (Mesa et al. 2002). Whereas in well studied parts of the Mediterranean the species has been

316   shown to prefer areas with a depth of up to 50 m (Heemstra and Randall 1993), our model

317   of the Southwest Atlantic waters suggested a higher likelihood of its occurrence in areas

318   with a depth of between 300 - 400 m. Although different *E. marginatus* populations

319   (Mediterranean x Southwest Atlantic) might be suited to different habitats, we cannot rule

320   out the possibility that overfishing or coastal degradation in the Southwest Atlantic plays a

321   role (Bruslé 1985; Morris et al. 2000; Begossi et al. 2012), thus making it harder to find

322   adults in shallower waters. Even in the Mediterranean the species can be found in deeper

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323   waters (up to 250 m), especially adults (Harmelin and Harmelin-Vivien 1999). We did not

324   have information on individual size, which may have supported the depth pattern observed

325   in the Mediterranean. Still, the habitat suitability comparison between regions is a rough

326   attempt, since this is the first prediction ever made for this species using similar models.

327   What is mainly innovative here is the suggestion that mere information provided by

328   geographically dispersed fishers on whether they catch this species in their customary

329   fishing grounds or not is enough to predict species distribution and achieve results similar

330   to those made with scientific data. This achievement is especially relevant given our

331   sampling limitations. For instance, our technique of allocating a random spot right across

332   from each fishing village where species occurrence was mentioned, to represent where the

333   species is caught, is an important caveat, because we did not consider ground specificities.

334   Clearly fishers go to specific grounds, such as rocky outcrops, to catch the dusky grouper

335   (Begossi and Silvano 2008; Begossi et al. 2012), but this information was not available in

336   most of the papers we reviewed. Nevertheless, even considering this limitation, the

337   predictive model was accurate enough to show between 71% and 91% of overlap with its

338   counterpart scientific model, in addition to performing well as a reliable predictive model

339   (see values of AUC and TSS). Similar to the scientific model, the ethnobiological model

340   also predicted *E. marginatus* occurrences in cooler and shallower waters. However, depth

341   in this case was just an artifact of our method of assigning a random point in the water to

342   represent its occurrence. On the other hand, for a species that seems to have a well-

343   established temperature range (Hereu et al. 2006), the simple information of village

344   location works as an accurate predictor of expected species occurrence, especially because

345   small-scale fishers do not venture to distant grounds with their small vessels.

346   Although overall the expected distribution was the same for both methods, there are

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347   some differences worth investigating. The first is the extended expected distribution down

348   south, towards Argentinian Patagonia, which is observed only in the scientific modeling

349   approach. We considered species occurrence along the southern coast of Brazil, close to the

350   mouth of Rio de la Plata in Uruguay, and in Argentinian Patagonian due to three reports

351   that expanded the geographical limit of the species occurrence in the last decades (Irigoyen

352   et al. 2005; Condini et al. 2016). The species is by no means common in those waters,

353   although the existence of suitable habitats in the region might represent a future

354   geographical range in a warming world (Perry et al. 2005). However, the ethnobiological

355   model could not predict *E. marginatus* occurrence south of Brazil because no study has

356   ever approached Uruguayan and Argentinian fishers about species occurrence in that

357   region.

358   Another striking difference between the two models was that the ethnobiological

359   model predicted the expected occurrence of the dusky grouper to be at the southern tip of

360   the northeastern Brazilian coast, which is north of what the scientific model predicted. With

361   the exception of three identical reports from the online databases that attributed the

362   presence of *E. marginatus* to the southern part of the Brazilian northeast, the scientific

363   model did not predict the northeast region to house the species (although there were some

364   reports of its presence in an offshore location in the north). In specific ethnobiological

365   studies designed to confirm its existence in the north with fishers, no such confirmation

366   was obtained above -12° degrees of latitude (Ribeiro 2016). However, the region between -

367   15° and -12° degrees seems to be a grayish zone, with some fishers reporting its occurrence

368   (Silvano et al. 2006). The northern limit of the *E. marginatus* distribution established from

369   information given by fishers seems to be close to a geographical barrier for some species,

370   such as some of the Dasyatidae rays (Costa et al. 2017). One possible explanation has to do

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371 with the San Francisco river; a large warm river that could be working as a barrier for

372 multiple species for various reasons, such as changes in temperature (Costa et al. 2017).

373 For *E. marginatus*, for instance, the warm river waters may make the northern coast unfit

374 for it. This break also broadly coincides with the breaks between the ecoregions of

375 Spalding et al. (2007).

376 However, it is not possible to rule out the possibility that fishers may have been

377 mistaken, confounding *E. marginatus* with another similar grouper that does occur in the

378 region, *E. morio* (Valenciennes, 1828) (Costa et al. 2003; Begossi et al. 2012). Another

379 possibility is that the fishers that mentioned the occurrence of *E. marginatus* north of its

380 known distribution are those relatively close to the expected distribution limit of this

381 species. They could be fishers that encounter the grouper when fishing slightly down south.

382 Although small-scale fishers in Brazil are not really mobile, performing mostly daily

383 fishing trips close to their villages (Lopes et al. 2009), some cross state borders to fish in

384 other regions (Silva, pers. comm.). This could represent an opportunity to come across

385 species not usually found close to their home villages. Finally, we should not disregard the

386 species' past presence in the area either, perhaps in lower abundances that were either

387 overfished or dislodged with warmer waters over the last decades.

388 It is also important to take into account larval dispersal distances where predictions

389 differed slightly between models. For the Mediterranean it is estimated that the median

390 distance for larval dispersal is 120km, but a maximum distance of 906 km is also possible

391 (Andrello et al. 2013). If larvae reach such distances, we could expect some sporadic

392 occurrences of the species outside its standard geographical limits when the larvae

393 eventually find some intermittent favorable conditions to settle. That would possibly allow

394 the maturation of a few extralimital adults beyond the species range, although not enough

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395   to form a viable fishing population.

396   Overall, this study points towards a promising low-cost approach (LEK) to provide

397   baseline data and to fill knowledge gaps to support not only fisheries management but also

398   marine conservation initiatives, such as marine spatial planning. Regardless if it is to be

399   used alone or as part of an integrative approach, we advise future studies on fishers' local

400   ecological knowledge to compile data on species occurrence per fishing ground with their

401   respective locations (e.g., Begossi et al. 2013). We also recommend future studies to be

402   specifically designed to collect LEK fish abundance data to support its reliability as a

403   conservation tool (Anadón et al. 2010). Finally, it is worth mentioning that this study also

404   shows that the integration of multiple sources of information into science and management

405   is a potentially invaluable tool. Such tool should not be overlooked, despite the limitations

406   of integrating information obtained through different sampling designs, which limit the

407   statistical methods that can be used. Indeed, further statistical advancements should work

408   towards developing single models that can account for all the variability that emerges

409   from combining data collected with different sampling techniques over distinct temporal

410   and spatial coverage. The Bayesian approach may provide the answer to combine datasets

411   from different sampling schemes while maintaining a sound statistical framework.

412   In conclusion, this is the first study to predict *E. marginatus* occurrence along the South

413   American coast, which is highly relevant given the threatened state of the species and its

414   importance as a fishing target (Sadovy de Mitcheson et al. 2013). Our study could also possibly be

415   hinting at an expected future distribution towards the southern cooler waters in the future (Perry

416   et al. 2005), by confirming the existence of suitable habitats close to Patagonia. Moreover, our

417   study demonstrated that with little data, provided by fishers, it is possible to get accurate species

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418 distributions at least for species limited by more general aspects such as temperature and

419 salinity, which are not expected to vary greatly between fishing grounds. For species whose

420 occurrence are affected by different requirements, more specific ethnobiological knowledge

421 might be necessary, whereas fishers may not be able to provide accurate information for species

422 they do not target regularly. Nevertheless, sampling ethnobiological data to predict the

423 distribution of target species (or species with which people interact regularly) is affordable and

424 allows the coverage of large geographical areas, which may be a considerable advancement for

425 data-poor countries with urgent needs for proper fisheries management. Ethnobiological data

426 can also be incorporated into integrative and potentially more informative approaches to

427 advance marine spatial planning and conservation in general.

428

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434

References

436 Agardy, T., di Sciara, G. N., and Christie, P. 2011. Mind the gap: Addressing the shortcomings of
437 marine protected areas through large scale marine spatial planning. Marine Policy 35:226–232.

438 Allouche, O., Tsoar, A., and Kadmon, R. 2006. Assessing the accuracy of species distribution
439 models: prevalence, kappa and the true skill statistic (TSS). Journal of Applied Ecology 43:1223–
440 1232.

441 Anadón, J. D., Giménez, A., and Ballestar, R. 2010. Linking local ecological knowledge and habitat
442 modelling to predict absolute species abundance on large scales. Biodiversity and Conservation
443 19:1443–1454.

187

444 Andrade, Á. B., Machado, L. F., Hostim-Silva, M., and Barreiros, J. P. 2003. Reproductive biology of
445 the dusky grouper Epinephelus marginatus (Lowe, 1834). Brazilian Archives of Biology and
446 Technology 46:373–382.

447 Andrello, M., Mouillot, D., Beuvier, J., Albouy, C., Thuiller, W., and Manel, S. 2013. Low
448 Connectivity between Mediterranean Marine Protected Areas: A Biophysical Modeling Approach
449 for the Dusky Grouper Epinephelus marginatus. PLOS ONE 8:e68564.

450 Barber, P. H., Ablan-Lagman, M. C. A., Ambariyanto, Berlinck, R. G. , Cahyani, D., Crandall, E.D.,
451 Ravago-Gotanco, R., Juinio-Meñez, M.A., Mahardika, I. N., Shanker, K., Starger, C.J, Toha, A. H. A.,
452 Anggoro, A. W., and Willette, D. A. 2014. Advancing biodiversity research in developing countries:
453 the need for changing paradigms. Bulletin of Marine Science 90:187–210.

454 Begossi, A., Camargo, E., and Carpi Jr, S. 2013. Os mapas da pesca artesanal: pesqueiros e
455 pescadores na costa do Brasil. Editora Rima, São Carlos.

456 Begossi, A., Lopes, P. F. M., and Silvano, R. A. M. 2012. Co-Management of Reef Fisheries of the
457 Snapper-Grouper Complex in a Human Ecological Context in Brazil. Pages 1–22 Global Progress in
458 Ecosystem-Based Fisheries Management. Alaska Sea Grant, University of Alaska Fairbanks, Alaska.

459 Begossi, A., Salivonchyk, S., Lopes, P. F. M., and Silvano, R. A. M. 2016. Fishers' knowledge on the
460 coast of Brazil. Journal of Ethnobiology and Ethnomedicine 12:20.

461 Begossi, A., and Silvano, R. A. M. 2008. Ecology and Ethnoecology of dusky grouper, garoupa,
462 [Epinephelus marginatus (Lowe, 1834)] along the coast of Brazi. Journal of Ethnobiology and
463 Ethnomedicine 4:1–20.

464 Bender, M. G., Machado, G. R., Silva, P. J. de A., Floeter, S. R., Monteiro-Netto, C., Luiz, O. J., and
465 Ferreira, C. E. L. 2014. Local Ecological Knowledge and Scientific Data Reveal Overexploitation by
466 Multigear Artisanal Fisheries in the Southwestern Atlantic. PLoS ONE 9:e110332.

467 Berkes, F. 2003. Alternatives to conventional management: lessons from small-scale fisheries.
468 Environments 31:5–19.

469 Besag, L. 1974. Spatial interaction and the statistical analysis of Lattice systems. Journal of the
470 Royal Statistical Society. Series B (Methodological) 36:192–236.

471 Bevilacqua, A. H. V., Carvalho, A. R., Angelini, R., and Christensen, V. 2016. More than anecdotes:
472 fishers' ecological knowledge can fill gaps for ecosystem modeling. PLOS ONE 11:e0155655.

473 Bouchereau, J. L., Body, P., and Chauvet, C. 1999. Growth of the dusky grouper Epinephelus
474 marginatus (Linnnaeus, 1758) (Teleostei, Serranidae), in the Natural Marine Reserve of Lavezzi
475 Islands, Corsica, France. Scientia Marina 63:71–77.

476 Bruslé, J. 1985. Exposé synoptique des données biologiques sur les mérous Epinephelus aeneus
477 (Geoffroy Saint Hilaire, 1809) et Epinephelus guaza (Linnaeus, 1758) de l'Océan Atlantique et de
478 la Méditerranée. FAO Syn. Pêches 129:1–64.

479 Castello, L., Viana, J. P., Watkins, G., Pinedo-Vasquez, M., and Luzadis, V. A. 2009. Lessons from
480 integrating fishers of arapaima in small-scale fisheries management at the Mamirauá Reserve,
481 Amazon. Environmental Management 43:197–209.

482 Chao, N. L., Frédou, F. L., Haimovici, M., Peres, M. B., Polidoro, B., Raseira, M., Subirá, R., and
483 Carpenter, K. 2015. A popular and potentially sustainable fishery resource under pressure–
484 extinction risk and conservation of Brazilian Sciaenidae (Teleostei: Perciformes). Global Ecology
485 and Conservation 4:117–126.

486 Cinner, J., and Huchery, C. 2014. A comparison of social outcomes associated with different
487 fisheries co-management institutions. Conservation Letters 7:224–232.

488 Cinti, A., Shaw, W., Cudney-Bueno, R., and Rojo, M. 2010. The unintended consequences of formal
489 fisheries policies: Social disparities and resource overuse in a major fishing community in the Gulf
490 of California, Mexico. Marine Policy 34:328–339.

491 Condini, M. V., Velez-Rubio, G. M, Fallabrino, A, and Garcia, A. M. 2016. First occurrence of dusky
492 grouper Epinephelus marginatus (Lowe, 1834) in a Marine Protected Area on the Uruguayan coast.
493 Cah. Biol. Mar:57.

494 Costa, P. A. S., Braga, A. D., and da Rocha, L. O. F. 2003. Reef fisheries in Porto Seguro, eastern
495 Brazilian coast. Fisheries Research 60:577–583.

496 Costa, T. L. A., Pennino, M. G., and Mendes, L. F. 2017. Identifying ecological barriers in marine
497 environment: The case study of Dasyatis marianae. Marine Environmental Research 125:1–9.

498 Damasio, L. de M. A., Lopes, P. F. M., Guariento, R. D., and Carvalho, A. R. 2015. Matching Fishers'
499 Knowledge and Landing Data to Overcome Data Missing in Small-Scale Fisheries. PLoS ONE
500 10:e0133122.

501 Davis, A., Hanson, J. M., Watts, H., and MacPherson, H. 2004. Local ecological knowledge and
502 marine fisheries research: the case of white hake (Urophycis tenuis) predation on juvenile
503 American lobster (Homarus americanus). Canadian Journal of Fisheries and Aquatic Sciences
504 61:1191–1201.

505 Davis, A., and Wagner, J. R. 2003. Who Knows? On the Importance of Identifying "Experts" When
506 Researching Local Ecological Knowledge. Human Ecology 31:463–489.

507 Dayton, P. K. 1998. Reversal of the burden of proof in fisheries management. Science 279:821–
508 822.

189

509 Di Dario, F., Alves, C. B. M., Boos, H., Frédou, F. L., Lessa, R. P. T., Mincarone, M. M., Pinheiro, M. A.
510 A., Polaz, C. N. M., Reis, R. E., Rocha, L. A., Santana, F. M., Santos, R. A., Santos, S. B., Vianna, M.,
511 and Vieira, F. 2015. A better way forward for Brazil's fisheries. Science 347:1079.

512 Eddy, T. D., Gardner, J. P. A., and Pérez-Matus, A. 2010. Applying fishers' ecological knowledge to
513 construct past and future lobster stocks in the Juan Fernández archipelago, Chile. PLoS ONE 5:1–
514 12.

515 Fielding, A. H., and Bell, J. F. 1997. A review of methods for the assessment of prediction errors in
516 conservation presence/absence models. Environmental Conservation 24:38–49.

517 Finkbeiner, E. M., and Basurto, X. 2015. Re-defining co-management to facilitate small-scale
518 fisheries reform: An illustration from northwest Mexico. Marine Policy 51:433–441.

519 Freeman, E. A., and Moisen, G. 2008. PresenceAbsence: An R Package for Presence Absence
520 Analysis. Journal of Statistical Software 23.

521 Gaines, S. D., White, C., Carr, M. H., and Palumbi, S. R. 2010. Designing marine reserve networks
522 for both conservation and fisheries management. Proceedings of the National Academy of
523 Sciences 107:18286–18293.

524 Gilles, A., Miquelis, A., Quignard, J.-P., and Faure, É. 2000. Molecular phylogeography of western
525 Mediterranean dusky grouper Epinephelus marginatus. Comptes Rendus de l'Académie des
526 Sciences - Series III - Sciences de la Vie 323:195–205.

527 Harmelin, J.-G., and Harmelin-Vivien, M. 1999. A review on habitat, diet and growth of the dusky
528 grouper Epinephelus marginatus (Lowe, 1834). Pages 11–20 Marine life. Institut océanographique
529 Paul Ricard, Les Embiez.

530 Heemstra, P. C., and Randall, J. E. 1993. FAO Fisheries Catalogue: An annotated and illustrated
531 catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO,
532 Rome.

533 Hereu, B., Diaz, D., Pasqual, J., Zabala, M., and Sala, E. 2006. Temporal patterns of spawning of the
534 dusky grouper Epinephelus marginatus in relation to environmental factors. Marine Ecology
535 Progress Series 325:187–194.

536 Hijmans, R. J., van Etten, J., Cheng, J., Mattiuzzi, M., Summer, M., Greenberg, J. A., and Ghosh, A.
537 2016. Package "raster". R package. R package.

