# PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)

## EFFECTS OF NO-TAKE MARINE RESERVES ON FISH ASSEMBLAGES IN BRAZIL: AN ASSESSMENT USING STEREO-VIDEOS

#### **FERNANDA ANDREOLI ROLIM**

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Zoologia).

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Orientador: Otto Bismarck Fazzano Gadig

Co-orientador: Tim Langlois

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TÍTULO DA TESE: EFFECTS OF NO-TAKE MARINE RESERVES ON FISH ASSEMBLAGES IN BRAZIL: AN ASSESSMENT USING STEREO-VIDEOS

**AUTORA: FERNANDA ANDREOLI ROLIM** 

ORIENTADOR: OTTO BISMARCK FAZZANO GADIG

**COORIENTADOR: TIMOTHY LANGLOIS** 

Aprovada como parte das exigências para obtenção do Título de Doutora em CIÊNCIAS BIOLÓGICAS (ZOOLOGIA), pela Comissão Examinadorá:

Prof. Dr. OTTO BISMARCK FAZZANO GADIG

Instituto de Biociências - Câmpus do Litoral Paulista / UNESP

Prof. Dr. DOMINGOS GARRONE NETO

Campus de Registro / UNESP

Prof. Dr. MARCOS CESAR DE OLIVEIRA SANTOS

Instituto Oceanográfico / USP/SP/

Prof. Dr. JUAN PABLO QUIMBAYO AGREDA

Centro de Biologia Marinha / USP

Prof. Dr. GUILHERME HENRIQUE PEREIRA FILHO

Instituto dd Mar / Universidade Federal Paulista/UNIFESP

Rio Claro, 03 de maio de 2019



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Resumo O estabelecimento de reservas marinhas de restrição total (No-take reserves - NTRs), ou seja, áreas com proibição total de pesca, tem sido uma alternativa mundial para preservar a biodiversidade e as funções dos ecossistemas. O Brasil tem importantes NTRs com poucos estudos descrevendo sua relevância e eficiência para a vida marinha. Com isso, o objetivo central desta tese foi avaliar os efeitos de NTRs em assembleias de peixes, testando a hipótese de que as diferenças em riqueza, abundância, biomassa e tamanho corporal dos peixes são mais explicadas pelo status de proteção do que por características ambientais. Para tanto, técnicas inovadoras no Brasil, estéreo-vídeos subaquáticos com isca (Baited Remote Underwater stereo-Videos - stereo-BRUVs) e estéreo-vídeos operados por mergulhador (Diver Operated stereo-Videos - stereo-DOVs) foram testados no Atlântico Sudoeste, comparando com os censos visuais tradicionalmente aplicados (estacionário e transecto), e utilizados para analisar os efeitos da Estação Ecológica Tupinambás e do Parque Nacional Marinho dos Abrolhos nas assembleias de peixes. Para isso, as características das assembleias de peixes em termos de riqueza, biomassa, abundância e tamanho corpóreo, foram comparadas com as áreas onde a pesca é permitida, e a complexidade do habitat foi estimada através das imagens. Em relação à comparação dos métodos, o estéreo-DOV apresentou maior eficiência, amostrando mais riqueza e abundância com menor esforço, e o estéreo-BRUV amostrou uma assembleia específica, composta principalmente por espécies de peixes móveis e de grande porte, geralmente alvos da pesca. Em ambas as NTRs avaliadas, as características das espécies-alvo foram explicadas pelo nível de proteção, enquanto as assembleias de espécies não-alvo foram mais relacionadas às características do habitat. Os resultados incluem a implementação de técnicas não-destrutivas de amostragem da ictiofauna no Brasil, uma geração de conhecimento sobre a ecologia dos peixes e a disseminação da importância dessas áreas protegidas, que auxiliarão nas medidas de manejo e estimularão a sua preservação.

**Palavras-chave** Ecologia marinha, área marinha protegida, peixes recifais, Atlântico Sul Ocidental, EventMeasure, TransectMeasure, GAMMs.

**Abstract** The establishment of no-take marine reserves (NTRs), *i.e.* areas with total fishing restrictions, has been an alternative worldwide aiming to preserve both biodiversity and ecosystem functions. Brazil has important NTRs with few studies describing their relevance and efficiency for marine life. With this, the central objective of this thesis was to evaluate the effects of NTRs on fish assemblage, testing the hypothesis that the differences in richness, abundance, biomass and fish body size is more explained by protection status than environmental characteristics. Thus, innovative techniques in Brazil, Baited Remote Underwater stereo-Videos (stereo-BRUVs) and Diver Operated stereo-Videos (stereo-DOVs) were tested in the Southwestern Atlantic, comparing with traditional visual census (stationary point count and belt transects), and used to analyze the effects of the Tupinambás Ecological Station and Abrolhos Marine National Park on fish assemblages. For this, fish assemblage characteristics, such as richness, abundance, biomass and body size, were compared to areas where fishing is allowed, and the habitat complexity was estimated through the footages. Concerning methods comparison, stereo-DOV showed to be more effective, sampling more richness and abundance within less effort, and Stereo-BRUVs showed to sample a very specific assemblage, comprised mostly by mobile and large bodied fish species, usually targeted by fisheries. Within both NTRs evaluated, target species characteristics was explained by protection status, while non-target species assemblage were more related to habitat characteristics. The results include an implementation of non-destructive ichthyofauna sampling techniques in Brazil, a generation of knowledge about fish ecology and the dissemination of the importance of these protected areas, which will assist management measures and encourage the preservation of the region.

**Key-words** Marine ecology, marine protected area, reef fish, Western South Atlantic, EventMeasure, TransectMeasure, GAMMs.

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#### - General Introduction -

# The development of non-destructive sampling techniques to assess effects of no-take marine reserves on reef fish assemblages

Reef ecosystems are considered one of the most productive and biologically rich ecosystems on the globe, supporting a high biomass of organisms and concentrating a wide range of endemic, threatened and rare species (BURKE et al., 2011). Due to these combination of factors these areas are regarded as hotspots of biodiversity and their conservation and management must be priorities (MYERS et al., 2016; BELLWOOD et al., 2004), especially in face of the numerous threats to which they are subject to, such as overfishing, pollution, oil exploration, coastal development and global warming (BURKE et al., 2011; HOEGH-GULDBERG et al., 2007; ROBERTS et al., 2002).

Reefs are essentially substrates rising above the sourrounding sea bottom to or nearly the surface, fomed either with biogenic origin (i.e. living organisms such as corals and calcareous algae), non-biogenic such as rocks and artificial, with manmade structures (FINKL, 2013; NEUENDROF; MEHL; JACKSON, 1997). These habitats can be found all around the globe and are known to aggregate high diversity and biomass of organisms, due to the high physical complexity (GRAHAM et al.; 2006; MCCOY; BELL, 1991; SALE, 1977; ST. PIERRE; KOVALENKO, 2014).

Rocky reefs are composed mainly by hard substrate of rocky composition with some species of corals and many algae that grow associated (Figure 1a). This environment is usually found on coastlines of islands and in rocky shores of the mainland, forming a complex wall that extends from the surface of the water to the unconsolidated bottom. These formations can be found on coasts of different latitudes worldwide (EBELING; HIXON, 1991).

On the other hand, coral reefs are characterized by a three dimensional continuous formation of stony corals, mainly scleractinians, growing patchily in shallow waters (CHOAT; BELLWOOD, 1991; EBELING; HIXON, 1991) (Figure 1b). Reef-building corals are usually sensible organisms concerning environmental conditions, in which most of the species grow in warm, shallow and oligotrophic/clear

waters. For these reasons, shallow coral reefs are mostly find in a narrow belt across the world's tropical oceans (SPALDING; RAVILIOUS; GREEN, 2001).

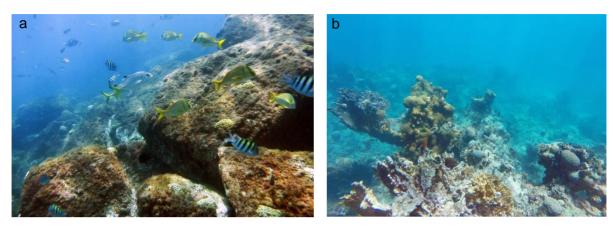


Figure 1. Examples of (a) rocky reefs (Alcatrazes Archipelago, SP, Brazil) and (b) coral reefs (Abrolhos, BA, Brazil) studied in this thesis.

Reef fish definition is not a consensus in the literature, whether it can be determined by specific taxonomic groups or its ecological function (BELLWOOD, 1998; BELLWOOD; WAINWRIGHT, 2002). Reef fish considered in the present study were the species that live or spend part of their life cycle associated with the reef, including those ones that occasionally shelter, reproduce, feed or search for cleaning services (BELLWOOD, 1988; BELLWOOD; WAINWRIGHT, 2002).

Overfishing represents one of the main threats to reefs worldwide, since it causes several direct effects and indirect effects of populations (JENNINGS; LOCK, 1996). Direct effects include the large removal of fish biomass affecting population structure, distribution, reproduction and growth aspects of target species; and indirect effects can be detected on non-target species trough the food web and alterations in the habitat (JENNINGS; LOCK, 1996; ROBERTS, 1995). Besides, reef fish are patchily distributed (FRANCISCO-RAMOS; ARIAS-GONZÁLEZ, 2013; YEAGER; LAYMAN; ALLGEIER, 2011), which makes them even more vulnerable to fisheries. Therefore, holistic management measures focused on the entire habitat are necessary and urgent.