538 Hijmans, R. J., Phillips, S., Leathwick, L., and Elith, J. 2011. Package "dismo."

539 Hilborn, R. 2007. Defining success in fisheries and conflicts in objectives. Marine Policy 31:153–
540 158.

190

541   Houk, P.,Rhodes, K., Cuetos-Bueno, J., Lindfield, S., Fread, V., and McIlwain, J. L. 2012. Commercial
542   coral-reef fisheries across Micronesia: A need for improving management. Coral Reefs 31:13–26.

543   Irigoyen, A. J., Galván, D. E., and Venerus, L. A. 2005. Occurrence of dusky grouper Epinephelus
544   marginatus (Lowe, 1834) in gulfs of northern Patagonia, Argentina. Journal of Fish Biology
545   67:1741–1745.

546   Jentoft, S. 2000. Legitimacy and disappointment in fisheries management. Marine Policy 24:141–
547   148.

548   Johannes, R. 1998. The case for data-less marine resource management: examples from tropical
549   nearshore finfisheries. Trends in Ecology & Evolution 13:243–246.

550   Johannes, R. E. 1981. Words of the lagoon: fishing and marine lore in the Palau district of
551   Micronesia. University of California Press, Berkeley.

552   Jones, J. P. G., Andriamarovololona, M. M. , Hockley, N., Gibbons, J. M., and Milner-Gulland, E. J.
553   2008. Testing the use of interviews as a tool for monitoring trends in the harvesting of wild
554   species. Journal of Applied Ecology 45:1205–1212.

555   Kaufman, L., Heneman, B., Barnes, T. J., and Fujita, R. 2004. Transition from low to high data
556   richness: an experiment in ecosystem-based fishery management from California. Bulletin of
557   Marine Science 74:693–708.

558   Kinas, P. G., and Andrade, H. A. 2014. Introdução à Análise Bayesiana (com R). Buqui Livros
559   Digitais, Porto Alegre.

560   Latimer, A. M., Wu, S., Gelfand, A. E., and Silander, J. A.. 2006. Building statistical models to
561   analyze species distributions. Ecological Applications: A Publication of the Ecological Society of
562   America 16:33–50.

563   Lopes, P. F. M., Francisco, A. S., and Begossi, A. 2009. Artisanal commercial fisheries at the
564   southern coast of São Paulo state, Brazil: Ecological, social and economic structures. Interciencia
565   34:536–542.

566   Lopes, P. F. M., Rosa, E. M., Salyvonchyk, S., Nora, V., and Begossi, A. 2013a. Suggestions for fixing
567   top-down coastal fisheries management through participatory approaches. Marine Policy 40:100–
568   110.

569   Lopes, P. F. M., Silvano, R. A. M., and Begossi, A. 2011. Extractive and Sustainable Development
570   Reserves in Brazil: resilient alternatives to fisheries? Journal of Environmental Planning and
571   Management 54:421–443.

572   Lopes, P. F. M., Silvano, R. A.M., Nora, V. A. and Begossi, A. 2013b. Transboundary Socio-Ecological
573   Effects of a Marine Protected Area in the Southwest Atlantic. AMBIO 42:963–974.

191

574 Machado, L. F., Andrade, A. B., Hostim-Silva, M., and Barreiros, J. P. 2003. Habitat use by the
575 juvenile dusky grouper Epinephelus marginatus and its relative abundance, in Santa Catarina,
576 Brazil. Journal of Ichthyology and Aquatic Biology 6:133–138.

577 Machado, L. F., Daros, F. A. M. L., Andrade Bertoncini, A., Hostim-Silva, M., and Barreiros, J. P.
578 2008. Feeding strategy and trophic ontogeny in Epinephelus marginatus (Serranidae) from
579 Southern Brazil. Cybium 32:33–41.

580 Marshall, N. A., and Marshall, P. A. 2007. Conceptualizing and operationalizing social resilience
581 within commercial fisheries in Northern Australia. Ecology And Society 12:1.

582 Mesa, G. L., Louisy, P., and Vacchi, M. 2002. Assessment of microhabitat preferences in juvenile
583 dusky grouper (Epinephelusmarginatus) by visual sampling. Marine Biology 140:175–185.

584 Moller, H., Berkes, F., Lyver, P. O., and Kislalioglu, M. 2004. Combining Science and Traditional
585 Ecological Knowledge: Monitoring Populations for Co-Management. Ecology and Society 9.

586 Mora, C., Myers, R. A., Coll, M., Libralato, S., Pitcher, T. J., Sumaila, R. U., Zeller, D., Watson, R.,
587 Gaston, K. J., and Worm, B. 2009. Management effectiveness of the world's marine fisheries. PLOS
588 Biology 7:e1000131.

589 Morris, A. V., Roberts, C. M., and Hawkins, J. P. 2000. The threatened status of groupers
590 (Epinephelinae). Biodiversity & Conservation 9:919–942.

591 Neis, B., Schneider, D. C., Felt, L., Haedrich, R. L., Fischer, J., and Hutchings, J. A. 1999. Fisheries
592 assessment: what can be learned from interviewing resource users? Canadian Journal of Fisheries
593 and Aquatic Sciences 56:1949–1963.

594 Pauly, D., and Zeller, D. 2016. Catch reconstructions reveal that global marine fisheries catches are
595 higher than reported and declining. Nature Communications 7:10244.

596 Pennino, M. G., Arcangeli, A., Fonseca, V. P., Campana, I., Pierce, G. J., Rotta, A., and Bellido, J. M.
597 2017. A spatially explicit risk assessment approach: Cetaceans and marine traffic in the Pelagos
598 Sanctuary (Mediterranean Sea). PLOS ONE 12:e0179686.

599 Pennino, M. G., Conesa, D., López-Quílez, A., Muñoz, F., Fernández, A., and Bellido, J. M. 2016.
600 Fishery-dependent and -independent data lead to consistent estimations of essential habitats.
601 ICES Journal of Marine Science 73:2302–2310.

602 Perry, A. L., Low, P. J., Ellis, J.R., and Reynolds, J. D. 2005. Climate change and distribution shifts in
603 marine fishes. Science 308:1912–1915.

604 Pinheiro, H. T., Dario, F. D., Gerhardinger, L. C., de Melo, M. R. S. , Moura, R. L. de , Reis, R. E.,
605 Vieira, F., Zuanon, J., and Rocha, L. A. 2015. Brazilian aquatic biodiversity in peril. Science
606 350:1043–1044.

607   Priolli, R. H. G., Bajay, M. M., Silvano, R. A. M., and Begossi, A. 2016. Population genetic structure
608   of an estuarine and a reef fish species exploited by Brazilian artisanal fishing. Scientia Marina
609   80:467–477.

610   R Development Team. 2017. R: A language and environment for statistical computing. R
611   Foundation for Statistical Computing, Vienna, Austria.

612   Ribeiro, A. R. 2016. Conhecimento ecológico local de pescadores sobre peixes recifais
613   (Epinephelidae) no litoral nordeste do Brasil. Master's, Universidade Federal do Rio Grande do Sul,
614   Porto Alegre.

615   Roos, N. C., Carvalho, A. R., Lopes, P. F. M., and Pennino, M. G. 2015. Modeling sensitive parrotfish
616   (Labridae: Scarini) habitats along the Brazilian coast. Marine Environmental Research 110:92–100.

617   Roos, N. C., Pennino, M. G., Lopes, P. F. M., and Carvalho, A. R. 2016. Multiple management
618   strategies to control selectivity on parrotfishes harvesting. Ocean & Coastal Management 134:20–
619   29.

620   Ruddle, K., and Davis, A. 2011. What is "Ecological" in Local Ecological Knowledge? Lessons from
621   Canada and Vietnam. Society & Natural Resources 24:887–901.

622   Sadovy de Mitcheson, Y., Craig, M.T., Bertoncini, A.A., Carpenter, K. E., Cheung, W. W. L., Choat, J.
623   H., Cornish, A. S., Fennessy, S. T., Ferreira, B. P., Heemstra, P. C., Liu, M., Myers, R. F., Pollard, D. A.,
624   Rhodes, K. L., Rocha, L. A., Russell, B. C., Samoilys, M. A., and Sanciangco, J. 2013. Fishing groupers
625   towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery.
626   Fish and Fisheries 14:119–136.

627   Sbrocco, E. J., and Barber, P. H. 2013. MARSPEC: ocean climate layers for marine spatial ecology.
628   Ecology 94:979–979.

629   Silvano, R. A. M., and Begossi, A. 2010. What can be learned from fishers? An integrated survey of
630   fishers' local ecological knowledge and bluefish (Pomatomus saltatrix) biology on the Brazilian
631   coast. Hydrobiologia 637:3–18.

632   Silvano, R. A. M., MacCord, P. F. L., Lima, R.V., and Begossi, A. 2006. When does this fish spawn?
633   Fishermen's local knowledge of migration and reproduction of Brazilian coastal fishes.
634   Environmental Biology of Fishes 76:371–386.

635   Silvano, R., and Valbo-Jorgensen, J. 2008. Beyond fishermen's tales: contribution of fishers' local
636   ecological knowledge to fish ecology and fisheries management. Environment Development and
637   Sustainability 10:657–675.

638   Spalding, M. D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S.,
639   Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., Mcmanus, E., Molnar, J., Recchia, C.A., and
640   Robertson, J. 2007. Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf
641   Areas. BioScience 57:573–583.

193

642 Spiegelhalter, D. J., Best, N.G., Carlin, B. P., and Van Der Linde, A. 2002. Bayesian measures of
643 model complexity and fit. Journal of the Royal Statistical Society: Series B (Statistical Methodology)
644 64:583–639.

645 Tesfamichael, D., Pitcher, T. J., and Pauly, D. 2014. Assessing Changes in Fisheries Using Fishers'
646 Knowledge to Generate Long Time Series of Catch Rates: a Case Study from the Red Sea. Ecology
647 and Society 19.

648 Vieilledent, G., Merow, C., Guélat, J., Latimer, A. M., Kéry, M., Gelfand,A. E., Wilson, A. M.,
649 Mortier, F., and Silander Jr, J.A. 2014. hSDM: hierarchical Bayesian species distribution models.

650 Warren, D. L., Glor, R. E., and Turelli, M. 2008. Environmental niche equivalency versus
651 conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883.

652 Worm, B., and Branch, T. A. 2012. The future of fish. Trends in Ecology & Evolution 27:594–599.

653 Zuur, A. F., Leno, E.N., and Elphick, C. S. 2010. A protocol for data exploration to avoid common
654 statistical problems. Methods in Ecology and Evolution 1:3–14.

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656 Table 1: Comparison of the most relevant *E. marginatus* scientific dataset models.

657 Deviance Information Criterion (DIC) scores measure the goodness-of-fit of each model.

658 Predictor acronyms are: sea surface temperature (SST), sea surface salinity (SSS), net

659 primary productivity (NPP), depth (D), slope (S) concavity of the seabed (C), spatial effect

660 (ρ), observer effect (Z). Relevant environmental predictors are indicated by *, i.e., those

661 predictors with 95% credibility intervals not covering zero. The best model is highlighted

662 in bold.

663

Models	DIC
$1 + SST^* + D^* + S + C + Z^* + \rho^*$	47.43
$1 + SST^* + D^* + S^* + Z^* + \rho^*$	**45.62**
$1 + D^* + S + Z^* + \rho^*$	50.58
$1 + D^* + C + Z^* + \rho^*$	52.36
$1 + SST^* + D^* + Z^* + \rho^*$	47.74
$1 + SSS + D^* + S + Z^* + \rho^*$	50.38
$1 + SSS + D^* + Z^* + \rho^*$	50.17
$1 + NPP + D^* + S + Z^* + \rho^*$	51.77
$1 + NPP + D^* + Z^* + \rho^*$	57.97
$1 + NPP^* + Z^* + \rho^*$	57.36
$1 + S + Z^* + \rho^*$	61.08
$1 + D^* + Z^* + \rho^*$	51.44
$1 + SST^* + Z^* + \rho^*$	57.19
$1 + SSS^* + Z^* + \rho^*$	57.66

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666 Table 2: Comparison of the most relevant *E. marginatus* ethnobiological dataset models.

667 Deviance Information Criterion (DIC) scores measure goodness-of-fit. Predictor acronyms

668 are: sea surface temperature (SST), sea surface salinity (SSS), net primary productivity

669 (NPP), depth (D), slope (S) concavity of the seabed (C), spatial effect (ρ), observer effect

670 (Z). Relevant environmental predictors are indicated by *, i.e., those predictors with 95%

671 credibility intervals not covering zero. The best model is highlighted in bold.

Models	DIC
1 + SST* + D*+ S + C* + Z* + ρ*	21.54
1 + SST + D + S + Z* + ρ*	19.61
1 + D + S + Z* + ρ*	18.57
1 + D* + C* + Z* + ρ*	17.12
1 + SST* + D* + Z* + ρ*	**14.89**
1 + SSS + D + C + Z + ρ*	15.13
1+ SSS +C* + Z* + ρ*	16.39
1 + SSS + D* + Z* + ρ*	18.70
1 + NPP + D* + Z* + ρ*	19.23
1 + NPP + Z* + ρ*	21.31
1 + C*+ Z* + ρ*	16.53
1 + D* + Z* + ρ*	18.26
1 + SST* + Z* + ρ*	19.24
1 + SSS + Z* + ρ*	21.48

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675   Figure legends

676   Figure 1: Sampling locations for the scientific (a) and ethnobiological (b) datasets. Red

677   dots indicate presences, while black dots reflect absences.

678

679   Figure 2. Smooth function of the predicted occurrence probability for depth (a), slope (b)

680   and SST (c), using the scientific dataset. The solid line is the smooth function estimate and

681   shaded regions represent the approximate 95% credibility interval (CI).

682

683   Figure 3. Mean occurrence probability of the *E. marginatus* species along South American

684   waters, obtained using the scientific dataset on its presence/absence.

685

686   Figure 4. Smooth function of the predicted occurrence probability of depth (a) and SST (b),

687   using the ethnobiological dataset. The solid line is the smooth function estimate. Shaded

688   regions represent the approximate 95% credibility interval (CI).

689

690   Figure 5. Mean occurrence probability of the *E. marginatus* species along South American

691   waters obtained using the ethnobiological dataset.

692

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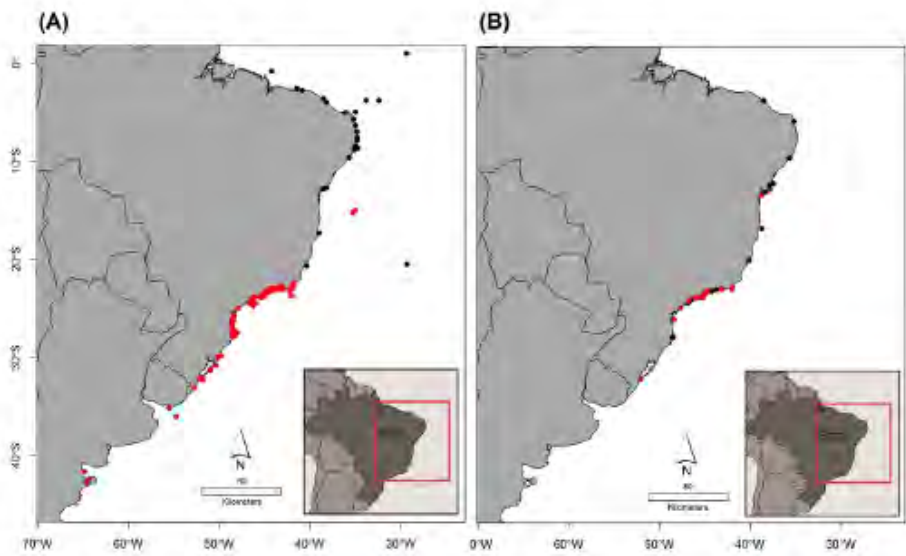
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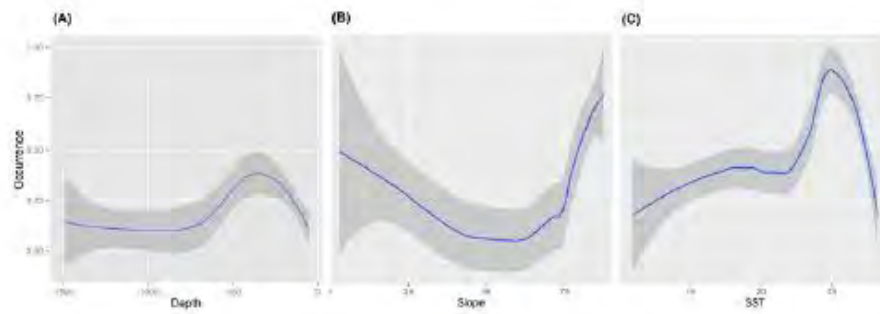
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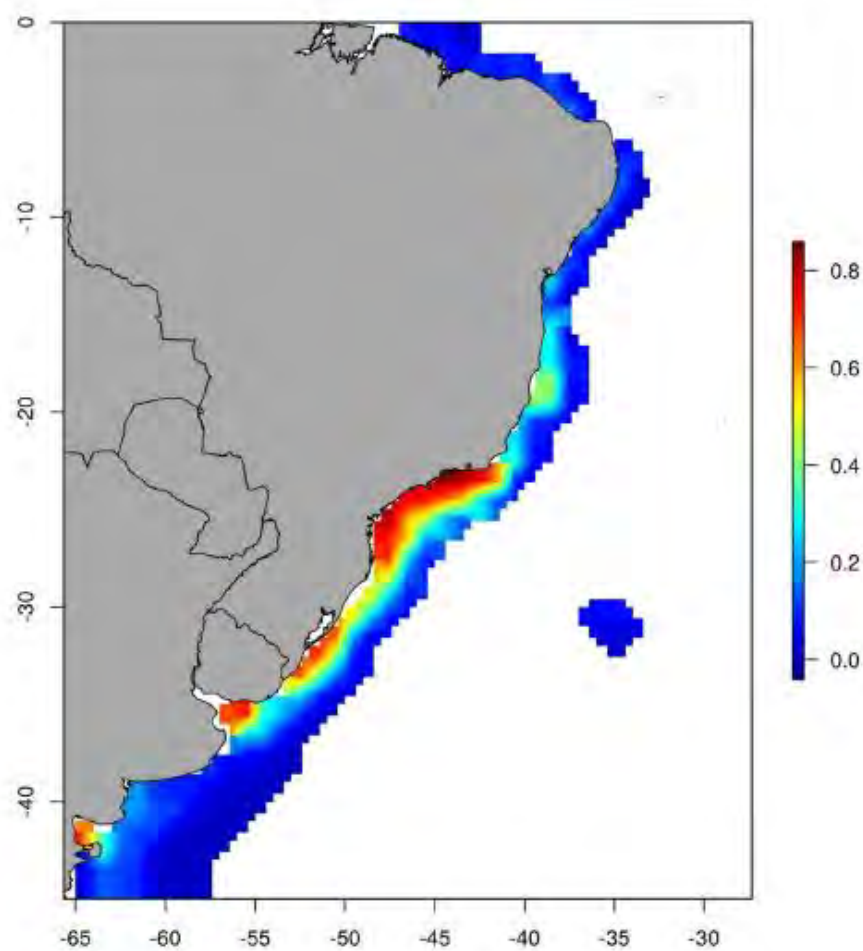
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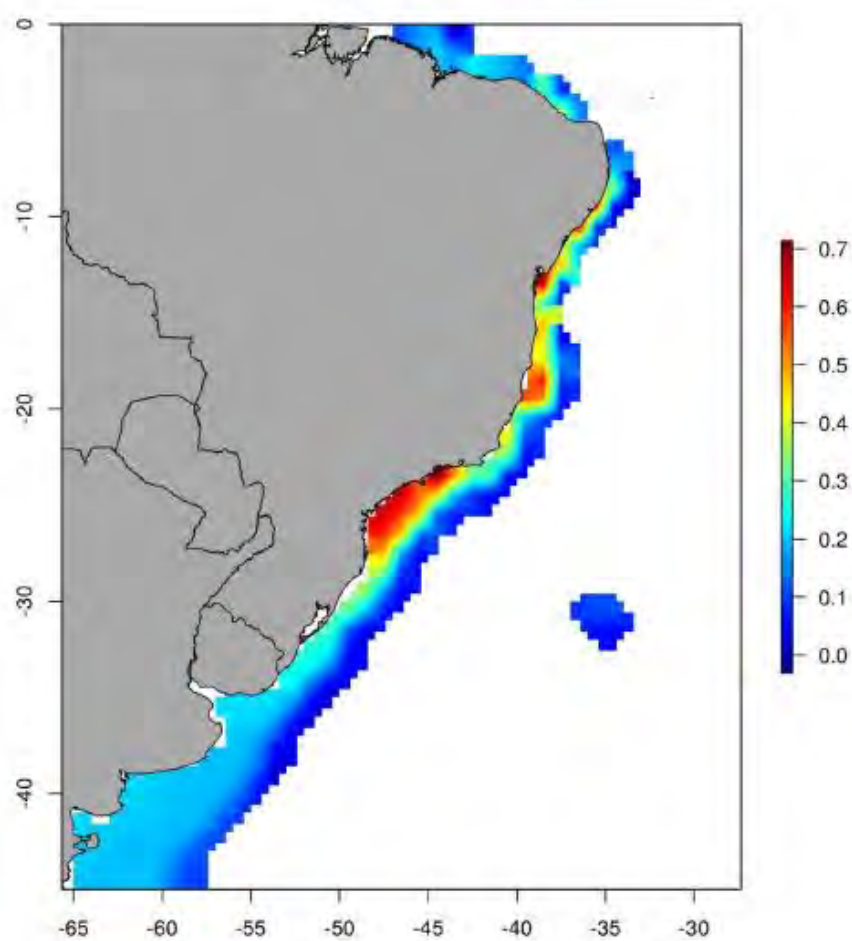
219x258mm (150 × 150 DPI)

RESEARCH ARTICLE

The largest fish in the world's biggest river: Genetic connectivity and conservation of *Arapaima gigas* in the Amazon and Araguaia-Tocantins drainages

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Abstract

Arapaima, pirarucu or paiche (*Arapaima gigas*) is one of the largest freshwater fish in the world, and has a long history of commercial exploitation in the Amazon region. To estimate levels of genetic variability and historical and recent connectivity in Arapaima, we examined variation in eleven microsatellite DNA markers in individuals from 22 localities in Brazil, Colombia, and Peru. The results of analysis of molecular variance, Bayesian clustering and discriminant analysis of principal components showed that *Arapaima* in our samples represents two major populations, one in the Amazonas and one in the Araguaia-Tocantins River basins. The Amazonas population is further structured by isolation-by-distance with the hydrologically largely unconnected Amapá locality representing the eastern-most extreme of this continuum; gene flow predominates at distances of less than 1500 km with localities separated by over 2000 km dominated by genetic drift and effectively forming different populations. We saw no evidence of multiple species of *Arapaima* in the Amazonas basin, and analysis of pairwise genetic divergence (F_{ST}) with Mantel tests and correlograms indicated that this largest population exhibits a large-scale pattern of isolation-by-distance, with which results from MIGRATE-N agreed. The degree and significance of genetic divergence indicates that most sampled localities represent demographically independent sub-populations, although we did identify several recent migration events between both proximal and more distant localities. The levels of genetic diversity were heterogeneous across sites, including low genetic diversity, effective population sizes, and evidence of genetic bottlenecks in several places. On average the levels of gene diversity and rarefied allelic richness were higher for localities along the Amazonas mainstem than in the tributaries, despite these being the areas of highest fishing pressure, while the lowest values were found in tributary

headwaters, where landscape modification is a significant threat. We recommend that managers consider the regional and local threats to these populations and tailor strategies accordingly, strategies which should ensure the ability of young *A. gigas* to disperse through floodplain corridors to maintain genetic diversity among otherwise sedentary adult subpopulations.

Introduction

The Amazon basin suffers from the myth of superabundance, wherein the natural resources present in this region, including its impressive diversity of fishes, are considered inexhaustible by any human demand [1, 2]. This myth derives in part from the huge scale of the Amazon, its high biodiversity, and its low relative human occupational density, since the perceived abundance of a region's natural resources is directly related to the intensity of exploitation [2]. Indeed this myth of superabundance has been promulgated since the Portuguese colonized the Amazon basin, and particularly during the "boom" of latex rubber export [3]. However, there are well documented cases of drastic reductions in exploited populations, including the black (*Melanosuchus niger*) and spectacled caimans (*Caiman crocodilus*) [4, 5], the Amazonian manatee (*Trichechus inunguis*) [6], numerous species of turtles (*Podocnemis* spp.) [7], and what may be South America's largest freshwater fish, the arapaima, pirarucu or paiche (*Arapaima gigas* Schinz 1822) [8]. Although the iconic arapaima continues to be exploited, it is described on the IUCN Red List as Data Deficient (IUCN, 2017), meaning there is insufficient knowledge of its biology, ecology, and genetics to effectively manage its conservation.

Another prominent uncertainty surrounding the arapaima is the number of species present in this genus and their distribution. In two recent publications, Stewart [9, 10] revised the taxonomy of the genus, revalidated several species and described a new species of *Arapaima* from the central Amazon [9]. Stewart [9, 10] puts forth an argument that at least six species of *Arapaima* exist: 1) *Arapaima gigas* (Schinz, in Cuvier 1822) described from near [Vila] Santarém, Para State, Brazil, only known from the holotype (MNHN A.8837); 2) *Arapaima mapae* (Valenciennes, in Cuvier and Valenciennes, 1847) described from Lago do Amapá or Lago Grande in Região dos Lagos in Amapá State, Brazil, only known from the holotype (MNHN A.8836); 3) *Arapaima arapaima* (Valenciennes, in Cuvier and Valenciennes, 1847) described from Guyana (Essequibo basin), only known from the holotype (BMNH 2009.1.19.1) but the holotype is misplaced or lost; 4) *Arapaima agassizii* (Valenciennes, in Cuvier and Valenciennes, 1847) described from "Brazilian Amazon", only known from an illustration of the holotype by Spix and Agassiz [11] and the holotype is lost; 5) *Arapaima leposoma* Steward 2013 described from one specimen collected from the Solimões River shortly upstream of the mouth of the Purus River, Amazonas State, Brazil, only known from the holotype (INPA 16847); and 6) *Arapaima* sp. *incertae sedis*, i.e. a species of uncertain taxonomic status, that apparently comprises all other Amazon basin *Arapaima* specimens deposited in scientific collections.

Günther in 1868 [12] synonymized all the species of arapaima described by Valenciennes, and Spix and Agassiz with *Arapaima gigas* (Schinz, in Cuvier 1822). In contrast to Günther [12], Stewart [9, 10] concluded that all former species should be considered valid, although they are known only from type specimens, their distributions are unknown, and all *Arapaima* specimens deposited in scientific collections—specimens sampled throughout the Amazon basin—are *Arapaima* sp. *incertae sedis*. Thus, although the specimens analyzed in this study may be *Arapaima* sp. *incertae sedis*, until further taxonomic clarification we take the conservative approach and refer to the specimens analyzed in this study as *Arapaima gigas*.

Arapaima gigas is one of the largest freshwater fishes in South America, competing only with the catfish *Brachplatystoma filamentosum* for that title, and can reach a length of three meters and weigh more than 200 kg [13, 14]. It is primarily piscivorous [15, 16], and the feeding habits of these large-bodied fishes provide top-down trophic regulation in floodplain ecosystems [17]. Although as adults *A. gigas* are primarily sedentary fish with low dispersal capability [18], they do make seasonal migrations between permanent wetlands and nearby floodplains (várzea or igapó). During the dry season, *A. gigas* inhabits permanent wetlands, such as slow-moving rivers and lagoons where adults develop their gonads, engage in courtship, build nests, and reproduce. During the rainy season when water levels rise, *A. gigas* migrate to the floodplain, where the males provide parental care including mouth brooding, and young exploit the abundant resources of the flooded zones. As the water levels fall, parental care ceases and adult *A. gigas* migrate back to the permanent wetlands. It is in the permanent wetlands where most fishing occurs, and *A. gigas* are particularly vulnerable to harpooning as these obligate air breathers surface to refresh their air bladder [19]. Individuals generally become reproductively mature after 3–5 years of age [20] and may live upwards of 15–20 years. Batch fecundity is low, however [21].

Arapaima gigas is native to, and historically common in, the lowland Amazonas basin and the Araguaia-Tocantins basin, the later of which is considered a separate drainage by some authors, although it is connected to the Amazonas by uninterrupted freshwater and exhibits a related biota [22]. In the late 1970's the species was also unintentionally introduced into the Bolivian Amazon [23]. In a pioneering study of *A. gigas* population genetics, Hrbek et al. [24] used mitochondrial DNA sequences from 120 individuals from six sites along the main channel of the Amazon basin and one site in the Araguaia-Tocantins basin and observed greater genetic diversity (haplotype diversity) in *A. gigas* far from large urban centers, where arapaima meat sales and distribution centers are concentrated. These data also suggested that the effective population size of this species had declined along with known decreases in census population density after two centuries of commercial exploitation, and that the Bolivian population was introduced from the Peruvian Amazon. Later, nuclear microsatellite data from the same sites revealed a pattern of isolation-by-distance along the Amazon River main stem [25]. Similar findings were reported by Araripe et al. [26].

In the present study, we expand on previous sampling to include sites throughout the Amazon basin, including the main axis of the Amazon basin and its main tributaries, and in the Araguaia-Tocantins watershed, to examine the distribution of genetic diversity and the pattern and magnitude of population structure. We sought to test if (i) the pattern of isolation-by-distance of *A. gigas* populations remains throughout the Amazon and Tocantins, (ii) if population genetic structure indicated that some areas exhibited unique genetic variation indicative of historical or sustained divergence, and (iii) whether indications of declines in population genetic diversity were only present near urban centers or were evident throughout the range of this fish.

Material and methods

Ethics statement

Permission to collect samples of was granted by IBAMA Permit Number 11325-1.

Sampling

We analyzed 517 individuals of *Arapaima gigas* sampled from 19 locations in the Amazon basin and three locations in the Araguaia-Tocantins basin (Fig 1 and Table 1). Samples were from natural populations and were collected by the fishing communities at each location.
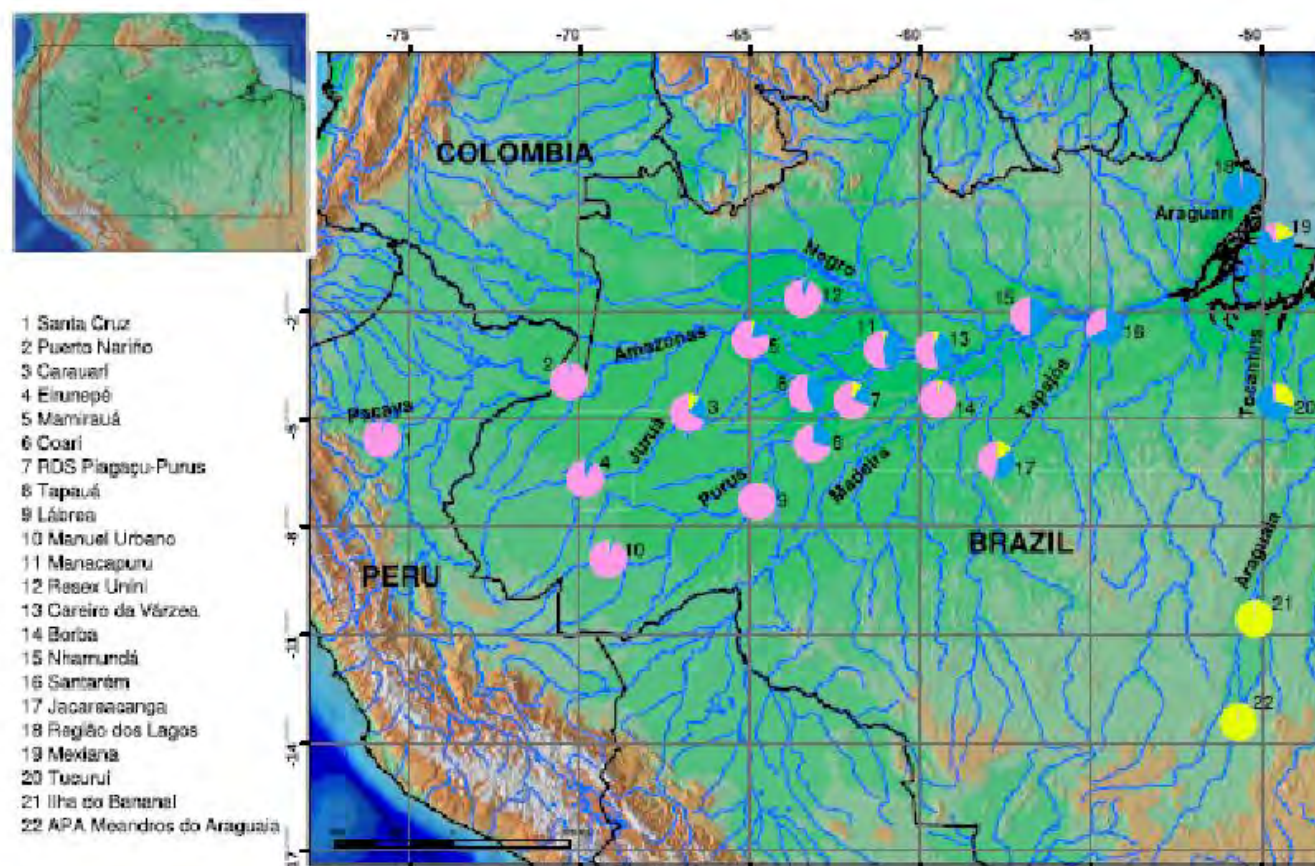
Fig 1. Map of collecting localities of *Arapaima gigas*. Pie plots indicate average population ancestry of each of the three main biological clusters detected in STRUCTURE analysis (see Fig 2). Color scheme is same as in Fig 2, K = 3.

1 Santa Cruz
2 Puerto Nariño
3 Carauari
4 Eirunepé
5 Mamirauá
6 Coari
7 RDS Piagaçu-Purus
8 Tapauá
9 Lábrea
10 Manuel Urbano
11 Manacapuru
12 Resex Unini
13 Careiro da Várzea
14 Borba
15 Nhamundá
16 Santarém
17 Jacareacanga
18 Região dos Lagos
19 Mexiana
20 Tucuruí
21 Ilha do Bananal
22 APA Meandros do Araguaia

Tissue samples were preserved in 95% alcohol and deposited in the Coleção de Tecidos da Genética Animal (CTGA) of the Laboratório de Evolução e Genética Animal (LEGAL) at the Universidade Federal do Amazonas (UFAM) in Manaus, Brazil.

DNA samples were extracted using the Qiagen extraction kit. Eleven microsatellite loci were amplified following Farias et al. [27] for *Arapaima gigas*. Loci used in this study were: CTm3, CTm4, CTm5, CTm7, CTm8, CAm2, CAm13, CAm15, CAm16, CAm20 and Cam26. PCR products were generated with labeled primers and visualized on a Megabace 1000 DNA automatic sequencer (GE-Healthcare). Allele sizes were scored against an internal ET-400 ROX size standard. Individuals were genotyped using the Genetic Profiler and Fragment Profiler (GE-Healthcare). MICRO-CHECKER v2.2.3 [28] was used to detect possible errors due to genotyping, null alleles or stutters. The matrix of genotypes is available at https://github.com/legalLab/publications.