In order to reverse this overfishing status and to overcome lack of sufficient information to apply more traditional fisheries management, marine protected areas (MPAs) have been widely implemented worldwide (LAUCK et al., 1998; SALA; GIAKOUMI, 2018). Basically, MPAs regulate human uses in order to promote the recovery of stocks, as well as to conserve and restore the ecosystems as a whole,

including habitat, biodiversity and food webs (LEENHARDT et al., 2015). It can be of different levels of restrictions, in which within a no-take reserve (NTR) all kind of extractive activity is prohibited; or it can regulate the uses, i.e. fishing, tourism and industrial activities (LEENHARDT et al., 2015). Brazil has several MPAs with few studies assessing its effectiveness in providing recovery of fish populations. Understanding the use of the reserves for the marine life becomes crucial to assess their environmental relevance, which raises discussions concerning the protection of other regions and, therefore, contributing to oceans management.

Several studies have tested the effects of no-take reserves to fish assemblage, and the responses can vary according to geographic location, commercial importance of the species. functional groups, level of enforcement/compliance as well as size and level of isolation of the NTR (CLAUDET et al., 2008; EDGAR et al., 2014; LESTER et al., 2009). Concerning biological effects, higher overall richness (COTE, 2001; GARCÍA-CHARTON et al., 2004; ILARRI; SOUZA; ROSA, 2017; KELAHER et al., 2014), abundance (GARCÍA-CHARTON et al., 2004; ILARRI; SOUZA; ROSA, 2017; KELAHER et al., 2014; VANDERKLIFT; BABCOCK; COOK, 2013) and biomass (ABURTO-OROPEZA et al., 2011; GARCÍA-CHARTON et al., 2004; ILARRI; SOUZA; ROSA, 2017; SALA et al., 2012) have been frequently registered within NTRs, but the evidences are stronger for biomass and abundances particularly for fisheries target species (CASTRO-SANGUINO et al., 2017; COTE, 2001; FÉLIX-HACKRADT et al., 2018; MCCLANAHAN; ARTHUR, 2001), highlighting direct fisheries effects and the role MPAs can play in providing recovering of reef fish populations.

The development of non-lethal sample methods is crucial since many species of organisms found in reefs are threatened with extinction. Several methods based on this principle have been developed worldwide with different objectives, such as the use of visual census (BROCK, 1954), underwater video (MALLET; PELLETIER, 2014), photo-identification (MARSHALL; PIERCE, 2012), genetic samples (CASTRO et al., 2007), ultrasound (CARRIER et al., 2003), blood test (AWRUCH et al., 2008), mark-recapture (HUSSEY et al., 2009), telemetry (COOKE et al., 2004; BROWNSCOMBE et al., 2019) and observation of physical characteristics such as size clasper condition to determine maturity in elasmobranchs (SIMPFENDORFER; UNSWORTH, 1998).

Use of videos to collect data has been increasingly adopted, especially in face of the fast advance of technology and the development of more accessible and high quality equipment (MALLET; PELLETIER, 2014). The use of video cameras can be associated with divers or act remotely, and they are usually passive, non-intrusive and non-lethal methods, and do not cause disturbance to the substrate (MALLET; PELLETIER, 2014). For this reason, they are perfectly suited for studies in marine protected areas (CAPPO et al., 2003).

The methods known as Baited Remote Underwater Video (BRUV) and Diver Operated Video (DOV) are being widely employed to assess diverse aspects of fish assemblages (CAPPO; HARVEY; SHORTIS, 2006; GOETZE et al., 2015). BRUV is basically characterized by a structure that supports a video camera in which the organisms are attracted to the field of view using a bait (MALLET; PELLETIER, 2014). This method has some advantages because it is not size selective, attracting most of the animals of the surroundings and can be applied in a wide variety of habitats and depths (CAPPO; HARVEY; SHORTIS, 2006). Also, it detects large and mobile animals, which usually avoid divers and/or active fishing gears (CAPPO; HARVEY; SHORTIS, 2006). However, it can underestimate small organisms (WATSON et al., 2005). On the other hand, DOVs are basically cameras carried by divers through transects. This method usually underestimates fish that avoid divers, however it covers better small benthic fish assemblages. Therefore, the combination of methods is always indicated to assess more effectively fish assemblages (WATSON et al., 2005).

When two cameras are installed in the same structure, it characterizes a stereo system, allowing measurements of the organisms through the videos. This system can be applied either remotely (stereo-BRUV) or with the aid of a diver (stereo-DOV) (Figure 2). Through photogrammetry, stereo methods provide accurate measurements because they display a three-dimensional image due to the binocular vision (HARVEY; SHORTIS, 1996). For better accuracy of measurements in the video, it is recommended to use cameras with a reduced rolling shutter effect, i.e. with high fps (frames per second), capturing the pixels quickly and reducing the distortion of images when the object is moving fast. In addition, it is important for the camera to display a large image sensor. For the use of two cameras, a calibration is required, since the cameras have a deviation from a perfect central projection which needs to be adjusted (HARVEY; SHORTIS, 1998). For this, the use of the calibration

cube and the CAL software of the company SeaGIS (www.seagis.com.au) is indicated (Figure 3).

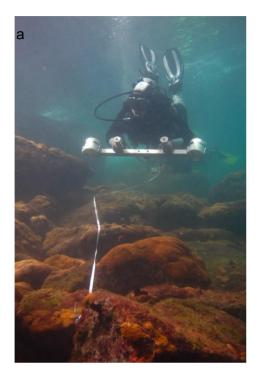




Figure 2. Stereo-video sampling methods used in this study. a. Diver Operated Stereo-video transect. b. Baited Remote Underwater Stereo-video deployment recording in the bottom.

Regarding the efficiency of the measurements made by stereo systems, the study by Langlois et al. (2012) did not find significant differences between the body size measurements made through longline and stereo-BRUVs for three species of fish (*Choerodon rubescens*, *Epinephelides armatus* and *Pagrus auratus*). The same precision was found by Harvey et al. (2003) for tunas (*Thunnus maccoyii*), demonstrating the efficiency and variety of information that can be obtained with minimal disturbance to the environment and to the organism itself.

These methods have been used in different parts of the world for several purposes (MALLET; PELLETIER, 2014; WHITMARSH; FAIRWEATHER; HUVENEERS, 2017), for example, Malcolm et al. (2007) and Kelaher et al. (2014) used BRUVs to compare fish assemblages in different marine reserves in Australia. In the same way Bond et al. (2012) used this method to compare relative abundance of sharks between marine reserves and non-restricted areas in Belize; and Brooks et al. (2011) used it to assess the diversity, distribution and abundance of sharks in the Bahamas. This latter work demonstrated that the method is suitable for surveys on

long-term data about relative abundance and richness of sharks, at different temporal and geographic scales. Concerning DOVs, Goetze et al. (2015) used this method to assess the impact of fishing on target species in areas with different management measures, and also compared the fish assemblage sampled by stereo-BRUVs and stereo-DOVs.

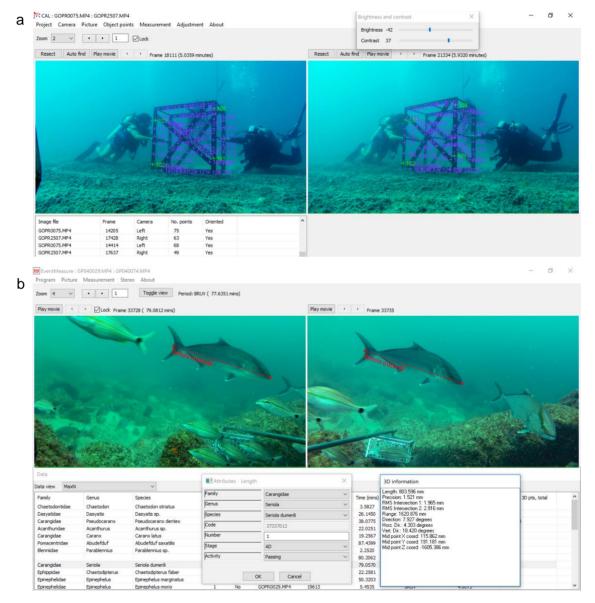


Figure 3. Images of the (a) calibration of the stereo systems using CAL software and (b) images analyzed in EventMeasure (SeaGIS) software in the process of measuring the fork length of individuals

In addition to the importance of deepening the knowledge about the ichthyofauna in NTRs in Brazil, the adaptation of non-destructive techniques of marine data sampling is indispensable. Stereo-DOV and stereo-BRUV fall into this

category and have been scarcely used in Brazil. These methods are already well developed worldwide and have been proved to be efficient in sampling valuable information. For these reasons, it is necessary to improve and expand their use.

#### General objective and structure of the thesis

Based on this, the general objective of the present Doctoral Thesis was to explore reef fish assemblage metrics using stereo-videos to assess the influence of no-take marine reserves and fisheries effects. It was hypothesized that reef fish species abundance, richness and biomass, as well as body size, especially for species targeted by fisheries, are better predicted by protection status than environmental characteristics.

The performance of stereo-methods to sample fish assemblage was tested in the Southwestern Atlantic and compared with traditional visual census methods, and the results are shown in Chapter 2. Moreover, stereo-videos were used to analyze the effects of NTRs in fish assemblages in rocky reefs (Chapter 3) and coral reefs (Chapter 4) in Southwestern Atlantic. Chapter three is already published and in this Thesis the text is presented as it is in the publication (ROLIM et al. 2019) (https://doi.org/10.1371/journal.pone.0204970).

As supplementary material, an occurrence of a semi-aquatic mammal, the Neotropical otter (*Lontra longicaudis*), in the marine environment using stereo-BRUVs, was reported, discussing the use of this method to assess other groups of organisms. This manuscript was published (ROLIM; RODRIGUES; GADIG, 2018) and the text presented here is also as it is in the publication.