Data analysis

Various genetic diversity parameters, including the observed heterozygosity, gene diversity (expected heterozygosity) and the number of alleles per locus was estimated using Arlequin 3.5 [29]. As richness estimates are constrained by sample size [30], we implemented the

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Table 1. Sampling areas of *Arapaima gigas* analyzed in the present study.

Map location	N	Locality	River location	Amazon Basin	Coordinate Lat/Lon
1	16	Santa Cruz	Pacaya River	Main channel	-5.50737°/-75.89130°
2	22	Puerto Nariño	Amazonas River	Main channel	-3.76613°/-70.37904°
3	18	Caramari	Middle Juruá River	Tributary	-4.93289°/-66.69848°
4	13	Eirunepé	Upper Juruá River	Tributary	-6.78775°/-69.81648°
5	32	Mamirauá	Confluence Solimões/Japurá Rivers	Main channel	-3.06471°/-64.80223°
6	9	Coari	Middle Solimões River	Main channel	-4.39622°/-63.47529°
7	20	RDS Piagaçu-Purus	Lower Purus River	Tributary	-4.14691°/-62.00704°
8	20	Tapaná	Middle Purus River	Tributary	-5.70652°/-63.20083°
9	15	Lábrea	Middle Purus River	Tributary	-7.30723°/-64.83540°
10	18	Manuel Urbano	Upper Purus River	Tributary	-8.93535°/-69.17622°
11	30	Manacapuru	Amazonas River	Main channel	-3.17434°/-60.79713°
12	21	Reserva Unini	Middle Negro River	Tributary	-1.59844°/-63.41299°
13	21	Careiro da Várzea	Amazonas River	Main channel	-3.23870°/-59.89439°
14	30	Borba	Lower Madeira River	Tributary	-4.37251°/-59.52051°
15	7	Nhamundá	Lower Nhamundá River	Tributary	-2.14754°/-56.75132°
16	31	Santarém	Amazonas River	Main channel	-2.69827°/-54.90179°
17	15	Jacareacanga	Tapajós River	Tributary	-6.21984°/-57.97813°
18	30	Região dos Lagos	Araguari River	Delta periphery	1.39913°/-49.61515°
19	17	Mexiana Island	Mouth of the Amazonas River	Delta	0.01254°/-49.75654°
Map location	N	Locality	River location	Araguaia-Tocantins Basin	Coordinate Lat/Lon
20	31	Tucuruí	Tocantins River	Main channel	-4.95746°/-49.65950°
21	15	Ilha do Bananal	Araguaia River	Main channel	-10.62590°/-50.43996°
22	80	APA Meandros do Araguaia	Araguaia River	Main channel	-13.33616°/-50.77259°
Total	511				

rarefaction analysis in the program HP-Rare [31] so that the number of alleles and allelic richness estimates could be compared between samples localities. Heterozygosity estimates are less influenced by sample size [32], so no correction was applied. Additionally, we estimated endogamy/inbreeding coefficient (F_{IS}) within each sampling site using Arlequin 3.5 [29].

To identify major population structuring patterns in the data, we utilized Bayesian clustering of individuals in the program STRUCTURE 2.3.3 [33]. This analysis clusters individual into a pre-defined number of populations (K) that minimize deviations from Hardy-Weinberg predictions and linkage disequilibrium among loci. We performed 10 independent runs for each predetermined number of biological groups (K = 1 to 22; considering that each location could be a different biological group), each run consisting of 1,000,000 MCMC chains after having discarded the first 100,000 chains as burn-in. We used the 'admixture' and 'correlated-allelic-frequencies' models with and without location information as a prior [34]. The location prior suggests that individuals sampled in the same locality are likely to belong to the same cluster, but it is considered a weak prior, while the admixture model allows individuals to have ancestry from multiple clusters. The optimal number of clusters was inferred based on changes in the precision of clustering with different K (delta K) [35]. While STRUCTURE clusters individuals, SAMOVA 2.0 [36] clusters *a priori* sampling groups (localities) into a pre-defined number of groups (K) to maximize the genetic variance among groups in a hierarchical AMOVA framework [37]. We clustered localities using 10,000 permutations from 100 starting groupings for K = 1 to 10 (upper value of K guided by STRUCTURE results) both with and without explicit geographical information. We quantified the genetic variance and significance

of the STRUCTURE and SAMOVA clusterings using hierarchical AMOVA in Arlequin 3.5 [29] using 10,000 permutations with genetic distance based on allele identity. Finally, we also used a multivariate ordination approach implemented in the Discriminant Analysis of Principal Components (DAPC) [38] using the R package Adegenet 2.1 [39] in R [40]. This procedure uses a discriminant analysis (DA) to maximize the among-group variance in components from a principal components analysis (PCA) of samples assigned to pre-defined groupings (here, sampling localities). This DAPC retained 22 PC axes and 4 discriminant axes.

Historical and contemporaneous gene flow and demography

We looked for additional spatial patterns of gene flow by testing for isolation-by-distance through correlation of genetic and geographic distances using the Mantel test [41] implemented in Arlequin 3.5 [29]. Here, geographical distances, in km, followed the course of rivers, and the genetic distance matrix contained Slatkin linear pair-wise F_{ST} values based on allele identity. The geographical distance between the sampling localities was estimated by tools available on Google Earth, version 4.2 beta 2008 (Google). We also calculated a Mantel correlogram using the *vegan* package 2.4–5 [42] in R. Distance classes used in the Mantel correlogram ranged from 0 to 5500 km in steps of 500 km, and 5500+ km. This analysis allows for discrimination of migration-dominated and genetic drift-dominated evolutionary processes across spatial scales.

Additionally, we estimated historical and recent gene flow. First, gene flow was estimated by Bayesian analysis using MIGRATE-N version 3.6.11 [43]. Recent dispersal or migration was estimated using STRUCTURE, and by population assignment in GENODIVE 2b 27 [44]. For MIGRATE-N we ran 10 short chains, sampling each chain 10,000 times. We then sampled 500,000 topologies from one long chain, discarding the first 5,000 samples as burn-in. Search of parameter space was improved though adaptive swapping among four heated chains. MIGRATE analyses were repeated three times with random seeds to assess convergence.

We used population assignment in STRUCTURE and GENODIVE v2b27 [44] to identify recent migrants. In STRUCTURE, we specified the population (sample site) origin of each individual, and estimated the probably of assignment to that cluster back two generations (USEPOPINFO = 1, GENSBACK = 2), with three different migration priors: 0.1, 0.05, and 0.01. Convergence of this chain was rapid, so we ran the analysis for 100k generations after equal burn-in, and made three replicate runs. Migrants were identified as individuals with probability less than 0.5 of being from their sampled site. In GENODIVE, we specified an alpha of 0.002 (0.05/22 populations) applied independently to each population, with those exceeding the likelihood ratio threshold identified as migrants. STRUCTURE and GENODIVE both identify recent migrants, but while the GENODIVE analysis has the advantage of providing a formal likelihood ratio comparison, this test effectively assumes that identified individuals are 1st generation migrants, a constraint that can lead to reduced sensitivity and mis-identification of the source population of 2nd or 3rd generation migrants.

In order to assess if the populations of *Arapaima* have experienced reductions in effective population size we used two moment-based methods implemented in the programs BOTTLENECK [45] and MValue [46], respectively. The program BOTTLENECK identifies populations that have experienced a reduction in effective population size by the presence of heterozygosity excess due to the loss of rare alleles, assuming an approximate infinite alleles model, wherein each mutation creates a new allele. The M-ratio implemented by Mvalue, which considers the range of repeat numbers of microsatellite alleles relative to richness, is considered more sensitive to recent reductions in effective population size, but requires assuming that surveyed microsatellites evolve by quasi-stepwise mutation [46].

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The two moment-based methods efficiently detect recent bottlenecks—population reductions with the last few generations; however, populations and species are also subject to historical demographic growth and/or reductions. Therefore we analyzed the data using the coalescent sampler implemented in the program MSVar v1.3 [47, 48]. We ran 10 independent parallel chains sampling every 1,000th proposal, collecting 20,000 proposals in the MCMC chain in each parallel run. Priors for current and historical population size means and variances were set equal, with variances encompassing three orders of magnitude. Prior for mean time of population size change was set at 1,000 generations ago with variance encompassing time range from 1,000,000 to 0 generations. The runs were evaluated for convergence and were pooled to provide an estimate of current and historical effective population size. Convergence was assessed using the Gelman–Rubin criterion [49] and the test of alternative hypotheses (population decline vs. stable population size) was carried out as suggested by Beaumont [47] using Bayes factors. Calculations and plots were performed in the R statistical programming language [40] using the packages CODA [50] and ggplot2 [51].

In addition to these tests, we also estimated the effective population size (Ne) for each population using the LDNe method [52] implemented in the program NeEstimator v2.0 [53], assuming a random mating model and allele frequencies cutoff of 0.02. This analysis, which estimates the number of individuals contributing to the sample based on allelic linkage, assumes that the sample is representative of the age structure of the population, and, when it is not, the Ne estimates are equivalent to the number of breeders that contributed offspring to the generations included the sample [54].

Results

We surveyed genotypes of 11 microsatellite loci in a total of 511 individuals from the mainstem and major tributaries of the Amazon River, the Araguaia-Tocantins River, and Região dos Lagos (Fig 1). MICRO-CHECKER suggested there was no evidence of null alleles in the data. After Bonferroni correction, linkage disequilibrium was observed in 1 to 4% of pairwise comparisons for most loci; however, loci CTm3 and CTm4 had more than 30% probability of being linked. Locus CTm3 was also in H-W disequilibrium in 4 of 22 populations, and so this locus was removed from population structure analyses. Genetic statistics per locus and per sampling locality are shown in S1 Table. Overall, various diversity parameters presented low values for the eastern end of the Amazon Basin (Mexiana and Região dos Lagos), as well as in the middle and upper Purus River (Lábrea, Manuel Urbano), and upper Araguaia River (Ilha do Bananal, APA Meandros do Araguaia). Statistics of genetic diversity by sampling area are listed in Table 2, which shows that the average gene diversity over loci ranged from 0.128 ± 0.111 (APA Meandros do Araguaia) to 0.649 ± 0.342 (Mamirauá). The average number of alleles varied from 6.35 in Mamirauá to 2.17 in Ilha do Bananal (S1 Fig). The inbreeding coefficient, F_{IS}, ranged from low in Nhamundá (0.00) to high in Ilha do Bananal (0.54) (Table 2); however, only 4 out of the 21 localities were significant (Santa Cruz, Manuel Urbano, and Manacapuru). Proportions of private alleles ranged from 0.02 in Região dos Lagos to 0.67 in Resex Unini, and eight localities presented frequencies of <0.10 of private alleles. Expected heterozygosity (H_E) varied from 0.26 in the specimens collected in the Araguaia-Tocantins drainage (APA Meandros do Araguaia) to 0.66 in the specimens collected near the main channel, at Mamirauá (S2 Fig). Considering only mainstem locations, H_E ranged from 0.50 in the specimens collected in Mexiana to 0.66 in the specimens collected in Mamirauá. Conversely, the specimens collected in the tributaries of the Amazon basin showed H_E of 0.26 in the APA Meandros do Araguaia in the upper Araguaia River, and 0.62 in Tapauá, in the middle Purus River. Five loci were monomorphic for specimens collected at Ilha do Bananal (Araguaia

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Table 2. Genetic characterization of *Arapaima gigas* sampled from 22 localities in Amazônia.

Localities	N	Average gene diversity over loci	N_A	A_R	P_A	Average HWE H_O–H_E	F_{IS}	Monomorphic loci
1. Santa Cruz	16	0.561 ± 0.304	5.45	3.78	0.27	0.449–0.561	0.201[*]	
2. Puerto Nariño	22	0.546 ± 0.295	5.27	3.63	0.18	0.503–0.546	0.079	
3. Caranari	18	0.514 ± 0.296	4.45	3.26	0.13	0.583–0.590	-0.021	
4. Eirunepé	13	0.594 ± 0.329	4.17	3.18	0.16	0.482–0.544	0.108	
5. Mamirauá	32	0.649 ± 0.342	6.36	3.95	0.20	0.693–0.660	-0.063	
6. Coari	9	0.602 ± 0.332	3.54	3.13	0.01	0.606–0.602	-0.007	
7. RDS Piagaçu-Purus	20	0.540 ± 0.296	4.54	3.23	0.06	0.586–0.569	-0.054	
8. Tapauá	20	0.547 ± 0.295	4.90	3.43	0.14	0.588–0.622	0.039	CAm20
9. Lábrea	15	0.343 ± 0.207	3.81	2.66	0.28	0.423–0.405	-0.075	
10. Manuel Urbano	18	0.421 ± 0.245	2.45	2.16	0.06	0.362–0.435	0.170[*]	CAm15, CAm20
11. Manacapuru	30	0.606 ± 0.321	6.27	3.57	0.15	0.554–0.620	0.098[*]	
12. Resex Unini	21	0.555 ± 0.310	5.27	3.60	0.67	0.558–0.617	-0.013	
13. Careiro da Várzea	21	0.559 ± 0.303	4.90	3.36	0.13	0.548–0.567	0.022	
14. Borba	30	0.540 ± 0.295	4.45	2.90	0.07	0.530–0.563	0.033	
15. Nhamundá	7	0.619 ± 0.350	3.81	3.44	0.24	0.606–0.606	-0.005	
16. Santarém	31	0.625 ± 0.330	5.54	4.57	0.10	0.608–0.635	0.036	
17. Jacareacanga	15	0.532 ± 0.302	3.81	2.93	0.12	0.632–0.556	-0.181	
18. Região dos Lagos	30	0.362 ± 0.204	2.81	2.32	0.02	0.431–0.407	-0.071	CTm5
19. Mexiana	17	0.489 ± 0.275	3.18	2.73	0.09	0.438–0.501	0.117	
20. Tucuruí	31	0.514 ± 0.277	3.54	2.65	0.02	0.478–0.515	0.072	
21. Ilha do Bananal	15	0.145 ± 0.107	2.17	1.57	0.04	0.647–0.459	-0.546	CAm16, CAm20, CTm3, CTm4, CTm8
22. APA Meandros do Araguaia	80	0.128 ± 0.111	3.10	1.79	0.10	0.258–0.262	-0.062	CAm15

Note: N = number of individuals analyzed; N_A = average number of alleles per locus; A_R = Allelic richness; P_A = Private Allelic richness; H_O = Observed heterozygosity; H_E = Expected heterozygosity; F_{IS} = Fisher's individual fixation index ("inbreeding coefficient")

[*] Indicates significant *P* value after Bonferroni correction.

River), two for specimens from the upper Purus River (Manuel Urbano) and one locus in the middle Purus River (Tapauá), Região dos Lagos (Amapá) and APA Meandros do Araguaia.

Distribution of genetic variability and population differentiation

Evaluation of clustering of individuals with STRUCTURE based on variance in likelihood among runs and across numbers of clusters (delta K) showed that two clusters (K = 2) was optimal, with an additional peaks at K = 3 and K = 6 (Fig 2 and S3 Fig). These clusters corresponded to geography. At K = 2, the clusters indicate the distinctness of fishes from the Araguaia-Tocantins system and its area of influence—and principally those of the upper Araguaia River (APA Meandros do Araguaia, Ilha do Bananal), and the rest of the Amazon basin. At K = 3, Amazonian populations show an east-west structuring gradient. At K = 6, it is also apparent that populations geographically distant from the mainstem of the Amazon River, or not directly connected to it, also show certain degree of reproductive divergence (Fig 2). At K = 6, STRUCTURE results emphasized the distinctness of location in the Purus drainage (Manuel Urbano, Lábrea), lower Madeira (Borba), the Negro (RESEX Unini), upper Tapajós (Jacareacanga) and Amapá (Região dos Lagos). Interestingly, SAMOVA with K = 2 or K = 3, emphasized the same groupings as STRUCTURE (Araguaia, Amapá), which explained 17% of the genetic variance (F_{CT} = 0.16748) by separating the Araguaia, and 18% (F_{CT} = 0.17894) by
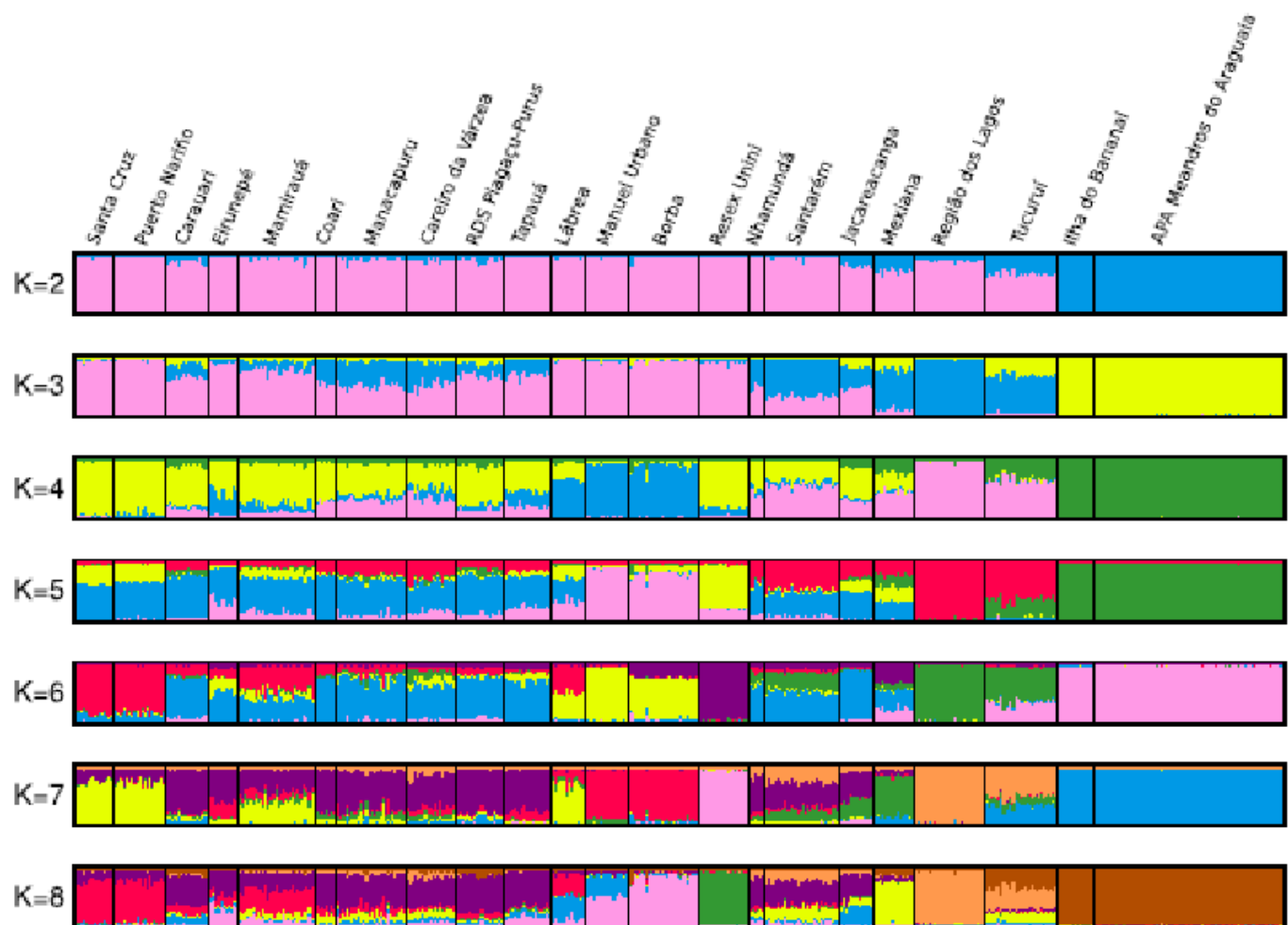
Fig 2. Graph of population structure of *Arapaima gigas* estimated in the program STRUCTURE. Each individual is represented by a vertical line.

separating both (with p<0.004). However, at K = 4 and K = 5, SAMOVA grouped the Amazon delta (Mexiana) and Negro (Unini) separately, which only provided marginal increases in the genetic variance explained (F_{CT} = 0.18538 and 0.19299, respectively). Finally, the DAPC showed contiguous overlap among most localities with the exception of the Araguaia and Amapá, and with the Tucuruí locality intermediate between these three groups (Fig 3). The congruency of these analyses with groupings at K = 2 and 3 and incongruence at larger K values indicate the robustness of this population structure.

Correlation between genetic divergence and geographic proximity

Despite forming an evolutionarily coherent population, the genetic structure among localities in the larger, Amazonas basin group was not insignificant, and F_{ST}-values ranged from 0.019 and non-significant (Alto Jurua x Nhamundá) to 0.475 and highly significant ($P < 0.0001$; Lábrea x Região dos Lagos in Amapá) (S2 Table and S4 Fig). A Mantel test indicated that this pattern of genetic divergence was significantly predicted by distance (r = 0.618174, P = 0.0003), suggesting that isolation-by-distance processes structure genetic diversity at the largest scale in
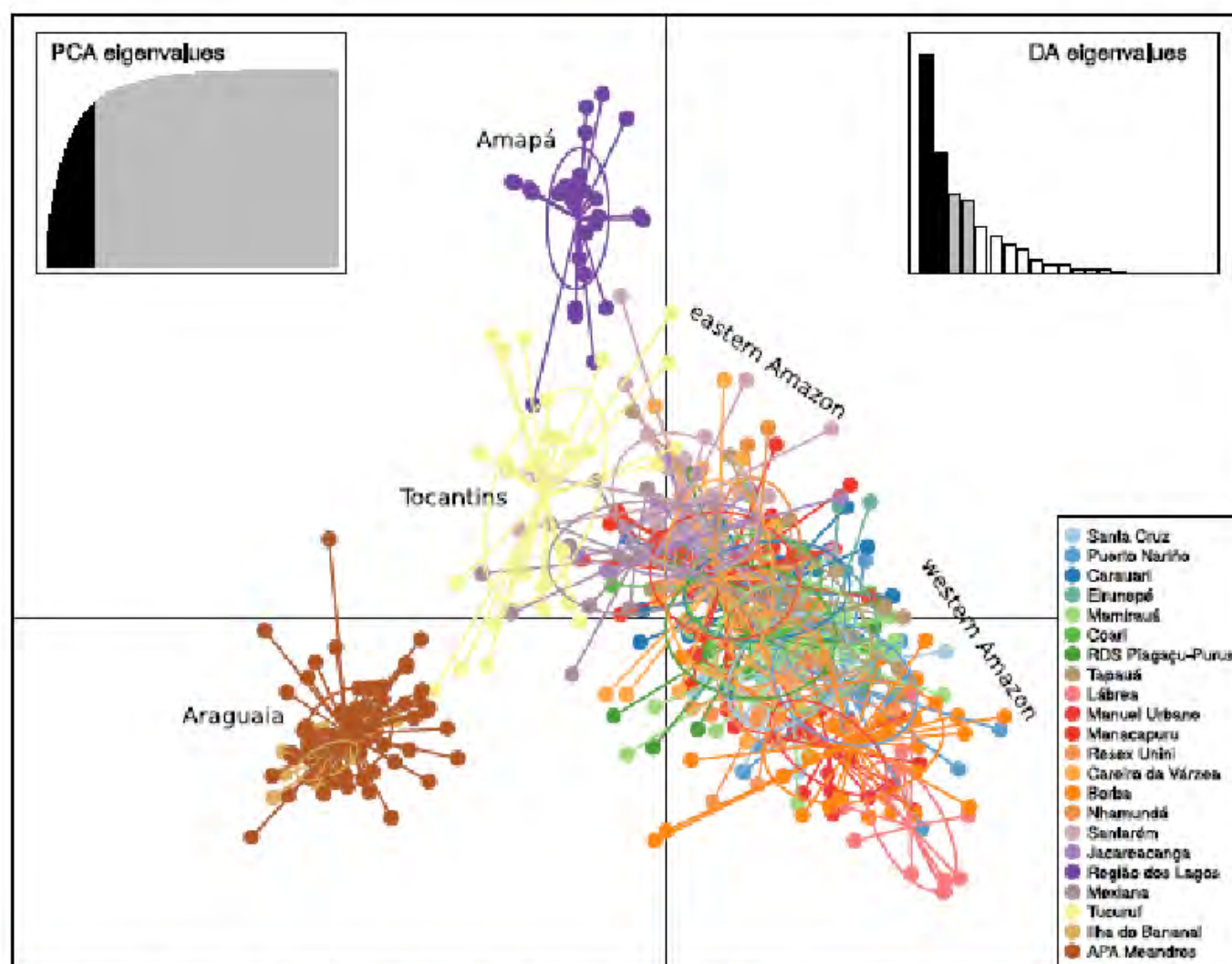
Fig 3. Results of DAPC analysis showing the scatterplot of the first two principal components based on 11 microsatellite loci of 511 individuals of *Arapaima gigas* from 22 sampling locations. The results are plotted showing an ellipse that shows one standard deviation of the variation of each population relative to its centroid. The eastern Amazon and western Amazon labels designate concentration of localities from these geographic regions.

the Amazonas group. A Mantel test was similarly positive including all samples ($r = 0.673128$, $P = 0.0001$) (Fig 4). Mantel correlograms showed positive spatial autocorrelation in genetic distance among localities up to 1,500 km, while localities separated by more than 2,000 km showed negative or non-significant spatial autocorrelation (Fig 5), indicating that neutral evolutionary processes across populations are dominated by gene flow up to 1,500 km, after which genetic drift plays a larger role between most populations. Importantly, these patterns were true considering all samples or without the Araguaia or Amapá samples.

Analysis of gene flow implemented in the program MIGRATE 3.6.11 [43] revealed a mixed pattern of gene flow among populations. The result indicated bidirectional gene flow between all localities (S2 Table), with all cases presenting more than 1 individual per generation. Although it is clear the reduction in *Nm* values when the populations of the Araguaia River are included.

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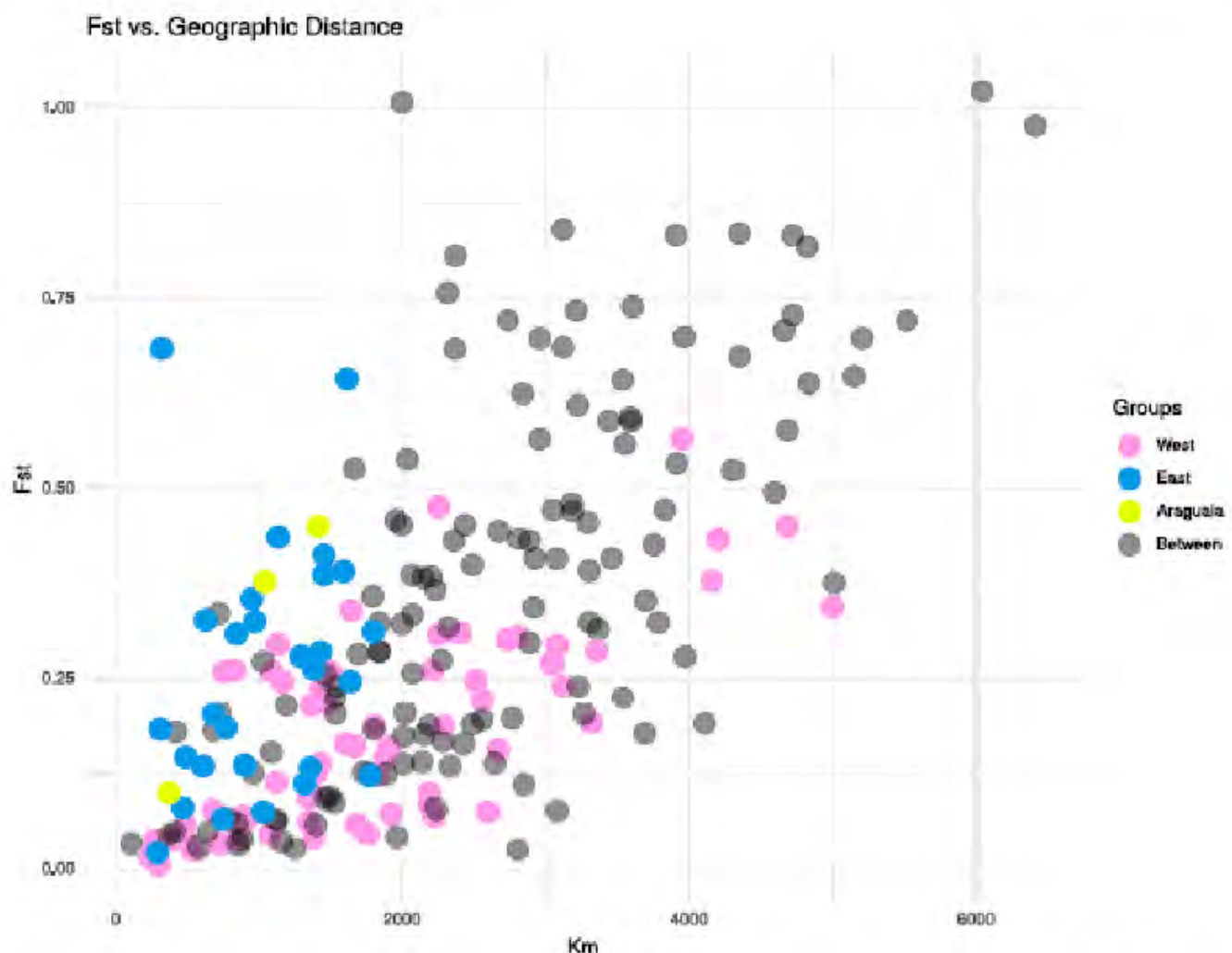
Fig 4. Graph of spatial autocorrelation analysis using linear pair-wise F_{ST} data and the distance between locations in kilometers (following the course of the rivers), all the distance categories showed significant correlations. The groups West, East and Araguaia refer to pair-wise comparisons between localities within each group, while Between are comparisons between localities of these groups. East and West localities are those that are east and west of the Madeira/Negro divide.

Likelihood tests in GENODIVE (Table 3) identified six migrants that were also corroborated by STRUCTURE: three of these with all migration priors, two with the two larger priors, and a single migrant only with the highest prior. Four additional individuals were identified by STRUCTURE with all priors as being 1st or later generation migrants, and a single individual was identified by GENODIVE as a migrant but was not corroborated in any STRUCTURE run (not shown). Although several of these migrants were from adjacent localities (e.g. Manacapuru and Nhamundá), several also suggested relatively distant dispersals, albeit potentially over several generations.

Evidence of population size changes

Results for analyses of a recent reduction in effective population size (bottleneck effect) are presented in Table 4. BOTTLENECK analyses detected significant deviations in observed

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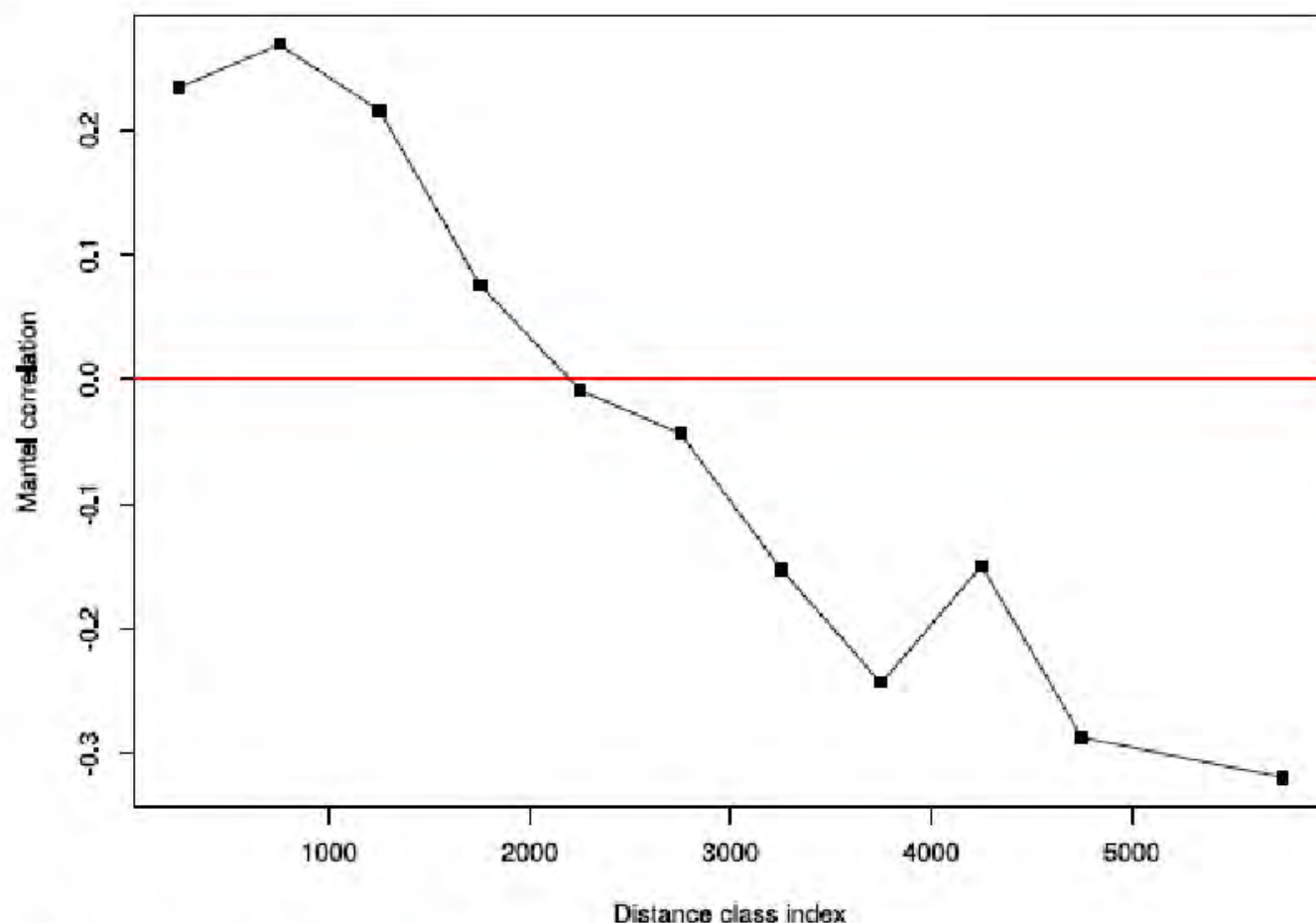
Fig 5. Graph of Mantel correlograms among localities.

https://doi.org/10.1371/journal.pone.0220882.g005

Table 3. Results of Likelihood test implemented in GENODIVE.

Migrant	Sampled at	Assigned to*	Generation
1	Jacareacanga	Santarém	1st
2	Manacapuru	Nhamundá	1st
3	Careiro da Várzea	Mamirauá	1st
4	Tucuruí	Borba	3rd
5	Borba	Tapauá	1st
6	Tapauá	Eirunepé	1st
7	Lábrea	RDS Piagaçu-Purus	2nd
8	Santa Cruz	Lábrea	3rd
9	Borba	Santarém	2nd
10	Manacapuru	Santarém	3rd

* From STRUCTURE with migration prior 5×10^{-2}.

https://doi.org/10.1371/journal.pone.0220882.t003

Table 4. Bottleneck metrics for *Arapaima gigas* by locality.