Finally, given the lack of availability of information and the small dissemination of the importance of Brazilian MPAs, a catalog of the ichthyofauna with basic information about the species found in the rocky reef of Tupinambás Ecological Station, São Paulo, was organized, disseminating to the community the importance of MPAs as well as the new methodologies applied. This catalogue was published as a book (ROLIM; RODRIGUES; GADIG, 2017) and the electronic version is available to download in the reference list.

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## Chapter 1

A comparison of four sampling methods for assessing rocky reef fish assemblages in the Southwestern Atlantic



**Abstract.** Visual census based on diver notes have been often complemented by stereo-videos based methods in marine reef fish assessments. However, four of the most employed methods have never been compared within the same study. Here, we compared rocky reef fish assemblage characteristics sampled by stationary point count and transect visual census, as well as Diver Operated stereo-Videos (stereo-DOVs) and Baited Remote Underwater stereo-Videos (stereo-BRUV) in a coastal island in Southwestern Atlantic. Diver based methods sampled more non-target species closely associated with the reef, as well as cryptic small-bodied species. Conversely, stereo-BRUVs sampled fisheries target, larger and mobile species such as from the Carangidae and Lutjanidae families. The stereo-DOV method presented a higher sample efficiency, registering higher abundance and richness within less sample effort in the field. Limitations of the four methods were discussed, however, the higher sampling efficiency of stereo-DOVs, combined with the diverse advantages video techniques present, such as permanent record of data and decreased interobserver variability for species identification and estimates of body size, indicates stereo-DOVs as a highly efficient method. Moreover, when associated with a remote technique, such as stereo-BRUVs, can provide efficiently capture estimates of both diversity metrics and the abundance and body size information for fisheries target species in rocky reefs.

**Key-words:** Marine Protected Area, stereo-videos, reef ecology, reef fish, Brazil.

#### Introduction

The access of information regarding population parameters of marine fishes (e.g., diversity, density and body length) is crucial to determine fisheries effects and other disturbances, being also essential information for management and conservation planning of the marine environment (CLAUDET et al., 2010; LANGLOIS; HARVEY; MEEUWIG, 2012; STUART-SMITH et al., 2008; TETREAULT; AMBROSE, 2007). The central issue is to determine which sampling methods to estimate these parameters, and the decision and robustness of the method depends on the objectives and hypothesis raised.

Regarding reef fish assemblages sampling, as many species in these habitats are under several threats, non-destructive and non-lethal methods have been widely developed and adopted. The decision on which method to apply must rely on different aspects of the study, such as overall objectives, level of precision required to detect changes, fish ecology and behavior, physical conditions of the area, the repeatability of the method, as well as financial and infrastructure resources (ANDREW; MAPSTONE, 1987; LANGLOIS et al., 2010; WILLIS; MILLAR; BABCOCK, 2000).

First introduced in a pioneering study by Brock (1954) the underwater visual census (UVCs) became popular for being efficient, rapid, fisheries-independent, nondestructive, and cost-effective methods. Several UVC methods have been developed, but the belt transect is still the most common method for studying shallow (< 20m) reef fish assemblages (CALDWELL et al., 2016). To encompass different types of environments, the stationary point count method, also called nested, also widely applied worldwide (BOHNSACK; BANNEROT, became 1986: COLVOCORESSES; ACOSTA, 2007; FRANCINI-FILHO et al., 2013; GRAHAM et al., 2007; MINTE-VERA; DE MOURA; FRANCINI-FILHO, 2008; WILLIAMS et al., 2015). However, these methods based on diver observations could lead to an underrepresentation concerning large and mobile fish species that usually avoid divers, or even overestimate some species that can be attracted (CAPPO; HARVEY; SHORTIS, 2006; GOETZE et al., 2015; WATSON; HARVEY, 2007). Besides that, diver's annotations can vary among observers (HARVEY et al., 2004; THOMPSON; MAPSTONE, 1997; THRESHER; GUNN, 1986).

The methods including video cameras have been progressively getting more attention in scientific studies to complement assessments and monitor fish assemblages (CAPPO et al., 2003; CAPPO; HARVEY; SHORTIS, 2006; MALLET; PELLETIER, 2014; MURPHY; JENKINS, 2010), especially since video cameras have become smaller, with better image quality and less expensive (CAPPO; HARVEY; SHORTIS, 2006; WHITMARSH; FAIRWEATHER; HUVENEERS, 2017). Underwater videos provide a permanent record of species composition, taxonomy and habitat characteristics simultaneously (BENNETT et al., 2016; MURPHY; JENKINS, 2010). It can be applied in different habitats and is highly replicable, demanding less training and time in the field (HOLMES et al. 2013). Additionally, fish counts, identification and measurements can be confirmed when analyzing the footages, decreasing the probability of interobserver variability (HARVEY et al., 2004; MURPHY; JENKINS, 2010). However, it still requires a significant amount of time to process the videos and the field of view of the cameras can be limited, specially in places with high fish densities, which can lead to underestimated abundances and also not allow the measurement and detection of all individuals (GOETZE et al., 2017; HARASTI; MALCOLM, 2013; HARVEY et al., 2010; HOLMES et al., 2013; SCHOBERND; BACHELER; CONN, 2014; SHERMAN et al., 2018; WATSON et al., 2010).

The difficulty of measuring fish lengths through the images has been addressed by the development of stereo-systems, allowing accurate measurements through the tridimensional view, both from the fish and sampling area (HARVEY; SHORTIS, 1996). These methods are being used remotely or with the aid of divers, and it can unbaited or baited, depending on the goals and target species (MALLET; PELLETIER, 2014).

Diver Operated stereo-Videos (stereo-DOVs) has been applied similarly to visual census, but the diver conducts the stereo-video system (SHEDRAWI et al., 2014; WATSON et al., 2010) filming a defined area. Despite of the advantages stereo-videos present, the precise identification of small-sized fish species using such method can be difficult, mainly due to image definition and the time the individual is within the camera's field of view (HOLMES et al., 2013).

Remote videos complement fish assemblage assessments where the access from divers is difficult due to adverse environmental conditions and/or greater depths (CAPPO; HARVEY; SHORTIS, 2006). More importantly, it can overcome the biases associated with the presence of divers (CAPPO; HARVEY; SHORTIS, 2006;

WATSON; HARVEY, 2007), recording species that usually avoid divers. Baited Remote Underwater stereo-Videos (stereo-BRUVs) usually employs oily bait to attract the individuals to the field of view and are being applied to sample fish assemblage with several objectives (CAPPO; HARVEY; SHORTIS, 2006). Due to the presence of the bait, it is highly effective to sample large and mobile carnivorous species (CAPPO et al., 2003; LANGLOIS et al., 2012; WATSON et al., 2010), without precluding herbivorous fish (HARVEY et al., 2007).

Despite the presence of studies comparing fish census methods in the literature (e.g. Harvey et al. (2004), Holmes et al. (2013), Langlois et al. (2010), Thanopoulou et al. (2018), Watson et al. (2010) and Willis; Millar; Babcock (2000), none have compared these four popular methods (Transect UVC, Stationary UVC, stereo-DOV and stereo-BRUV). Besides that, results of methods comparisons can change dramatically depending on the region (LANGLOIS et al., 2010). Specifically for Southwestern Atlantic, stereo-videos have been scarcely used and, to date, marine protected areas have mostly been monitored using traditional visual census (ANDERSON et al., 2014; FLOETER; HALPERN; FERREIRA, 2005; FRANCINI-FILHO; MOURA, 2008; ILARRI; SOUZA; ROSA, 2017). Therefore, in face of these lack of studies with stereo-videos in the Southwestern Atlantic, we tested the implementation of these novel techniques, both remotely and with divers, to complement and monitor reef fish assemblage within this area.

With this, the present study aimed to quantitatively and qualitative compare the performance of the four most employed reef fish assemblage sampling methods (stereo-BRUV, stereo-DOV, Stationary and Transect), in order to evaluate the biases related to each method and determine the most suited ones to monitor rocky reefs fish assemblages. We predict that: 1. Stereo-videos samples higher total abundance; 2. Visual census based on divers observations registers more efficiently cryptic and site-attached species usually non-targeted by fisheries; 3. stereo-BRUVs samples a fish assemblage mainly composed by target and larger species.

#### Methods

#### Study area

The study was conducted in Palmas island, located in the northern coast of São Paulo state, Brazil, Southwestern Atlantic. This island is located 7 kilometers from the mainland (23° 32´S; 45° 01´W) (Figure 1) and it is about 950 m long and 300 m wide, surrounded by rocky shore. All samples were performed at the leeward side of the island in the interface area, where the rocky reef reaches the sandy bottom. The depth was similar in all the sites, ranging from 10 to 12 m deep. The sampling was conducted in three consecutive days between 11 and 13 of January 2017, repeating the design each day. Fish were identified at the lowest taxonomic level, and characterized as targeted and non-targeted by fisheries according to Carvalho-Filho (1999), Rolim et al. (2017), Floeter et al. (2007) and Begossi and Richerson (1993).

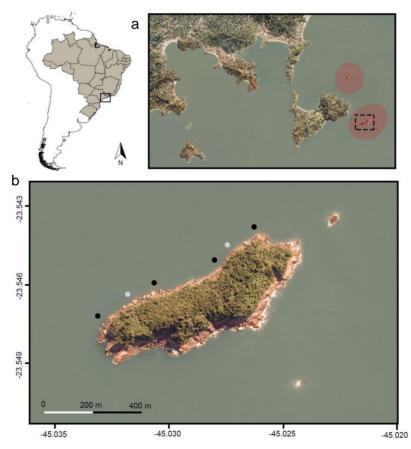


Figure 1. Study area depicting (a) the no-take marine reserve in red and (b) Palmas Island with stereo-BRUVs deployments location, represented by the black dots, and dive locations (Stereo-DOVs, Transect UVC and Stationary UVC), represented by grey dots. Source of pictures: EMPLASA, 2010.