Map location	N	Localities	IAM	TPM	SMM	M value (P)	Ne (95% CI)
1	16	Santa Cruz	0.89844	0.41309	**0.02100**	0.776 (0.0494)	95 (23 – Inf)
2	22	Puerto Nariño	0.27832	0.96582	0.24023	0.742 (0.0093)	14.1 (9.3–23)
3	18	Caranari	**0.00342**	0.08301	0.36523	**0.623 (< 0.0001)**	7.4 (3.3–136)
4	13	Eirunepé	0.32031	0.63770	0.14746	**0.646 (0.0006)**	13.3 (4.7–103.3)
5	32	Mamirauá	**0.00049**	0.05371	0.17480	0.760 (0.0154)	21.6 (14.5–34.6)
6	9	Coari	0.05371	0.36523	0.89844	**0.632 (0.0001)**	41.8 (6.7–Inf)
7	20	RDS Piagaçu-Purus	0.17481	0.32031	0.70020	**0.658 (< 0.0001)**	5.9 (3.0–100)
8	20	Tapauá	**0.03223**	0.69531	0.01896	0.705 (0.0017)	31.6 (15.1–173.3)
9	15	Lábrea	0.27832	0.14746	0.02100	**0.587 (< 0.0001)**	1.1 (0.8–15)
10	18	Manuel Urbano	0.20313	1.00000	0.65234	**0.646 (< 0.0001)**	1.5 (1.0–2.4)
11	30	Maracapuru	0.41309	0.46484	0.00684	0.757 (0.0123)	18.8 (12.4–30.7)
12	21	Resex Unini	0.20606	0.76465	0.57715	**0.658 (0.0001)**	21.9 (11.3–67.9)
13	21	Careiro da Várzea	0.27832	0.89844	0.12305	**0.670 (0.0004)**	10.2 (6.0–177)
14	30	Borba	**0.00342**	0.96582	0.02100	0.694 (0.0005)	23.8 (14.4–47.2)
15	7	Nhamundá	0.96582	0.57715	0.41309	**0.651 (0.0005)**	Inf (20.6—Inf)
16	31	Santarém	**0.01611**	0.89844	0.36523	0.755 (0.0131)	48.8 (25.8–160.1)
17	15	Jacareacanga	**0.01221**	0.46484	0.10156	0.686 (0.0013)	2.0 (1.5–29)
18	30	Região dos Lagos	0.10547	0.55664	0.49219	0.694 (0.0003)	11.8 (6.0–24.3)
19	17	Mexiana	**0.02100**	0.27832	0.70030	**0.576 (< 0.0001)**	14.8 (6.1–687)
20	31	Tucuruí	**0.03483**	0.34907	0.57445	0.735 (0.0151)	5.6 (3.1–109)
21	15	Ilha do Bananal	**0.03125**	**0.03125**	**0.04688**	0.662 (0.0064)	Inf (2.0–Inf)
22	80	APA Meandros do Araguaia	0.12891	**0.00977**	**0.00977**	**0.601 (< 0.0001)**	0.8 (0.5–12)
Total	511						

Note: N = number of individuals analysed; IAM = Infinite Alleles Model; TPM = Two Phase Model; SMM = Stepwise Mutation Model; M value = ratio of number of alleles and allelic spread, see [46]; Ne = Effective population size estimated from linkage disequilibrium. Values in bold are significant. Significance of M values is based on simulations; however, empirical studies suggest that populations that have suffered recent bottlenecks have < 0.68 [46].

https://doi.org/10.1371/journal.pone.0220882.t004

heterozygosity in 12 groups of individuals. Reduction in number of alleles implemented in the program MValue also indicated that 12 *Arapaima* localities experienced a significant reduction in size ($M < 0.68$), according to Garza & Williamson [46], eight of them from tributaries. Areas which showed significant reduction were not necessarily the same in both analysis. Additionally, estimates of effective number of breeders were relatively low for many sites (Table 4).

In addition to recent population declines, coalescent analyses implemented in the program MSVar [55] indicate long-term decline as well (Figs 6 and 7 and Table 5). Analyses partitioned into two (Araguaia-Tocantins and Amazon) or three (Araguaia-Tocantins, lower Amazon and upper Amazon) groups show the same pattern. Historical population sizes of all groups were approximately equal (Ne 4.71, 95% HPD 3.26–6.15 vs. 4.62, 95% HPD 3.28–6.01 and 4.87, 95% HPD 3.51–6.27; Theta 0.98, 95% HPD 0.23–1.77 vs. 1.13, 95% HPD 0.59–1.65 and 1.43, 95% HPD 0.85–2.01) and began to decline at approximately the same time (4.49, 95% HPD 3.09–5.89 vs. 4.78, 95% HPD 3.39–6.15 and 4.65, 95% HPD 3.28–6.04). However, while populations declines centered on an order of magnitude in the Amazon basin (1.11, 95% HPD 1.62–0.60 and 1.33, 95% HPD 1.91–0.71), they were over two orders of magnitude in the Araguaia-Tocantins basin (2.09, 95% HPD 2.89–1.25).
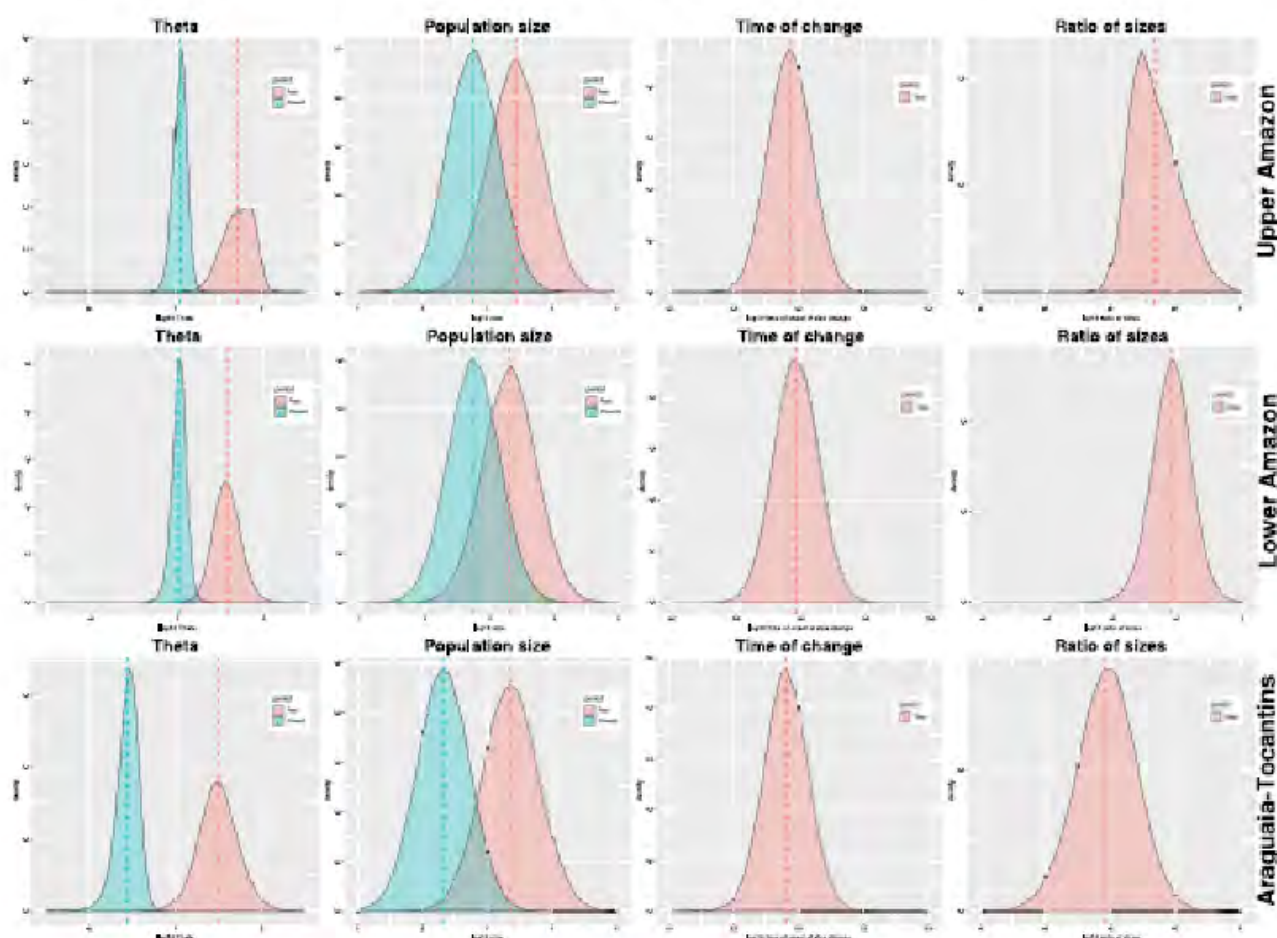
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Arapaima gigas
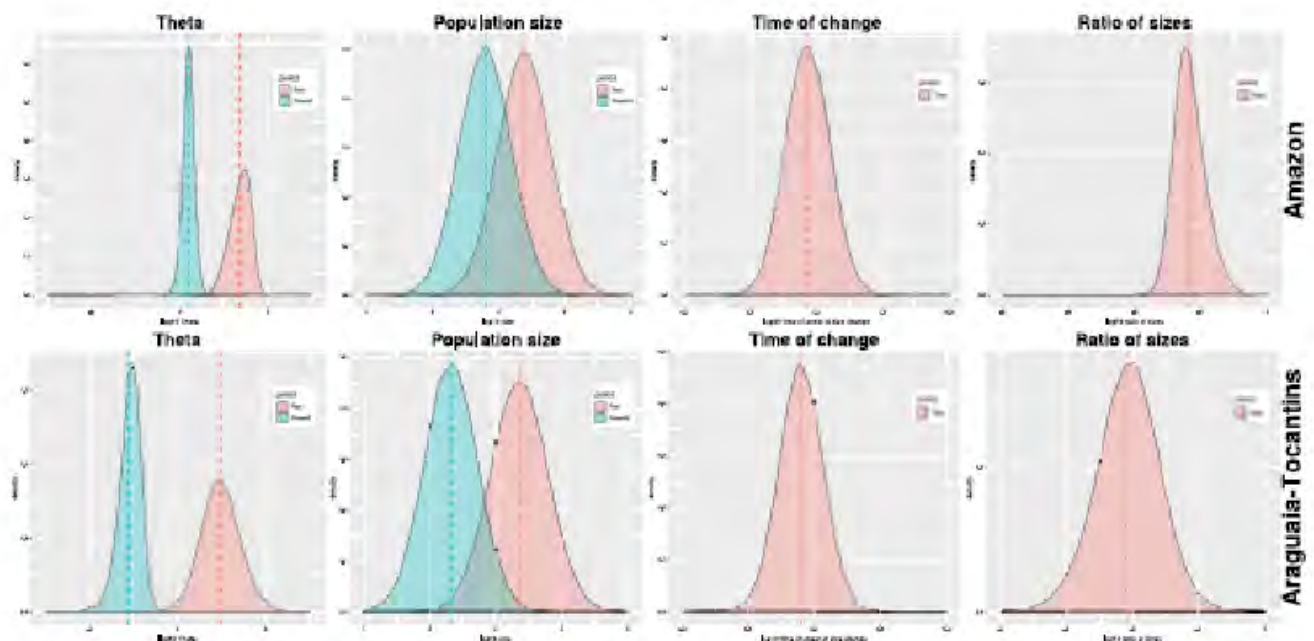
Fig 6. Coalescent population size change, Araguaia-Tocantins vs. Amazon. Note all metrics are log10 scale. Theta–historical and current theta (4Neμ); Population size–historical and current effective population size (Ne); Time of change–onset of population size change from historical to current theta; Ratio of sizes–ratio of current to historical theta.

Discussion

The arapaima is a charismatic fish of special cultural and socioeconomic significance to riverine communities of the Amazon, as well as occupying an apex ecological role in aquatic habitats [15, 16]. As obligate air breathing fish, an adaptation which allows them to exploit hypoxic floodplain environments, *Arapaima gigas* must regularly surface to renew the air in its highly vascularized swim bladder, but this dynamic also makes them especially vulnerable to human exploitation [56]. *Arapaima gigas* has been part of the diet of the riparian inhabitants of Amazonia since the early 18th century [56, 57] and gradually gained significant commercial importance. However, catches began to decline at least as early as the 1960s, and by the 1980s *Arapaima gigas* was commercially extinct close to major urban centers [56, 58, 59]. In 1975, *Arapaima gigas* was listed in Appendix II of CITES (Convention on International Trade in Endangered Species) as a species not necessarily under threat of extinction, but for which commercial use must be controlled to avoid utilization incompatible with its survival; yet it is still considered data deficient.

Population structure and isolation-by-distance in a complex river system

One of the most prominent uncertainties surrounding *Arapaima* is the number of species present in this genus and their distribution. Contrary to the assertions of Stewart [9] [10], we find no evidence for multiple species of *Arapaima* existing or co-existing in the Amazon basin and satellite river basins. Our sampling does not include, however, samples from the

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Arapaima gigas

Fig 7. Coalescent population size change, Araguaia-Tocantins vs. lower Amazon vs. upper Amazon. Note all metrics are log10 scale. Theta–historical and current theta (4.Neμ); Population size–historical and current effective population size (Ne); Time of change–onset of population size change from historical to current theta; Ratio of sizes–ratio of current to historical theta.

Rupununi, a floodplain in the headwaters of the Essequibo River, and an area of occurrence of *Arapaima arapaima* (Valenciennes, in Cuvier and Valenciennes, 1847). However, based on extensive ichthyofaunal sharing between the Essequibo and Branco (Amazon) basin—254 freshwater fish species representing ~73.8% of the total species sampled [60], and the mid-Pleistocene separation of these basins [61, 62], we view it unlikely that the population from the Rupununi is not *A. gigas*.

While we find no evidence for the existence of multiple species of arapaima in the Amazonian ecosystem, we observed population structuring. We found that the greatest structure in our genetic data reflected geographic disjunction of *A. gigas* in the upper Araguaia-Tocantins —a basin that become effectively isolated from the Amazon basin at the Plio-Pleistocene boundary [63], while the remaining fishes from the Amazonas basin reflected a single, albeit

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Table 5. Population decline coalescent metrics for *Arapaima gigas* by region.

Region	Metric	log value (95% HPD)
Araguaia	Current N_E	2.62 (1.27–3.99)
	Past N_A	4.71 (3.26–6.15)
	Beginning of decline	4.49 (3.09–5.89)
	Current Theta	-1.10 (-1.50 – -0.65)
	Past Theta	0.98 (0.23–1.77)
	Magnitude of decline	-2.09 (-2.89 – -1.25)
Amazon	Current N_E	3.61 (2.30–4.92)
	Past N_A	4.79 (3.48–6.15)
	Beginning of decline	4.65 (3.29–6.03)
	Current Theta	0.18 (-0.04–0.40)
	Past Theta	1.36 (0.96–1.74)
	Magnitude of decline	-1.18 (-1.58 – -0.78)
lower Amazon	Current N_E	3.51 (2.21–4.84)
	Past N_A	4.62 (3.28–6.01)
	Beginning of decline	4.78 (3.39–6.15)
	Current Theta	0.02 (-0.26–0.30)
	Past Theta	1.13 (0.59–1.65)
	Magnitude of decline	-1.11 (-1.62 – -0.60)
upper Amazon	Current N_E	3.54 (2.24–4.86)
	Past N_A	4.87 (3.51–6.27)
	Beginning of decline	4.65 (3.28–6.04)
	Current Theta	0.10 (-0.15–0.35)
	Past Theta	1.43 (0.85–2.01)
	Magnitude of decline	-1.33 (-1.91 – -0.71)

Note: Beginning of decline is reported in years based on generation time of five years; Magnitude of decline is ratio of current and past Theta (and N_E).

structured population along the east-west axis, with fishes of the Região dos Lagos in Amapá representing eastern-most extreme of this gradient. This was consistent between the STRUCTURE, SAMOVA, and DAPC analyses. While it may be tempting to hypothesize that the Araguaia-Tocantins populations reflect undescribed species, we note that the degree of genetic divergence of these populations (e.g. as measured by F_{ST}) falls within the range of many widespread but cohesive species e.g. Hey & Pinho [64], and moreover, although contemporary gene flow between Araguaia-Tocantins and Amazon may be restricted, the admixture evident in localities in the eastern Amazon reflects historical gene flow between these areas (Fig 2). Thus, we continue to refer to all individuals as part of a single species (*Arapaima gigas* Schinz 1822), though we note that our sampling did not include the region from which *A. arapaima* is described (Guyana).

Within the Amazon—independent whether or not Amapá and/or Araguaia-Tocantins, the two regions not draining directly into the Amazon, were included—there was a strong and significant association between genetic divergence and geographical proximity, indicating that the attenuation of gene flow by distance (isolation-by-distance) is the major process structuring Amazon *A. gigas* populations on a large geographical scale. This agrees with the results of Hrbek et al. [24, 25] who surveyed additional loci but fewer and more distantly spaced localities. Intriguingly, positive spatial autocorrelation, reflecting the distance across which the homogenizing effects of gene flow are expected to dominate the diversifying effects of genetic

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drift, was significant up to 1500 km, a notable distance considering that adult *A. gigas* are known to be largely sedentary, only making small-scale annual migrations to and from the floodplain. We did, however, also discover a number of recent-generation migrants, some between relatively distant localities (Table 3), an inference supported by field and telemetry data [65]. If indeed adults are generally sedentary and show site fidelity, this may suggest an important role for juvenile dispersal as a means of conveying gene flow between sub-populations.