#### Sampling methods

#### a. Diver observations

Estimates of abundance, richness and fork length of fish were made by divers, categorizing the individuals in size classes (< 2, < 10, 10-20, 20-30, 30-40 and > 40 cm). The stationary (nested) point count method consists in the diver sampling within an estimated cylinder with two different radii of 2 and 4 m (Figure 2a) (MINTE-VERA; DE MOURA; FRANCINI-FILHO, 2008). Abundance of each species and body length is estimated in a period of 5 minutes. In the 2 m radii only individuals smaller than 10 cm were counted while in the 4 m radii all the individual larger than 10 cm were counted. In the belt transect method, the diver covers through the 20x2 m transect path twice, first looking to the fish in the water column and then returning recording small species in the sand/rocky bottom, writing down the abundance and fork length for each fish specimen in a drawing board (Figure 2b). A total of 30 transects were made for each method.

#### **b.** Stereo-video systems

The stereo-systems used in this study comprise a metal structure coupled with two GoPro Hero 3+ cameras inside a water and pressure resistant housing. The cameras were set up to 60 frames per second at 1080p and placed 0.7m apart from each other converging with an angle of 7° degrees. Before sampling, the stereo system was calibrated with the calibration routine provided by SeaGis (www.seagis.au), trough software CAL. The description of the design and calibration of the stereo-video in detail can be found in (HARVEY; SHORTIS, 1996, 1998). The video footages were analyzed using EventMeasure software, where fish were identified, counted and the fork length was measured.

As the transect method, Diver Operated stereo-Videos (stereo-DOVs) were executed by a single scuba diver who conducted the stereo system through 20x2m transects (Figure 2c). The diver runs through the section forward and back, searching for fishes in the water column and in the bottom. A total of six dives were made resulting in 37 transects. The abundance was estimated counted all fish recorded, and whenever possible, the fork length was measured. Only fish within five meters facewarding the camera were counted and measured.

Baited Remote Underwater stereo-Videos (stereo-BRUVs) consist of the stereo-system fixed and involved in a larger metal structure (Figure 2d) aimed to protect the system from physical impact and stabilize the camera in the bottom. Coupled to the metal structure there is the bait cage placed 1.5m from the cameras. The bait consisted of 0.8 kg of sardine (*Sardinella brasiliensis*). The sardine was chosen as bait due to their oiliness, dispersing the odor plume more efficiently (WALSH; BARRET; HILL, 2016). A total of four replicates separated by 250 m were deployed each day, resulting in 12 deployments in the current study. The abundance was estimated counted the maximum number of each species that appear at the same frame (MaxN) within 7 m of the cameras, and the fork length of fish were measured only at the moment of MaxN.

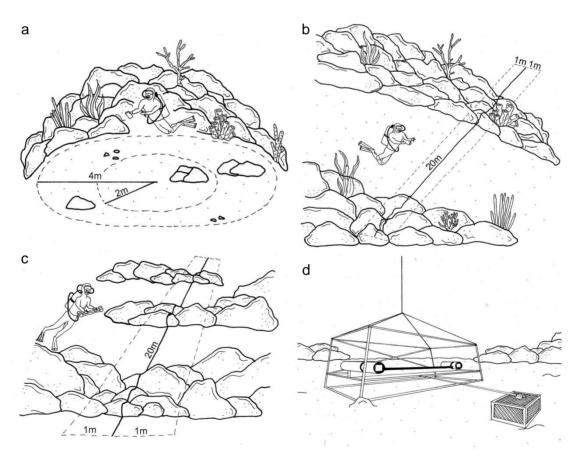


Figure 2. Schematic representation of the four methods used in the present study to assess reef fish assemblage. The visual census methods based on diver observations represented by (a) Stationary UVC and (b) Transect UVC, and the stereo-video methods: (c) Diver Operated stereo-Video (stereo-DOV) and (d) Baited Remote Underwater stereo-Video (stereo-BRUV).

#### Statistical analysis

In order to make the abundance estimates comparable among methods for multivariate analysis, counts of fish for each sample were standardized, resulting in relative abundance estimates of each of the species detected by each sample. This routine was also performed for fish family abundance, as well as for target and nontarget species abundance. These four data matrices of relative abundances were Modified Gower Log<sub>10</sub> resemblance transformation transformed using a (ANDERSON; ELLINGSEN; MCARDLE, 2006). Principal Coordinates analysis (PCO) was used to visualize multivariate data in two dimensions and a canonical analysis of principal coordinates (CAP) was applied to compare fish family relative abundance sampled by the four methods. A PERMANOVA model was constructed in which the two factors were: method (four levels, stereo-BRUV, stereo-DOV, Transect and Stationary, as a fixed factor) and sample day (three levels, as a random factor). Sample day was treated as a random factor, as primary analysis was aimed at detecting differences in fish assemblage recorded by method rather than differences among days.

For univariate analysis comparing total richness and abundance, PERMANOVAs were used to test for method and sample day effects with the same model described above, in an approach similar to parametric ANOVA. Univariate PERMANOVA tests were run on Euclidean distances matrices. PERMANOVA was chosen for univariate analyses because it allows for two-factor designs, considers an interaction term and does not assume a normal distribution of errors. All PERMANOVAs were run with 9999 permutations adding results of Monte-Carlo (MC) tests, to be considered in the event of there being too few possible permutations for a meaningful test in PERMANOVA. For fish fork length, Kruskal-Wallis test was applied comparing the methods, with KruskalMC as a post hoc test (SIEGEL; CASTELLAN, 1988).

Taxon sampling curves, both sample-based and individual-based, were estimated for the four methods (COLWELL et al., 2012; COLWELL; MAO; CHANG, 2004; GOTELLI; COLWELL, 2001). Rarefaction curves with expected number of species by sample and by number of individuals were determined and plotted with Chao1 richness estimator (CHAO, 1984).

The univariate and multivariate analysis were run in the software package PRIMER-E v6 (CLARKE; GORLEY, 2006) with the PERMANOVA extension. EstimateS software was used (COLWELL, 2013) to determine richness estimates and R Statistical Language (R CORE TEAM, 2018) was used for data tidying and Kruskal-Wallis test with the package pgirmess (GIRAUDOUX, 2017).

#### Results

A total of 4775 fish individuals from 72 species and 44 families were recorded. The five most abundant species for all four methods combined were Haemulon aurolineatum (n= 1556), Chaetodipterus faber (n= 506), Mycteroperca marginatus (n= 293), Coryphopterus spp. (n= 198) and Priacanthus arenatus (n= 160). The most frequent species were Haemulon aurolineatum (96.3%), Mycteroperca marginatus (82.6%), Coryphopterus spp. (64.2%), Holocentrus adscensionis (62.4%), Anisotremus virginicus (61.5%), and Pomacanthus paru (57.8%). Stereo-BRUV recorded the largest number of species that were not detected by the other methods (e.g., Calamus pennatula, Caranx crysos, Caranx hippos, Lutjanus jocu, Myliobatis sp., Lutjanus chrysurus and Sphyraena guachancho), followed by stereo-DOV (Acanthurus coeruleus and Haemulon parra) and Stationary UVC (Bothus ocellatus and Chromis jubauna) with two species each and the Transect UVC with only one species (Gymnothorax funebris).

Fish species composition in all methods showed significative distinction in the assemblage sampled (Table 1, Figure 3). These patterns were reflected in the constrained CAP ordinations of the species composition (Figure 3b). The CAP ordination of the abundance data with the four methods found a clear separation between three different groups, in which the first consisted by stereo-BRUV samplings, the second with the Stationary UVC e the third was comprised by stereo-DOV and Transect UVC samplings. Stereo-BRUV presented the most constant composition, being characterized by pelagic and commercially important groups, such as *Pseudocaranx dentex*, *Caranx latus*, *Epinephelus morio* and *Lutjanus jocu* (Figures 3b and d). On the other hand, Stationary UVC method had a higher representation of smaller sized pelagic species, such as *Anisotremus virginicus* (Figure 3b). Transect and stereo-DOV presented similar assemblages, focusing also

on smaller-sized species associated with the reef, such as *Halichoeres poeyi* and *Coryphopterus* spp. (Figure 3b). In relation to family abundance composition, all methods presented differences, except for Transect and Stationary UVCs (Table 1, Figure 3). As shown in the CAP ordination, these UVCs methods, together with stereo-DOV presented higher representation of fish from the Gobiidae family, whilst stereo-BRUVs presented a very distinctive assemblage represented by the families Lutjanidae, Carangidae, Monacanthidae and Mugilidae (Figure 3d).

Table 1. Permutational multivariate analysis of variance (PERMANOVA) testing for method (fixed) and sample day (random) differences on fish relative abundance at species and family levels, as well as for fisheries target and non-target species relative abundance. The test statistic (F) is a pseudo-F value and the probability values (P) are computed by the PERMANOVA routine and Monte-Carlo test (P(MC)) with 9999 permutations.

Species r	elativ	e abund	dance d	compos	sition								
Permanova							Pairwise						
Source	df	SS	MS	F	Р	Perms	P(MC)	Groups	t	Р	Perms	P(MC)	
Me	3	12.485	4.162	4.378	0.0004	9930	0.0001	BRUV, Transect	3.291	0.1636	30	0.0001	
Da	2	3.393	1.697	2.329	0.0001	9858	0.0001	BRUV, Stationary	2.632	0.1615	30	0.0008	
MexDa	5	4.816	0.963	1.322	0.0100	9765	0.0214	BRUV, DOV	2.275	0.1652	30	0.0007	
Res	96	69.938	0.729					Transect, Stationary	1.667	0.1560	360	0.0082	
Total	106	90.713						Transect, DOV	1.782	0.1294	360	0.0016	
								Stationary, DOV	2.063	0.0867	360	0.0001	
Family re	Family relative abundance composition												
Permanova						Pairwise							
Source	df	SS	MS	F	Р	Perms	P(MC)	Groups	t	Р	Perms	P(MC)	
Me	3	12.314	4.105	4.895	0.0002	9933	0.0001	BRUV, Transect	3.999	0.0979	30	0.0002	
Da	2	3.339	1.670	2.561	0.0002	9894	0.0005	BRUV, Stationary	2.740	0.1649	30	0.0008	
MexDa	5	4.245	0.849	1.302	0.0450	9837	0.0563	BRUV, DOV	2.637	0.1620	30	0.0024	
Res	96	62.585	0.652					Transect, Stationary	1.494	0.1622	360	0.0561	
Total	106	82.657						Transect, DOV	1.785	0.1244	360	0.0095	
								Stationary, DOV	2.156	0.0904	360	0.0010	

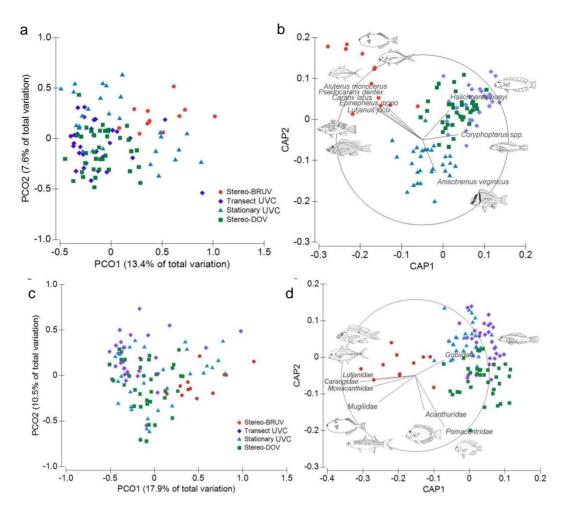


Figure 3. Multivariate analysis for (a,b) species and (c,d) family relative abundances. Principal coordinates analysis (PCO) (a,c) and Canonical analysis of principal coordinates (CAP) (b,d), using Modified Gower Log base 10 dissimilarity measure, showing the species with vector lengths (Pearson correlation) higher than 0.35. Fish drawings were based on Carvalho-Filho (1999).

Stereo-BRUVs detected a higher abundance and richness of the assemblage with lower sample effort, however the maximum sampling power was limited in terms of richness. On the other hand, stereo-DOVs requires less sample effort to record higher number of species and abundance (Figure 4). Transect and Stationary UVC methods showed the lowest number of species sampled, and requires more samples to be more representative when sampling the assemblage.

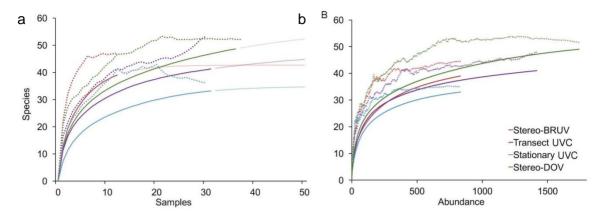


Figure 4. Taxon sampling curves comparing the four methods. a. Rarefaction sample-based curves of richness (strong continuous line), estimated until 50 samples (translucid continuous line), and also Chao1 estimator (dashed lines) for the four methods. b. Rarefaction individual-based curves (strong continuous line), and Chao1 estimator (dashed lines).

In Stationary and Transect UVC methods, the fork length measurements of all individuals were sampled; on the other hand, stereo-BRUV presented measurements of 94.6% of individuals sampled and stereo-DOV presented 85.0%. Methods were significantly different (*H*=411.45; p<0.001) concerning fish body size recorded of total sample (Figure 5a), in which Transect UVC recorded smaller fish (median=150mm, mean=163\(\pi\)SD84.9mm), followed by stereo-DOV (median=164mm; mean= 185∓SD80.4mm). The largest fish sampled Stationary were by UVC (median=250mm; mean=245+SD98.2mm) and Stereo-BRUVs (median=211mm; mean=228+108.5mm) (Figure 5a). Concerning only fisheries target species, the results were similar (H=133.99; p<0.001), in which Stationary UVC sampled the largest fish (median=250mm; mean= 255+SD96.3mm), followed by stereo-BRUV (median=211mm; mean= 228+SD110.9mm), stereo-DOV (median=169mm; mean= 2027SD70.2mm) and Transect UVC (median=150mm; mean= 1977SD82.8mm).

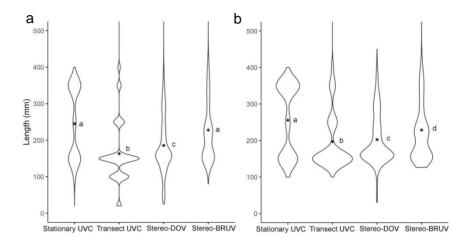


Figure 5. Fish fork length distributions for the four different methods for the (a) total sample and for (b) target species. With Kruskal-Wallis test results. \*=mean.

#### **Discussion**

Herein is presented the first study comparing these four highly employed methods, stereo-DOV, stereo-BRUVs, Stationary UVC and Transect UVC, to assess fish assemblage. Moreover, despite being widely adopted worldwide, stereo-video methods have not been consistently used in the Southwestern Atlantic, therefore, these comparisons and validations are crucial for the region.

Non-destructive methods are important to being developed specially in sensible areas that harbours a rich biodiversity and significant amount of endangered and endemic species, such as reefs (PINHEIRO et al., 2018). However, potential significant differences among techniques should be considered as it can result in different estimates of assemblage. All the bias should be well understood and considered so the method chosen satisfies the desired goal. Here, we aim to discuss our results to access the limitations of each method, in order to have a more efficient and complete assemblage estimates to monitor rocky reef fish assemblages.

Even though the sampling of fish assemblage using the four methods within a specific rocky reef area may limit the generalizability of our findings, this approach provides a decreased number of variables, being able to compare and highlight the differences in the estimates by each technique. With this, monitoring programs and fish ecology studies in rocky reefs in coastal islands might incorporate our results to define the best combination of methods to apply and accomplish their aims.

The Stationary and Transect UVC methods sampled a varied assemblage encompassing small-sized fish species mostly associated with the reef, such as the Blenniidae family (gobies). This is expected since diver based methods allow to focus on cryptic species and accurate differentiate similar species (BORTONE; MARTIN; BUNDRICK, 1991; HOLMES et al., 2013; TESSIER et al., 2013). Divers can follow individuals and focus upon small characteristics that are not detected in the camera, as well as to look underneath rocks, inside burrows and crevices. Despite of this advantage, in the present study, these methods sampled fewer number of species within the same effort, resulting in lower sampling efficiency when compared to the other methods. This indicated that these methods require more time in the field to have a more representative sampling of the assemblage.

One possible limitation involving the Transect and Stationary UVC methods is regarding length accuracy and determination of sampling area. As observed by Harvey et al. (2004), the error in visual distance estimates made by both novice and highly experienced scientific divers can occur, potentially affecting the size of the sample and consequently affecting the number of fish counted by census and the comparison of the data collected. The inaccuracy in estimate the lengths and distance of fish is also discussed by Bohnsack and Bannerot (1986), Thresher and Gunn (1986) and Harvey et al. (2000). Based on this, even with appropriate training, inaccuracies are likely to occur; therefore, more samples are necessary to decrease this variation.

Stereo-DOV method applied in this study followed the same protocol of the transect that is often applied in rocky reefs (ABURTO-OROPEZA; BALART, 2001; FLOETER et al., 2007; NEVES et al., 2016; TEIXEIRA-NEVES; NEVES; ARAÚJO, 2015), in which the path is runned by the diver back and forth, also searching for small species closely associated with the reef. This fact explains the high efficiency and similar results to the other diver-based techniques in the present study. This is not the same protocol usually applied for stereo-video transects – e.g., Goetze et al. (2015), Langlois; Harvey; Meeuwig (2012) and Watson et al. (2010). However we decided to adapt the method to the rocky reefs, since the high number of endemic small species associated with the reefs needed to be considered (PINHEIRO et al., 2018). This different approach, combined with the ability of the diver to also focus on individuals that have details that need to be assessed to identify the species, made stereo-DOV the method that detected higher abundance and richness within fewer

samples in the present study, enhancing the transect method sampling within less time in the field. Due to the limitation of the traditionally applied stereo-DOV protocol to detect cryptic species, some other studies detected lower richness estimates with this method when comparing with transects (HOLMES et al., 2013; PELLETIER et al., 2011), which can be overcomed by this different approach. Based on these findings, we recommend further research to investigate the efficiency of this stereo-DOV protocol in other habitat types.

On the other hand, stereo-BRUVs detected a very specific assemblage with a high similarity of samples, as well as a high abundance and richness in average by sample. This efficiency has the potential to improve the statistical power of analysis, specially when comparing habitats and detection changes in fish assemblage. In the present study this assemblage was mainly composed by larger fish targeted by fisheries and that usually avoid divers. Indeed, it was noticed that several fish may be attracted to or repelled from divers, particularly large carnivorous (COLE et al., 2007; FRANCOUR; LIRET; HARVEY, 1999; WATSON; HARVEY, 2007), which contributes for stereo-BRUVs detect a higher abundance of this feeding guild by different studies (COLTON; SWEARER, 2010; WATSON; HARVEY, 2007; WILLIS; BABCOCK, 2000). Therefore, this method is also perfectly suited to detect fisheries effects with higher accuracy, but is not efficient to record small cryptic species, such as blennies and gobies.