It would be convenient to assume that the weak population structure is the result of contemporary demographic and evolutionary processes as mediated by current landscape structure, but it is well known that the Amazon landscape itself has had a dynamic history. In the area occupied by the larger *A. gigas* population, the Amazonas River in its current west-to-east format is understood to have only formed around 10–11 million years ago (mya) with the breaching of the "Purus Arch", a basement arch now largely buried and so named for its location along the Solimões near the Purus River [66, 67]. Prior to this breaching, the "Proto-Solimões" and western Amazon drained northward to the Caribbean and separately from more eastern Atlantic versants [67]. Indeed, previous studies have inferred an effect of the Purus arch not only on species distributions but on contemporary population structuring of wide-spread species as well. For example, Farias and Hrbek [68], in their analysis of the genus *Symphysodon*, inferred lineage distributions consistent with the Purus arch. Similarly, Willis et al. [69] discovered that genetic diversity in *Cichla monoculus* west of the Purus Arch was a subset of that found farther east, consistent with ancient east-to-west colonization. Importantly, *Arapaima* fossils similar to *A. gigas* have been discovered in the Miocene age La Venta formation of Colombia (~13 mya) [70], an area that would have been part of the northward-draining "Lago Pebas" system that shortly preceded the current west-to-east arrangement [66, 71]. So, *Arapaima* may have been present but separated on both sides of the Purus Arch, or colonized the eastern Amazon from the Lago Pebas system in the west. However, we saw no clear indications of diminished genetic diversity in eastern populations (apart from localized depletions discussed below), and plots of F_{ST} vs. geographic distance were fairly continuous among localities on either side of the Purus Arch (not shown). Thus, if the biogeographic history of *Arapaima* was significantly influenced by the Purus Arch, gene flow in the intervening period appears to have largely obscured these effects (see also Hrbek et al. [24]).

The degree of population genetic structure in *Arapaima gigas*, with significant genetic divergence among most localities (S2 Table), is notable for Amazonian fishes, whose continued study has revealed important variance in population structuring patterns. For example, examining the mitochondrial control region at locations on the Amazonas River mainstem, Santos et al. [72] and Farias et al. [73] found high genetic variability for tambaqui (*Colossoma macropomum*) and relatively low population structuring over vast distances. An analysis of nuclear microsatellites of *C. macropomum* populations from the Amazon mainstem and its main tributaries by Santos et al. [74] confirmed the mtDNA pattern, however, populations from tributaries and principally those close to headwaters also showed certain degree of differentiation. Similarly, Batista & Alves-Gomes [75], who examined the control region of the catfish *Brachyplatystoma rousseauxii*, also found high levels of genetic polymorphism and the absence of structuring. Similar patterns have been found with mtDNA or microsatellite markers for *Brachyplatystoma platynemum* [76], *Brycon amazonicus* [77], and *Prochilodus nigricans* [78]. Notably, these species share a migratory or semi-migratory and broadcast-spawning life history, with no parental care. In contrast, the patterns discovered for *A. gigas* are more similar to other species with sedentary adults, limited batch fecundity, and/or significant parental care, including the cichlids *Cichla* [79, 80] and *Symphysodon* [81] and the freshwater ray *Paratrygon aiereba* [82]. Thus, it appears there is a general trend in which genetic variability and the

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degree of population structure is strongly determined by life history strategy, an observation that may assist in defining effective management strategies in the absence of more detailed information [78][83]. However, we recommend that additional studies to determine the contributions of variation in body size, habitat preferences, and biogeographic history would be prudent [84].

Implications for *Arapaima* conservation in a threatened river system

The floodplain has been reported as the environment with the highest productivity in the Amazon drainage basin, and is the most common environment of the Solimões-Amazonas axis. The flood-ebb system of the flooded forest, or 'flood pulse', provides exceptional availability of diverse habitats and seasonal resource abundance [85]. However, the floodplain is also the most threatened habitat [86][87]. *Arapaima gigas* is a floodplain specialist, inhabiting lakes and lagoons that are connected by channels to the riverine network of the Amazonas basin. Although *A. gigas* is not a "migratory" species, these fishes do make small lateral movements through the network of lakes and channels of the Amazon floodplain, which provide abundant food for growing young [18]. These rich floodplains also provide important corridors for linear migration of individuals along and among river courses [18].

Although, as demonstrated here, while the major population structure of *Arapaima gigas* are the differences among the Araguaia and Amazon basins and the hydrologically isolated Região dos Lagos (Amapá), the Amazonas population is structured by isolation-by-distance at the largest scale—with the Região dos Lagos representing its eastern-most extreme, we discovered statistically significant genetic divergence (i.e. F_{ST}) among most localities (S3 Table); in fact, few localities were not significantly divergent, although few F_{ST} values were greater than 0.2—at mutation-drift equilibrium equivalent to $Nm = 1$. These results indicate that at the smallest sampled scales, sub-populations of *A. gigas* are likely to be demographically somewhat independent, and cannot be assumed to compensate for exploitation or replenish one another over fishery-relevant timescales (e.g. Waples [88]). As such, *A. gigas* at these, and potentially smaller, spatial scales should be considered separate Management Units [89]. This would explain why population trends are different between the Araguaia-Tocantins and Amazon basins, and also some of the differences between the upper and lower Amazon basins.

In this context, the results indicating recent population declines (genetic bottlenecks) compounded onto long-term demographic declines potentially associated with the extent and distribution of Amazonian floodplains [90, 91] and low effective number of breeders is concerning (Table 4), since it appears that demographic recovery will largely depend on local recruitment rather than immigration. Indeed, the majority of localities exhibited effective population sizes well below the "50/500" threshold for limiting inbreeding depression (50) and loss of genetic diversity due to excessive genetic drift (500) [92], guidelines that have even been suggested as needing upward revision [93]. We note, however, that basing management goals from meta-analyses can be risky [94], and species with high parental investment and smaller lifetime fecundity ("K" or "equilibrium" strategists; [95, 96]) often have higher census to effective population size ratios. It is interesting to note recent indications that the socially monogamous *A. gigas* may regularly engage in polygamous reproduction [97], thereby reducing potential inbreeding and subsequent genetic erosion. Moreover, for species with sedentary adults and potentially significant small-scale spatial genetic (family) structure, it is unclear what an expected effective population size should be at any given spatial scale, and low numbers may be a natural aspect of species that otherwise effectively purge deleterious recessive alleles [98]. However, small populations with low growth rates may nonetheless also experience higher rates of population extirpation in the face of environmental variation [99], and

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these dynamics may be exacerbated by human exploitation [100]. Given this uncertainty, we suggest that studies examining the minimum viable population size for *A. gigas* are warranted.

To curb continuing population declines, in 2001 fishing of *Arapaima gigas* was banned by IBAMA (Brazilian Institute of Environment and Renewable Natural Resources), except in management areas such as the Mamirauá and Piagaçu-Purus Sustainable Development Reserves (RDS) where fishing is controlled, preventing local overexploitation [101, 102]. Additional successes in sustainable exploitation of *A. gigas* have been reported for community based management initiatives in which individual communities or families restrict access to spatially delimited populations of *A. gigas* e.g. [103]. Whether these successes can be replicated more widely and ensure long-term viability of *A. gigas* remains to be seen, especially with regard to evidence of low effective population sizes, genetic bottlenecks, and the potential for erosion of genetic diversity recovered here (Table 4). Whether or not these genetic patterns are the result of documented overexploitation, small populations depend on dispersal and immigration to maintain long-term genetic diversity e.g. [103]. Genetic diversity is the raw material upon which evolution acts, enabling populations to evolve in response to environmental changes, and without which a population may be more susceptible to extirpation or extinction [103, 104]. Here, our observation of recent migrants among populations is important, since it is these individuals that boost genetic variation in populations otherwise limited by local abundance. However, to effectively increase local diversity, these individuals must first successfully disperse. Despite localized sustainable initiatives and a ban on *A. gigas* fishing in Brazil, illegal fishing continues and creates risk even for dispersers among sustainably-managed areas. Moreover, the floodplain habitats of the Amazon basin through which these dispersers move have been reported as the most threatened in South America due to logging, forest clearing for cattle-ranching, construction of hydroelectric dams, and other disturbances [86]. The long-term viability of *A. gigas* fisheries will ultimately depend on addressing these significant regional challenges as well.

Some sub-populations of *Arapaima gigas* also face more localized hurdles to viability. We observed that statistics of genetic diversity were variable across localities, a result also observed by Hrbek et al. [24] with mtDNA. Intriguingly, although localities along the Amazon mainstem (e.g. Santarém, Carreiro da Varzea, Manacapuru, Coari) are those facing the greatest fishing pressure, they are also among the localities with the highest genetic diversity (Table 2), a feature we hypothesize to relate to their positions near the intersections of the river network. Despite their genetic diversity, continued illicit exploitation suggest that the longevity of these populations may depend on management regimes than ensure minimum viable populations. On the other hand, several locations in the upstream portions of tributaries (Eirunepé, Manuel Urbano, Meandros do Araguaia) exhibit lower genetic diversity, a worrisome trend considering that, even though these are the sub-populations that currently face lower fishing pressure, they are increasingly subjected to major habitat changes in Brazil's "arc of deforestation." As such, these localities may be those for which inbreeding and loss of genetic diversity would be most problematic, and for which immigration may be the most beneficial. Thus, one size does not fit all, and management strategies for the management units will need to be tailored to local challenges.

Arapaima from the unique extra-Amazon populations

Although the population in the Amazonas basin *sensu stricto* contains the majority of individuals and greatest fishing pressure, populations in the Araguaia-Tocantins and Amapá deserve special consideration. The divergence and genetic poverty of the *Arapaima gigas* in the Araguaia River (APA Meandros do Araguaia and Ilha do Bananal) observed here corroborate the findings of Vitorino et al. [105], who showed low values of genetic diversity and structuring

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between populations of four localities in Araguaia and Tocantins Rivers, and those of Hrbek et al. [25], who discovered a number of mtDNA haplotypes endemic to the Tocantins basin. The Araguaia-Tocantins River basin is connected to the Amazonas basin by uninterupted freshwater, albeit in the form of small meandering channels, and many researchers do not consider the Araguaia-Tocantins part of the Amazonas basin, since it drains primarily through the "Pará River" to the south of Marajó Island. However, as the presence of *A. gigas* in both basins implies, they share a close biogeographic history and exhibit similar icthyofaunas [106], along with several other adjacent Atlantic versants (e.g. Araguari, Oiapoque, Parnaíba). However, although numerous species are apparently distributed in both basins, several recent studies have shown that the Araguaia and/or Tocantins exhibit endemic lineages of fishes e.g. [69, 107, 108] and other aquatic organisms, including the Araguaian river dolphin *Inia araguaiaensis* [109]. The confirmation of an Araguaian population of *A. gigas* is in keeping with these trends.

The most likely feature promoting divergence of aquatic organisms in the upper Tocantins basin is, ironically, one that no longer exists, the Itaboca waterfalls that were submerged when the Tucuruí hydroelectric dam was constructed. As such, the Tucuruí population of *A. gigas*, which presented as a genetic intermediate between the Araguaia and eastern Amazon subpopulation in STRUCTURE and DAPC analyses, presents something of a conundrum. The sampling locations in the upper Araguaia River (AEP Meandros do Araguaia and Ilha do Bananal), in addition to being about 1,340 and 890 km, respectively, from the reservoir, are also separated by numerous rapids which may limit the gene flow between these areas, implying that the pattern of admixture may be natural. However, the dam itself was also built downstream of the historical barrier (Itaboca), trapping some of the downstream fauna within the flooded region. Thus, the admixture of lower Amazon/lower Tocantins and upper Tocantins lineages may instead be an anthropogenic effect of reservoir construction. Indeed, several other studies have suggested that aquatic organisms of Tucuruí exhibit unique patterns of admixture or hybridization different from that of the lower Tocantins e.g. [69]. In the face of planned and ongoing construction of hydroelectric dams on numerous rivers in Brazil, this trend should serve as a cautionary tale.

The Araguaia-Tocantins basin is considered highly threatened not only by hydroelectric projects in the headwaters of tributary rivers, but it is also among the areas in Brazil with the highest rates of landscape modification for cattle ranching, road construction, and agriculture [22]. The unique population of *A. gigas* in this region, despite relatively low fishing pressure, should thus be considered highly threatened, especially considering these fishes exhibited the lowest observed levels of genetic diversity. Whether this reduced diversity results from natural (e.g. Pleistocene-age colonizations or bottlenecks) or anthropogenic effects, the ability of this unique population to remain viable in the face of ongoing habitat modifications should be closely monitored.

The population of *Arapaima gigas* from the Região dos Lagos in Amapá are significant as well. This region, which is technically connected to the Amazonas basin by freshwater from the Amazonas outflow, exhibits an icthyofauna with affinities both for that of the Amazon, as well as those of coastal Guyana drainages to the north [106]. The presence of *A. gigas* here is thus not a surprise, although, considering the strong and turbulent current that runs along the coast and probably limits dispersal, nor is their distinctness from the other populations. Fortunately, a significant portion of the Região dos Lagos is protected by a state park which limits exploitation, although it remains unknown if *A. gigas* from the Araguari River are part of this population as well. In either case, habitat degradation from cattle ranching and urban development continues to encroach upon the watersheds were this population is found, and being limited to such a relatively small area, this unique population could easily be placed at risk if current protections were to prove inadequate.

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Conclusions

The findings presented here should be seen as an important warning about the fragility of *Arapaima gigas* populations, given the evidence of reduced genetic capacity and the intersection of threats against them. These data should contribute toward the design of management and conservation programs for this species in the Amazon, Amapá, and Araguaia-Tocantins regions.

Ethics statement

Permits for field collection and molecular analyses were given by IBAMA/CGEN 11325–1. This study involved no experimentation, manipulation or euthanasia of any animals on part of the researchers. We simply collected tissue samples of exemplars fished by local communities for food and as fishes were brought to market. In these instances our institution (UFAM) does not require IACUC approval. Finally, this research could not have been submitted to IACUC for analysis since this committee became operational only after the completion of this study.

Supporting information

S1 Fig. Graphic representation of the average number of alleles per population of *Arapaima gigas*.
(EPS)

S2 Fig. Graphic representation of expected heterozygosity (H_E) per population of *Arapaima gigas*.
(EPS)

S3 Fig. Absolute values of the 2nd order rate of change of mean likelihoods of each K.
(EPS)

S4 Fig. Graphic representation of pairwise F_{ST} values among groups of individuals of *Arapaima gigas*.
(EPS)

S1 Table. Characteristics of the 11 microsatellite loci analyzed for *Arapaima gigas* considering separately the groups of individuals grouped by collection site. N_A = Total number of alleles; A_R = Allelic richness; H_O = Observed Heterozygosity; H_E = Expeted Heterozygosity; mono = Monomorphic locus * significant *P* value for deviation from HWE after Bonferroni correction (P = 0.00455).
(DOCX)

S2 Table. Number of migrants per generation Nm = $M\theta/2$. Row localities are sending individuals, while column localities are receiving individuals. Locality codes are: 1- Santa Cruz, 2- Puerto Nariño, 3- Carauari, 4- Eirunepé, 5- Mamirauá, 6- Coari, 7- RDS Piagaçu-Purus, 8- Tapauá, 9- Lábrea, 10- Manuel Urbano, 11- Manacapuru, 12- Resex Unini, 13- Careiro da Várzea, 14- Borba, 15- Nhamundá, 16- Santarém, 17- Jacareacanga, 18- Região dos Lagos, 19- Mexiana, 20- Tucuruí, 21- Ilha do Bananal, 22- APA Meandros do Araguaia.
(XLSX)

S3 Table. Matrix of pairwise F_{ST} values among localities sampled for *Arapaima gigas*. Note: significant differences at p < 0.05 and after Bonferroni correction are in bold.
(XLSX)

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Acknowledgments

Author Contributions

References

1. Araújo-Lima CARM, Goulding M. Os Frutos do Tambaqui. Ecologia, Conservação e Cultivo na Amazônia. Belem, Brazil: Sociedade Civil Mamirauá –MCT—CNPq; 1998.

2. Goulding M, Smith NJH, Mahar DJ. Floods of Fortune: Ecology and Economy along the Amazon. New York, NY: Columbia University Press; 2000.

3. Prang G. A caboclo society in the middle Rio Negro basin: ecology, economy, and history of an ornamental fishery in the state of Amazonas, Brazil. Wayne State University. 2001.

4. Smith NJH. Caimans, capybaras, otters, manatees, and man in Amazônia. Biol Conserv. 1980; 19: 177–187.

5. Medem F. Los Crocodylia de Sur America. Vol. 2. Bogota, Colombia: Ed. Carrera; 1983.

6. Best RC. Apparent dry-season fasting in Amazonian manatees (Mammalia: Sirenia). Biotropica. 1983; 15: 76–78.

7. Best RC. The aquatic mammals and reptiles of the Amazon. In: Sioli H, editor. The Amazon Limnology and Landscape Ecology of a Mighty Tropical River and its Basins. Dodrecht, The Netherlands: Dr. Junk W Publisher; 1984. pp. 371–412.

8. Goulding M. Fishes and the Forest: Explorations in Amazonian Natural History. Los Angeles, CA: University of California press; 1980.

9. Stewart DJ. A new species of *Arapaima* (Osteoglossomorpha: Osteoglossidae) from the Solimões River, Amazonas State, Brazil. Copeia. 2013; 2013: 470–476. https://doi.org/10.1643/CI-12-017

10. Stewart DJ. Re-description of *Arapaima agassizii* (Valenciennes), a rare fish from Brazil (Osteoglossomorpha: Osteoglossidae). Copeia. 2013; 2013: 38–51. https://doi.org/10.1643/CI-12-013

11. Spix JB von, Agassiz L. Selecta genera et species piscium Brasiliensium: Quos in itinere per Brasiliam annis MDCCCXVII-MDCCCXX jussu et auspiciis Maximiliani Josephi I. Munich, Germany: Bavariae regisaugustissimiperacto collegit et pingendos curavit Dr. J. B. de Spix; 1829.