The use of bait can also present biases, particularly regarding the bait plume dispersion, which is related to physical aspects of the sampling site, as well as to currents, wave action, topography, fish appetite, feeding activity, bait type, amongst others (STOBART et al., 2015), making the area covered by BRUV difficult to estimate. On the other hand, stereo-DOV, Transect and Stationary methods have a predetermined area, which allows the estimation of density of fish and make these methods more suitable to assess fish species that are not repelled by divers.

The larger variation of fish body sizes was found in stereo-BRUVs, but still limited to detect fish larger than 8 cm, on the other hand, size distribution of diver based methods included more small-sized fish, with the transect detecting the smaller ones. This is expected since diver based observations encompasses a larger bottom area, and consequently records more efficiently small species that are closely related to the reef. Stereo-videos, on the other hand, present greater accuracy when estimating size, however the individual is often partially obstructed, or does not

always appear in the field of view of the two cameras, and measurement can not be performed. This possibly leads to fewer measurements when compared to diver annotations in the census, but at the same time offers greater precision in detecting variations in body size and biomass of fish assemblages (FRANCOUR; LIRET; HARVEY, 1999; HARVEY; FLETCHER; SHORTIS, 2000).

#### **Conclusions**

The advantages of stereo-video techniques include higher accuracy in length estimates (HARVEY et al., 2010; HARVEY; FLETCHER; SHORTIS, 2000), also decreasing substantially the effect of observer influence and variability (HARVEY; FLETCHER; SHORTIS, 2000; THOMPSON; MAPSTONE, 1997; THRESHER; GUNN, 1986). Besides, the footages can be revisited when necessary, both to check data or to provide data for different studies, and does not require a fish specialist in the field. Another reason is concerning the accuracy in determining sample boundaries through the stereo-video (HARVEY et al., 2010), which therefore leads to more precise abundance estimates within those limits. And lastly, habitat characteristics can be also classified through the images (BENNETT et al., 2016; COLLINS et al., 2017), decreasing time in the field. This indicates that the use of videos can bring more advantages than limitations when assessing and monitoring rocky reef fish assemblages.

Therefore, as a conclusive remark, to sample rocky reef fish assemblages, the efficiency of each method depends mostly on the group of fish the study aims to record. However, the present study suggests that the adapted stereo-DOV method is highly efficient to sample rocky reef fish assemblages, because it recorded the higher number of species and abundance of a varied assemblage with lower effort. Besides that, the efficiency is also due to the many advantages that cameras can offer, such as a more precise length measurement and the possibilities of many different types of analysis with the footages available. To complement the fish assessment sampling highly target fish species that usually avoid divers, stereo-BRUV is recommended, being especially important in studies that aim to test direct fisheries effects. Therefore, to sample diverse aspects of fish assemblage specially aiming monitoring programs in rocky reefs, the combination of methods is highly recommended, mainly encompassing a diver based with a remote technique.

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# Supplementary material

Table S1 - List of species registered by the four methods. \*Endemic from Brazilian biogeographic province (FLOETER; GASPARINI, 2000; JOYEUX et al. 2001; GASPARINI; JOYEUX; FLOETER, 2003; PINHEIRO et al. 2018);  $VU_I = Vulnerable$  by International Union for Nature Protection Red List (IUCN, 2018);  $NT_I = Near$  threatened by IUCN;  $VU_{Br} = Vulnerable$  by Brazilian legislation (MMA, 2014);  $CR_{Br} = Critically$  endangered by Brazilian Legislation; Y = Target; N = Non-target; N = Abundance; N = Frequency.

Family Chanica		BRUV		DOV		Stationary		Transect		TOTAL	
Family	Species	N	F%	N	F%	N	F%	N	F%	TOTAL	
Myliobatidae	Myliobatis spp.	1	8.3	-	0.0	-	0.0	-	0.0	1	
Muraenidae	Gymnothorax funebris	-	0.0	-	0.0	-	0.0	1	3.3	1	
Muraenidae	Gymnothorax moringa	2	16.7	-	0.0	-	0.0	3	10.0	5	
Muraenidae	Gymnothorax spp.	1	8.3	-	0.0	-	0.0	-	0.0	1	
Synodontidae	Synodus intermedius	-	0.0	1	2.7	-	0.0	1	3.3	2	
Synodontidae	Synodus spp.	-	0.0	-	0.0	1	3.3	-	0.0	1	
Ogcocephalidae	Ogcocephalus vespertilio	-	0.0	2	5.4	-	0.0	3	10.0	5	
Holocentridae	Holocentrus adscensionis	27	58.3	57	70.3	17	53.3	27	63.3	128	
Holocentridae	Myripristis jacobus	60	8.3	2	5.4	-	0.0	-	0.0	62	
Serranidae	Serranus baldwini	-	0.0	1	2.7	15	23.3	42	43.3	58	
Epinephelidae	Epinephelus morio (NTı;VU <sub>Br</sub> )	3	25.0	-	0.0	-	0.0	-	0.0	3	
Epinephelidae	Mycteroperca marginatus (EN <sub>I</sub> ;VU <sub>Br</sub> )	48	75.0	84	83.8	47	70.0	108	96.7	287	
Epinephelidae	Mycteroperca spp.	-	0.0	-	0.0	-	0.0	2	3.3	2	
Epinephelidae	Mycteroperca acutirostris	4	33.3	16	29.7	12	33.3	18	36.7	50	
Priacanthidae	Heteropriacanthus cruentatus	-	0.0	2	2.7	-	0.0	-	0.0	2	
Priacanthidae	Priancanthus arenatus	-	0.0	161	13.5	-	0.0	-	0.0	161	
Malacanthidae	Malacanthus plumieri	-	0.0	1	2.7	2	6.7	-	0.0	3	
Carangidae	Caranx crysos	64	16.7	-	0.0	-	0.0	-	0.0	64	
Carangidae	Caranx hippos	2	16.7	-	0.0	-	0.0	-	0.0	2	
Carangidae	Caranx latus	65	83.3	4	8.1	5	6.7	-	0.0	74	
Carangidae	Pseudocaranx dentex	33	75.0	-	0.0	-	0.0	-	0.0	33	
Lutjanidae	Lutjanus analis (NT <sub>I</sub> )	5	33.3	-	0.0	2	6.7	3	10.0	10	
Lutjanidae	Lutjanus jocu	2	16.7	-	0.0	-	0.0	-	0.0	2	
Lutjanidae	Lutjanus chrysurus	5	16.7	-	0.0	-	0.0	-	0.0	5	
Haemulidae	Anisotremus spp.	-	0.0	2	2.7	-	0.0	-	0.0	2	
Haemulidae	Anisotremus surinamensis	1	8.3	11	27.0	2	6.7	20	33.3	34	
Haemulidae	Anisotremus virginicus	1	8.3	64	83.8	28	60.0	25	56.7	118	
Haemulidae	Haemulon aurolineatum	268	91.7	536	100.0	216	93.3	541	96.7	1561	
Haemulidae	Haemulon parra	-	0.0	1	2.7	-	0.0	-	0.0	1	
Haemulidae	Haemulon plumieri	-	0.0	-	0.0	-	0.0	1	3.3	1	
Haemulidae	Haemulon steindachneri	5	33.3	64	75.7	-	0.0	28	56.7	97	
Sparidae	Calamus pennatula	1	8.3	-	0.0	-	0.0	-	0.0	1	
Sparidae	Diplodus argenteus	10	58.3	15	32.4	12	30.0	7	10.0	44	
Sciaenidae	Odontoscion dentex	-	0.0	24	24.3	-	0.0	13	10.0	37	
Sciaenidae	Pareques acuminatus	-	0.0	2	5.4	4	6.7	41	66.7	47	
Mullidae	Pseudupeneus maculatus	3	16.7	8	5.4	26	26.7	4	10.0	41	
Kyphosidae	Kyphosus spp.	10	16.7	54	29.7	13	10.0	16	6.7	93	
Chaetodontidae	Chaetodon striatus	57	58.3	19	29.7	16	30.0	23	36.7	115	
Pomacanthidae	Holacanthus tricolor	-	0.0	2	5.4	-	0.0	-	0.0	2	
Pomacanthidae	Pomacanthus paru	14	66.7	55	75.7	20	23.3	38	66.7	127	
Pomacentridae	Abudefduf saxatilis	20	33.3	102	56.8	1	3.3	21	30.0	144	
Pomacentridae	Chromis jubauna*	-	0.0	-	0.0	3	6.7	- 25	0.0	100	
Pomacentridae	Chromis multilineata	1	8.3	68	56.8	15	36.7	25 17	43.3	109	
Pomacentridae	Stegastes fuscus*	-	0.0	29	43.2	4	6.7	17	26.7	50	
Pomacentridae	Stegastes pictus*	-	0.0	9	16.2		0.0	4	13.3	13	

Pomacentridae	Stegastes variabilis*	-	0.0	2	5.4	3	10.0	-	0.0	5
Mugilidae	Mugil spp.	55	83.3	47	21.6	20	6.7	15	10.0	137
Sphyraenidae	Sphyraena guachancho	1	8.3	-	0.0	-	0.0	-	0.0	1
Labridae	Bodianus pulchellus	1	8.3	1	2.7	-	0.0	-	0.0	2
Labridae	Bodianus rufus	5	41.7	9	21.6	8	23.3	4	13.3	26
Labridae	Halichoeres bivittatus	1	8.3	-	0.0	-	0.0	-	0.0	1
Labridae	Halichoeres brasiliensis*	-	0.0	2	5.4	5	16.7	1	3.3	8
Labridae	Halichoeres poeyi	6	25.0	21	37.8	-	0.0	54	70.0	81
Labridae	Halichoeres sazimai*	-	0.0	4	2.7	-	0.0	-	0.0	4
Labridae	Scarus spp.	-	0.0	1	2.7	-	0.0	-	0.0	1
Labridae	Scarus zelindae* (VU <sub>Br</sub> )	3	16.7	-	0.0	-	0.0	1	3.3	4
Labridae	Sparisoma amplum*	2	16.7	4	8.1	-	0.0	1	3.3	7
Labridae	Sparisoma axillare* (VU <sub>Br</sub> )	1	8.3	10	18.9	4	10.0	4	10.0	19
Labridae	Sparisoma frondosum (VU <sub>Br</sub> )	-	0.0	1	2.7	3	3.3	-	0.0	4
Labridae	Sparisoma spp.	1	8.3	2	5.4	-	0.0	1	3.3	4
Blenniidae	Parablennius marmoreus	-	0.0	-	0.0	1	3.3	8	10.0	9
Blenniidae	Parablennius sp.	-	0.0	6	13.5	-	0.0	-	0.0	6
Gobiidae	Coryphopterus glaucofraenum	-	0.0	2	2.7	-	0.0	-	0.0	2
Gobiidae	Coryphopterus spp.	-	0.0	81	73.0	35	56.7	163	86.7	279
Gobiidae	Elacatinus figaro* (VU <sub>Br</sub> )	-	0.0	-	0.0	-	0.0	9	23.3	9
Microdesmidae	Ptereleotris randalli	-	0.0	2	2.7	-	0.0	7	6.7	9
Ephippidae	Chaetodipterus faber	25	75.0	106	10.8	291	40.0	109	26.7	531
Acanthuridae	Acanthurus bahianus*	-	0.0	19	21.6	1	3.3	3	6.7	23
Acanthuridae	Acanthurus chirurgus	-	0.0	2	2.7	1	3.3	-	0.0	3
Acanthuridae	Acanthurus spp.	5	16.7	13	13.5	-	0.0	-	0.0	18
Bothidae	Bothus ocellatus	-	0.0	-	0.0	2	6.7	-	0.0	2
Balistidae	Balistes vetula (NT <sub>I</sub> )	3	25.0	6	13.5	-	0.0	1	3.3	10
Monacanthidae	Aluterus monoceros	11	41.7	2	5.4	-	0.0	2	6.7	15
Tetraodontidae	Sphoeroides spengleri	-	0.0	1	2.7	-	0.0	-	0.0	1
Tetraodontidae	Sphoeroides spp.	-	0.0	-	0.0	-	0.0	2	3.3	2

# Chapter 2

Network of small no-take marine reserves reveal greater abundance and body size of fisheries target species



**Abstract.** No-take marine reserves (NTRs), i.e. areas with total fishing restrictions, have been established worldwide aiming to promote biodiversity and ecosystem conservation. Brazil has 3.3% of its Exclusive Economic Zone protected by 73 different NTRs, however, most of them currently lack scientific knowledge and understanding of their ecological role, particularly regarding rocky reefs in subtropical regions. In this context, this study aimed to contrast a network of NTRs with comparable fished sites across a coastal biogeographic gradient to investigate the effect of fishing and habitat variability on the abundance and body size of rocky reef fish. We used Baited Remote Underwater stereo-Video (stereo-BRUV) and Diver Operated stereo-Video (stereo-DOV) systems to simultaneously sample reef fish and habitat. Model selection and results identified habitat and biogeographic variables, such as distance from shore, as important predictor variables, explaining several aspects of the fish assemblage. The effect of protection was important in determining the abundance and body size of targeted species, in particular for epinephelids and carangids. Conversely, species richness was correlated with habitat complexity but not with protection status. This is the first study using these survey methods in the Southwestern Atlantic, demonstrating how a network of NTRs can provide benchmarks for biodiversity conservation and fisheries management.

**Key-words** Marine reserves, fisheries effects, functional groups, GAM, TransectMeasure, EventMeasure.

#### Introduction

No-take marine reserves (NTRs) have been established worldwide as an important management strategy, mostly aiming to protect marine biodiversity from the effects of fishing and other human disturbances (FLOURNOY, 2003; FOX et al., 2012). It is well documented that these NTRs can provide refuge to marine life, increasing local abundance, species richness, body size and the reproductive capacity of fish (EDGAR et al., 2014; GELL; ROBERTS, 2002; LESTER et al., 2009; WORM et al., 2006). Networks of NTRs can be used to investigate effects of fishing across biogeographic gradients, with the aim of estimating benchmarks for conservation and fisheries management. Increased biomass of target species has been recorded inside NTRs, contrasting with open areas where the removal of large carnivores can result in higher abundance of prey species, leading to a trophic reorganization. (GRAHAM; EVANS; RUSS, 2003; MICHELI et al., 2004; STENECK, 1998).

Extensive research has documented that fish assemblage structure varies with physical, chemical and biological factors across biogeographic and habitat gradients (KREBS, 1972; LANGLOIS et al., 2012; TOKESHI; ARAKAKI, 2012). In particular, distance from the coast and topographic complexity have shown increase of species richness, abundance and biomass of reef fish (FLOETER et al., 2007a; GARCÍA-CHARTON et al., 2004; MORAIS; FERREIRA; FLOETER, 2017; NEVES et al., 2016; PARSONS et al., 2016; PINHEIRO; MARTINS; JOYEUX, 2013; TEIXEIRA-NEVES; NEVES; ARAÚJO, 2015). It is therefore important for any investigation of the effects of fishing to control for covariates across NTRs and open areas.

Brazil has 8500 km of coastline and a territorial sea that, together with the Exclusive Economic Zone, encompasses 4 million km². Of this area, 26.4% is currently protected by 177 marine protected areas (MPAs), of which 73 are NTRs, representing 3.3% of the country's marine waters (ICMBIO, 2018). However, the majority of this protection is in large and remote offshore areas, with only 0.3% of these NTRs occurring in small to medium-sized protected areas (1-100km²) in coastal waters (ICMBIO, 2018). The effectiveness of these remote NTRs in terms of achieving conservation objectives has been questioned due to the difficulties of enforcement and monitoring of offshore waters (GIGLIO et al., 2018; MAGRIS;

PRESSEY, 2018). Despite the relatively small sizes of these coastal networks of NTRs, they have potentially high ecological and social value given the greater human impacts occurring in these coastal waters (IMOTO; CARNEIRO; ÁVILA-DA-SILVA, 2016; TEIXEIRA-NEVES; NEVES; ARAÚJO, 2015; VON GLASOW et al., 2013).

Coastal habitats along the northern coast of Brazil (north of 19°S) are dominated by coral reefs, whereas southern regions (between 19-28°S) are typified by rocky reefs. In general, the Brazilian province shelters a high number of endemic species and biomass of marine organisms (FLOETER et al., 2001; MOURA, 2000; PINHEIRO et al., 2018; ROCHA, 2003). In the transition zone between tropical and subtropical-temperate environments (20°S to 23°S), the mosaic of habitat types results in one of the highest species diversity of benthic (AUED et al., 2018) and reef fish species recorded in Brazil (FLOETER et al., 2001; PINHEIRO et al., 2015, 2018). These transitional reefs are biologically rich and complex environments, where it is vitally important to establish, enforce and understand the benefits of NTRs. However, the few studies available about the effects of Brazilian NTRs on fish assemblage are concentrated in the northern (FRANCINI-FILHO; MOURA, 2008a) and southern region (ANDERSON et al., 2014, 2018) of the country's coastline, or in offshore islands (FLOETER; HALPERN; FERREIRA, 2005; ILARRI; SOUZA; ROSA, 2017), with a lack of studies in the transition zones between tropical and subtropical realms of coastal NTR networks.

Historically, NTRs and reef ecosystems in the Southwestern Atlantic have been assessed using underwater visual census (UVC) [e.g., Anderson et al. (2014), Floeter; Halpern; Ferreira (2005); Francini-Filho and Moura (2008a); Ilarri; Souza; Rosa (2017)]. Despite the benefits of UVCs, such as being a rapid and effective tool in providing precise data especially about conspicuous and sedentary fish species (GOETZE et al., 2015; MINTE-VERA; DE MOURA; FRANCINI-FILHO, 2008; MURPHY; JENKINS, 2010), biases involving interobserver variability, underrepresentation of large and mobile species targeted by fisheries, as well as inaccuracy of abundance and size estimates can occur (HARVEY et al., 2004; ST. JOHN; RUSS; GLADSTONE, 1990; THOMPSON; MAPSTONE, 1997). In order to mitigate some of these issues and complement fish assemblage assessments, the use of video-based methods to collect data has been increasingly adopted; aided by rapid advancements in video technology and accessibility to cheaper and higher quality equipment (HARVEY et al., 2004; HARVEY; FLETCHER; SHORTIS, 2000).

Importantly, methods using such technologies create a permanent record allowing fish identification to be confirmed by experts and revisited when necessary.