12. Günther A. Catalogue of the fishes in the British Museum, Volume 7. London, England: The British Museum (Natural History); 1868.

13. Saint-Paul U. Potential for aquaculture of South American freshwater fishes: a review. Aquaculture. 1986; 54: 205–240.

226

3

36. Dupanloup I, Schneider S, Excoffier L. A simulated annealing approach to define the genetic structure of populations. Mol Ecol. 2002; 11: 2571–2581. https://doi.org/10.1046/j.1365-294X.2002.01650.x PMID: 12453240

37. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics. 1992; 131: 479–491. Available: https://www.ncbi.nlm.nih.gov/pubmed/1644282 PMID: 1644282

38. Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 2010; 11: 94. https://doi.org/10.1186/1471-2156-11-94 PMID: 20950446

39. Jombart T. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 2008; 24: 1403–1405. https://doi.org/10.1093/bioinformatics/btn129 PMID: 18397895

40. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2018. Available: http://www.r-project.org

41. Mantel N. The detection of disease clustering and a generalized regression approach. Cancer Res. 1967; 27: 209–220. Available: https://www.ncbi.nlm.nih.gov/pubmed/6018555 PMID: 6018555

42. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package [Internet]. 2018. Available: https://cran.r-project.org/package=vegan

43. Beerli P, Felsenstein J. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proc Natl Acad Sci U S A. 2001; 98: 4563–4568. https://doi.org/10.1073/pnas.081068098 PMID: 11282657

44. Meirmans PG, Van Tienderen PH. GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes. 2004; 4: 792–794. https://doi.org/10.1111/j.1471-8286.2004.00770.x

45. Piry S, Luikart G, Cornuet J-M. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. J Hered. 1999; 90: 502–503. https://doi.org/10.1093/jhered/90.4.502

46. Garza JC, Williamson EG. Detection of reduction in population size using data from microsatellite loci. Mol Ecol 2001; 10: 305–318. https://doi.org/10.1046/j.1365-294X.2001.01190.x PMID: 11298947

47. Beaumont MA. Detecting population expansion and decline using microsatellites. Genetics. 1999; 153: 2013–2029. Available: https://www.ncbi.nlm.nih.gov/pubmed/10581303 PMID: 10581303

48. Storz JF, Beaumont MA. Testing for genetic evidence of population expansion and contraction: an empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. Evolution (N Y). 2002; 56: 154–166. https://doi.org/10.1111/j.0014-3820.2002.tb00857.x

49. Gelman A, Rubin DB. Inference from iterative simulation using multiple sequences. Stat Sci. 1992; 7: 457–472.

50. Plummer M, Best N, Cowles K, Vines K. CODA: Convergence diagnosis and output analysis for MCMC. R News. 2006; 6: 7–11. Available: https://journal.r-project.org/archive/

51. Wickham H. ggplot2: Elegant Graphics for Data Analysis [Internet]. Springer-Verlag New York; 2009. Available: http://ggplot2.org

52. Waples RS, Do C. Ldne: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour. 2008; 8: 753–6. https://doi.org/10.1111/j.1755-0998.2007.02061.x PMID: 21585883

53. Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol Ecol Resour. 2014; 14: 209–214. https://doi.org/10.1111/1755-0998.12157 PMID: 23992227

54. Palstra FP, Fraser DJ. Effective/census population size ratio estimation: a compendium and appraisal. Ecol Evol. 2012; 2: 2357–2365. https://doi.org/10.1002/ece3.329 PMID: 23139893

55. Storz JF, Beaumont MA, Alberts SC. Genetic evidence for long-term population decline in a savannah-dwelling primate: inferences from a hierarchical Bayesian model. Mol Biol Evol. 2002; 19: 1981–1990. https://doi.org/10.1093/oxfordjournals.molbev.a004022 PMID: 12411607

56. Verissimo J. A Pesca na Amazônia. Rio de Janeiro, Brazil: Livraria Clássica, de Alves & C.; 1895.

57. Kurlansky M. Cod: A Biography of the Fish that Changed the World. London, England: Jonathan Cape; 1998.

58. Goulding M. Ecologia de Pesca do Rio Madeira. Manaus, Brazil: INPA; 1979.

59. Crossa M. NM, Petrere M Jr. Morphometric relationships and indirect determination of the length frequency structure of the pirarucu, *Arapaima gigas* (Cuvier), in Brazilian Amazonia. Fish Manag Ecol. 1999; 6: 233–240.

228

60. Souza LS de, Armbruster JW, Werneke DC. The influence of the Rupununi portal on distribution of freshwater fish in the Rupununi district, Guyana. Cybium. 2012; 36: 31–43.

61. Schaefer CEGR, Vale Júnior JF. Mudanças climáticas e evolução da paisagem em Roraima: uma resenha do Cretáceo ao recente. In: Barbosa RI, Ferreira EJG, Castellón EG, editors. Homem, Ambiente e Ecologia no Estado de Roraima. Manaus, AM: Instituto Nacional de Pesquisas da Amazônia (INPA); 1997. pp. 231–265.

62. Cremon ÉH, Rossetti D de F, Sawakuchi A de O, Cohen MCL. The role of tectonics and climate in the late Quaternary evolution of a northern Amazonian River. Geomorphology. Elsevier B.V.; 2016; 271: 22–39. https://doi.org/10.1016/j.geomorph.2016.07.030

63. Rossetti D de F, Valeriano M de M. Evolution of the lowest Amazon basin modeled from the integration of geological and SRTM topographic data. Catena. 2007; 70: 253–265. https://doi.org/10.1016/j.catena.2006.08.009

64. Hey J, Pinho C. Population genetics and objectivity in species diagnosis. Evolution (NY). 2012; 66: 1413–1429. https://doi.org/10.1111/j.1558-5646.2011.01542.x PMID: 22519781

65. Campos-Silva JV, Hawes JE, Peres CA. Population recovery, seasonal site fidelity, and daily activity of pirarucu (*Arapaima* spp.) in an Amazonian floodplain mosaic. Freshw Biol. 2019; 64: 1255–1264. https://doi.org/10.1111/fwb.13301

66. Bemerguy RL, Costa JBS. Considerações sobre a evolução do sistema de drenagem da Amazônia e sua relação com o arcabouço tectônico-estrutural. Bol do Mus Para Emílio Goeldi, Série Ciências da Terra. 1991; 3: 75–97.

67. Rossetti D de F, Cohen MCL, Tatumi SH, Sawakuchi A de O, Cremon ÉH, Mittani JCR, et al. Mid-Late Pleistocene OSL chronology in western Amazonia and implications for the transcontinental Amazon pathway. Sediment Geol. Elsevier B.V.; 2015; 330: 1–15. https://doi.org/10.1016/j.sedgeo.2015.10.001

68. Farias IP, Hrbek T. Patterns of diversification in the discus fishes (*Symphysodon* spp. Cichlidae) of the Amazon basin. Mol Phylogenet Evol. 2008; 49: 32–43. https://doi.org/10.1016/j.ympev.2008.05.033 PMID: 18762435

69. Willis SC, Macrander J, Farias IP, Ortí G. Simultaneous delimitation of species and quantification of interspecific hybridization in Amazonian peacock cichlids (genus *Cichla*) using multi-locus data. BMC Evol Biol. 2012; 12: 96. https://doi.org/10.1186/1471-2148-12-96 PMID: 22727018

70. Lundberg JG, Chernoff B. A Miocene fossil of the Amazonian fish *Arapaima* (Teleostei: Arapaimidae) from the Magdalena River region of Colombia: biogeographic and evolutionary implications. Biotropica. 1992; 24: 2–14.

71. Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, et al. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science (80-). 2010; 330: 927–931. https://doi.org/10.1126/science.1194585 PMID: 21071659

72. Santos M da CF, Ruffino ML, Farias IP. High levels of genetic variability and panmixia of the tambaqui *Colossoma macropomum* (Cuvier, 1818) in the main channel of the Amazon River. J Fish Biol 2007; 71A: 33–44. https://doi.org/10.1111/j.1095-8649.2007.01514.x

73. Farias IP, Torrico JP, García-Dávila C, Santos M da CF, Hrbek T, Renno J-F. Are rapids a barrier for floodplain fishes of the Amazon basin? A demographic study of the keystone floodplain species *Colossoma macropomum* (Teleostei: Characiformes). Mol Phylogenet Evol. 2010; 56: 1129–1135. https://doi.org/10.1016/j.ympev.2010.03.028 PMID: 20362063

74. Santos M da CF, Hrbek T, Farias IP. Multilocus approach to understand the historical and contemporary genetic connectivity of the keystone floodplain species *Colossoma macropomum* (Teleostei: Characiformes) in the Amazon basin. Front Genet. 2018; 9: 263. https://doi.org/10.3389/fgene.2018.00263 PMID: 30154822

75. Batista J da S, Alves-Gomes JA. Phylogeography of *Brachyplatystoma rousseauxii* (Siluriformes—Pimelodidae) in the Amazon Basin offers preliminary evidence for the first case of "homing" for an Amazonian migratory catfish. Genet Mol Res. 2006; 5: 723–740. PMID: 17183183

76. Ochoa LE, Pereira LHG, Costa-Silva GJ, Roxo FF, Batista J da S, Formiga K, et al. Genetic structure and historical diversification of catfish *Brachyplatystoma platynemum* (Siluriformes: Pimelodidae) in the Amazon basin with implications for its conservation. Ecol Evol. 2015; 5: 2005–2020. https://doi.org/10.1002/ece3.1486 PMID: 26045952

77. Oliveira RC de, Santos M da CF, Bernardino G, Hrbek T, Farias IP. From river to farm: an evaluation of genetic diversity in wild and aquaculture stocks of *Brycon amazonicus* (Spix & Agassiz, 1829), Characidae, Bryconinae. Hydrobiologia. 2018; 805: 75–88. https://doi.org/10.1007/s10750-017-3278-0

78. Machado VN, Willis SC, Hrbek T, Farias IP. Population genetic structure of the Amazonian black flannelmouth characin (Characiformes, Prochilodontidae: *Prochilodus nigricans* Spix & Agassiz, 1829):

229

contemporary and historical gene flow of a migratory and abundant fishery species. Environ Biol Fishes. 2017; 100: 1–16. https://doi.org/10.1007/s10641-016-0547-0

79. Willis SC, Winemiller KO, Montaña CG, Macrander J, Reiss P, Farias IP, et al. Population genetics of the speckled peacock bass (*Cichla temensis*). South America's most important inland sport fishery. Conserv Genet. 2015; 16: 1345–1357. https://doi.org/10.1009/s10592-015-0744-y

80. Willis SC. One species or four? Yes!. . .and, no. Or, arbitrary assignment of lineages to species obscures the diversification processes of Neotropical fishes. PLoS One. 2017; 12: e0172349. https://doi.org/10.1371/journal.pone.0172349 PMID: 28235096

81. Amado MV, Hrbek T, Farias IP. A molecular perspective on systematics, taxonomy and classification Amazonian discus fishes of the genus *Symphysodon*. Int J Evol Biol. 2011; 2011: 360654. https://doi.org/10.4061/2011/360654 PMID: 21811676

82. Frederico RG, Farias IP, Araújo MLG, Charvet-Almeida P, Alves-Gomes JA. Phylogeography and conservation genetics of the Amazonian freshwater stingray *Paratrygon aiereba* Müller & Henle, 1841 (Chondrichthyes: Potamotrygonidae). Neotrop Ichthyol. 2012; 10: 71–80. https://doi.org/10.1590/S1679-62252012000100007

83. Winemiller KO. Life history strategies, population regulation, and implications for fisheries management. Can J Fish Aquat Sci. 2005; 62: 872–885. https://doi.org/10.1139/f05-040

84. Meirmans PG, Goudet J, Gaggiotti OE. Ecology and life history affect different aspects of the population structure of 27 high-alpine plants. Mol Ecol. 2011; 20: 3144–3155. https://doi.org/10.1111/j.1365-294X.2011.05164.x PMID: 21689194

85. Lowe-McConnell RH. Ecological aspects of seasonality in fishes of tropical waters. Symp Zool Soc London. 1979; 44: 219–241.

86. Castello L, McGrath DG, Hess LL, Coe MT, Lefebvre PA, Petry P, et al. The vulnerability of Amazon freshwater ecosystems. Conserv Lett. 2013; 6: 217–229. https://doi.org/10.1111/conl.12008

87. Castello L, Macedo MN. Large-scale degradation of Amazonian freshwater ecosystems. Glob Chang Biol. 2016; 22: 990–1007. https://doi.org/10.1111/gcb.13173 PMID: 26700407

88. Waples RS. Evolutionary significant units, distinct population segments, and the Endangered Species Act: reply to Pennock and Dimmick. Conserv Biol. 1998; 12: 718–721. https://doi.org/10.1111/j.1523-1739.1998.97524.x

89. Palsbøll PJ, Bérubé M, Allendorf FW. Identification of management units using population genetic data. Trends Ecol Evol. 2007; 22: 11–16. https://doi.org/10.1016/j.tree.2006.09.003 PMID: 16982114

90. Behling H. Holocene environmental dynamics in coastal, eastern and central Amazonia and the role of the Atlantic sea-level change. Geogr Helv Swiss J Geogr. 2011; 66: 208–216.

91. Rossetti D de F, Gribel R, Toledo PMde, Tatumi SH, Yee M, Tudela DRG, et al. Unfolding long-term Late Pleistocene–Holocene disturbances of forest communities in the southwestern Amazonian lowlands. Ecosphere. 2018; 9: e02457. https://doi.org/10.1002/ecs2.2457

92. Franklin IR. Evolutionary change in small populations. In: Soule ME, Wilcox BA, editors. Conservation Biology: An Evolutionary-Ecological Perspective. Sunderland, MA, USA: Sinauer Associates, Inc.; 1980. pp. 135–140.

93. Frankham R, Bradshaw CJA, Brook BW. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol Conserv. Elsevier Ltd; 2014; 170: 56–63. https://doi.org/10.1016/j.biocon.2013.12.036

94. Jamieson IG, Allendorf FW. How does the 50/500 rule apply to MVPs? Trends Ecol Evol. Elsevier Ltd; 2012; 27: 580–584. https://doi.org/10.1016/j.tree.2012.07.001 PMID: 22868005

95. Pianka ER. On r- and K-selection. Am Nat. 1970; 104: 592–597. https://doi.org/10.1086/282697

96. Winemiller KO, Rose KA. Patterns of life-history diversification in North American fishes: implications for population regulation. Can J Fish Aquat Sci. 1992; 49: 2196–2218.

97. Farias IP, Leão AS de A, Almeida YS, Verba JT, Crossa M, Honczaryk A, et al. Evidence of polygamy in the socially monogamous Amazonian fish *Arapaima gigas* (Cuvier, 1817). Neotrop Ichthyol. 2015; 13: 195–204. https://doi.org/10.1590/1982-0224-20140010

98. García-Dorado A. On the consequences of ignoring purging on genetic recommendations for minimum viable population rules. Heredity (Edinb). 2015; 115: 185–187. https://doi.org/10.1038/hdy.2015.28 PMID: 25873145

99. Frankham R. Genetics and extinction. Biol Conserv. 2005; 126: 131–140. https://doi.org/10.1016/j.biocon.2005.05.002

100. Purcell SW, Polidoro BA, Hamel J-F, Gamboa RU, Mercier A. The cost of being valuable: predictors of extinction risk in marine invertebrates exploited as luxury seafood. Proc R Soc B Biol Sci. 2014; 281: 20133296–20133296. https://doi.org/10.1098/rspb.2013.3296 PMID: 24598425

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101. Queiroz HL de, Crampton WGR. Estratégias para Manejo de Recursos Pesqueiros em Mamirauá. Brasília, Brazil: Sociedade Civil Mamirauá, MCT—CNPq; 1999. p. 208.

102. Castello L. A method to count pirarucu *Arapaima gigas*: fishers, assessment, and management. North Am J Fish Manag. 2004; 24: 379–389.

103. Campos-Silva JV, Peres CA. Community-based management induces rapid recovery of a high-value tropical freshwater fishery. Sci Rep. Nature Publishing Group; 2016; 6: 34745. https://doi.org/10.1036/srep34745 PMID: 27731319

104. Reed DH, Frankham R. Correlation between fitness and genetic diversity. Conserv Biol. 2003; 17: 230–237.

105. Vitorino CA, Nogueira F, Souza IL, Araripe J, Venere PC. Low genetic diversity and structuring of the arapaima (Osteoglossiformes, Arapaimidae) population of the Araguaia-Tocantins basin. Front Genet. 2017; 8: 1–10. https://doi.org/10.3389/fgene.2017.00001

106. Jégu M, Keith P. Lower Oyapock River as northern limit for the Western Amazon fish fauna or only a stage in its northward progression. C R Biol. 1999; 322: 1133–1143.

107. Willis SC, López-Fernández H, Montaña CG, Farias IP, Ortí G. Species-level phylogeny of "Satan's perches" based on discordant gene trees (Teleostei: Cichlidae: *Satanoperca* Günther 1862). Mol Phylogenet Evol. 2012; 63: 798–808. http://doi.org/10.1016/j.ympev.2012.02.018 PMID: 22406409

108. Amaral CRL, Brito PM, Silva DA, Carvalho EF. A new cryptic species of South American freshwater pufferfish of the genus *Colomesus* (Tetraodontidae), based on both morphology and DNA data. PLoS One. 2013; 8: e74397. https://doi.org/10.1371/journal.pone.0074397 PMID: 24040239

109. Hrbek T, da Silva VMF, Dutra N, Gravena W, Martin AR, Farias IP. A new species of river dolphin from Brazil or: how little do we know our biodiversity. PLoS One. 2014; 9: e0083623. https://doi.org/10.1371/journal.pone.0083623 PMID: 24465386

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