Baited Remote Underwater stereo-Video (stereo-BRUV) and Diver Operated stereo-Video (stereo-DOV) are being widely employed to assess diverse aspects of fish assemblages (BOND et al., 2018; CAPPO; HARVEY; SHORTIS, 2006; GOETZE et al., 2015; MALLET; PELLETIER, 2014; WHITMARSH; FAIRWEATHER; HUVENEERS, 2017). Stereo-video techniques provide accurate body size and range measurements of individuals from the three-dimensional calibration of imagery (HARVEY; SHORTIS, 1996). Stereo-BRUV have been found to sample a wide range of species without precluding estimates of herbivorous species (HARVEY et al., 2007) and can be applied across a wide variety of habitats and depths (WHITMARSH; FAIRWEATHER; HUVENEERS, 2017). Also, as a remote sensing technique, it detects large and mobile animals which usually avoid divers and active fishing gears (CAPPO; HARVEY; SHORTIS, 2006; GOETZE et al., 2015), but has a range of acknowledged biases and limitations related to the presence of the bait and potential underrepresentation of small-bodied fish species [see Langlois et al. (2015) and Goetze et al. (2015)]. Conversely, the presence of a diver may impact the abundance of fish recorded using stereo-DOVs (GOETZE et al., 2015; HOLMES et al., 2013), suggesting that the combination of methods is more effective to sample fish assemblages (GOETZE et al., 2015; WATSON et al., 2005).

In order to expand knowledge about the ichthyofauna of the Southwestern Atlantic, we applied novel non-destructive methods that complement the traditionally used visual sampling techniques, offering potentially more robust estimates of targeted species among protected and fished areas. The improvement of non-lethal and non-destructive techniques to assess fish assemblage is crucial, especially for sensitive habitats inside protected areas such as reef environments, which shelter a significant amount of endangered and endemic species (PINHEIRO et al., 2018). Thus, this study is the first assessing fish assemblages using stereo-BRUVs and stereo-DOVs in the Southwestern Atlantic, and aims to contribute to the conservation and fisheries management in the region. Based on this, we aim to investigate the response of the fish assemblage to environmental and habitat variables, as well as the effect of protection among NTRs. We hypothesize that: (1) abundance and body size of targeted fish groups will be greater inside NTRs; whereas (2) non-target fish

abundance and species richness will be explained better by habitat and biogeographic variables.

#### Material and methods

This study was conducted in accordance with all Brazilian government legislation. This includes Federal Government authorization to observe and assess images within the Tupinambás Ecological Station under the permits #48259-1, and also authorization from the São Paulo State government (Fundação Florestal), by the Comissão Técnico Científica - COTEC, to develop the research project.

## Study site

The Ecological Station (ESEC) of Tupinambás is a no-take marine reserve (NTR) (corresponding to IUCN Category Ia) located on the northern coast of São Paulo State, Brazil, Southwestern Atlantic. The ESEC was established in 1987 (BRAZIL, 1987) and is divided into two sectors. Sector I is in the archipelago of Alcatrazes (24.101° S; 45.692° W), which is located approximately 43 km from of São Sebastião, São Paulo. This sector has six protected localities, each of them with 1km of buffer area. Two sets of two of these sites are close enough to overlap, creating four primary areas of protection (Figure 1). Sector II protects Palmas Island (23.547° S; 45.029° W) including two nearby reefs (Palmas Reef and Forno Reef) and Cabras Island (23.517° S; 45.041° W), located 5.7 km and 3.6 km respectively from the coast of Ubatuba, São Paulo.

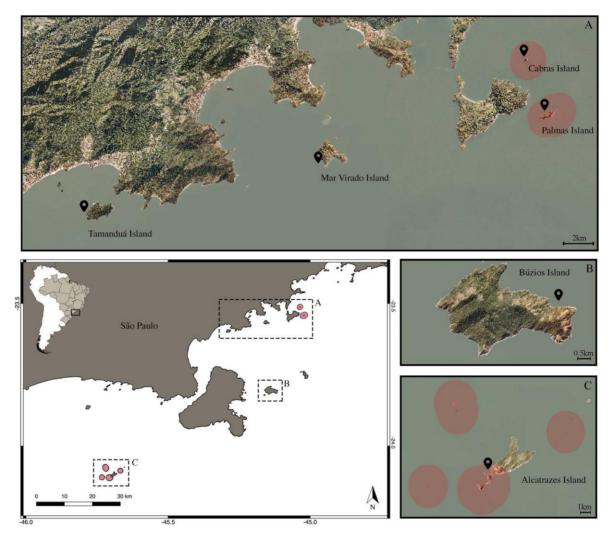


Figure 1. Map of the study area on the southeastern Brazilian coast with the no-take marine reserve Tupinambás Ecological Station in red. The control islands, where fishing activity is permitted (Tamanduá, Mar Virado and Búzios), are also displayed (a,b). No-take areas in detail in the islands of Cabras and Palmas (a) and in Alcatrazes Archipelago (c) with the sample sites represented by the black spots. Source of pictures: (EMPLASA, 2010).

The open-fishing areas used to test the effects of protection on fish assemblage included Búzios (23.804° S; 45.139° W), Mar Virado (23.567° S; 45.156° W) and Tamanduá (23.597° S; 45.289° W) islands. These islands are part of a multiple use marine protected area established in 2008 (Environmental Protection Area - corresponding to IUCN category V). They are located 34 km, 2 km, and 0.5 km respectively from the mainland. Small scale fishing, such as angling, spearfishing, longlines, fixed traps and gillnetting, is permitted around Mar Virado and Tamanduá islands, but no industrial fishing that uses pair trawling, driftnet vessels above 20

gross tonnage (GT) or trawling vessels up to 10 GT is allowed. However, only pair trawlers are excluded from fishing in Búzios Island.

## Sampling

Samples were collected using stereo-DOVs and stereo-BRUVs. Both equipment types are comprised of a metal base bar with two underwater SeaGIS housing (www.seagis.com.au), each with a digital video camera inside. Housings are positioned approximately 700 mm apart, each inwardly converged at 8 degrees. Stereo-BRUVs were deployed from a boat connected by a rope with a surface float, and left on the seafloor for 90 minutes to record fishes and habitat characteristics. The camera base bar was enclosed within a stainless steel frame, and a bait cage with 800 g of mashed sardine (*Sardinella brasiliensis*) was positioned at the end of a bait arm approximately 1.5 m away from the cameras. Oily bait provide greater sampling efficiency (DORMAN; HARVEY; NEWMAN, 2012; WHITELAW et al., 1991) due to the odor plume dispersion. Stereo-DOVs used the same camera base bar setup, with the addition of a handle, allowing divers to swim along a transect. These standard survey methods have been developed and used by many authors worldwide (CAPPO; HARVEY; SHORTIS, 2006; LANGLOIS et al., 2018; WHITMARSH; FAIRWEATHER; HUVENEERS, 2017).

Stereo-BRUV and stereo-DOV sampling was undertaken bimonthly at each island for a year (2016 - March, May, July, October, November; and 2017 - January). Each expedition was approximately 4-6 days long, covering all six islands. Due to the small size of islands and to maintain independence among samples, (suggested minimum distance between replicates is at least 250 m (HARVEY et al., 2007)), only two stereo-BRUV samples were collected on the leeward side of the islands, totaling 12 stereo-BRUVs at each island after six expeditions. Each stereo-BRUV was deployed at the interface of the rocky reef with the sandy bottom. Water depth ranged from 2-17 m depending on the location of the rock-sand interface at each island and the average water depth sampled was 8.3±3.6 m.

Stereo-DOV transects were 25 m long and 5 m wide, and swum at the interface of the rocky shore with the sandy bottom, as well as at the shallow zone above the reef. Due to the small size of the islands, sampling was restricted to three

transects at the rock-sand interface and three in the shallow zone on each island at each expedition, totaling 36 transects per island at the end of six expeditions. Stereo-DOV transects were surveyed twice. During the first survey, the observer filmed conspicuous species in the water column; during the second survey, the observer focused on the substrate to detect cryptic species (families Blenniidae, Gobiidae, Labrisomidae and Chaenopsidae). The sampling unit therefore included the number and size of both conspicuous and cryptic fish species per transect. This protocol is comparable to that used for underwater visual census in the region to ensure that the species that are more likely to avoid divers are recorded first, whilst small cryptic species are also sampled (ABURTO-OROPEZA; BALART, 2001; FLOETER et al., 2007a; NEVES et al., 2016). In stereo-DOV samplings, the interface zone presented an average depth of 8.9±3.8 m and the shallow zone 4.2±1.9 m.

#### Video analysis

# a. Fish assemblage

Stereo-video systems were calibrated using the CAL software and video analysis was carried out in the EventMeasure software (www.seagis.com.au). The description of the design and calibration of stereo-videos can be found in Harvey and Shortis (1996, 1998). Fish were identified to the finest taxonomic level possible, counted and measured if they were within 7m of the stereo-BRUVs and 5m for stereo-DOVs.

The relative abundance of each species filmed on stereo-BRUVs was recorded as MaxN, defined as the maximum number of individuals of the same species recorded in a single frame from the left camera. This is a conservative approach in order to avoid counting and measuring the same individual more than once. The fork length of individual fish contributing to a species' MaxN was measured when the fish was straight and no more than 45 degrees perpendicular to the cameras. In the stereo-DOV, all fish filmed on the left camera were counted and measured using the same rules. These data are stored on GlobalArchive (LANGLOIS et al., 2017) (globalarchive.org).

Biomass was calculated for all species using measured fish lengths and length-weight relationship referenced in the FishBase database (FROESE; PAULY, 2018). If equations for fork length of a species were not available, length-length conversions were used if available. Biomass of species without length-weight information was calculated using equations from a similar species from the same family.

Fish species were classified by broad functional groups based on diet, using information available in the literature (FERREIRA; GONÇALVES; COUTINHO, 2001; GIBRAN; MOURA, 2012) and FishBase (FROESE; PAULY, 2018). Groups included: carnivores, piscivores, planktivores, roving herbivores, territorial herbivores, omnivores, sessile invertebrate feeders, mobile invertebrate feeders. Piscivores were pooled with carnivores because there were not enough individuals for statistical analysis. Species were categorized in target and non-target for fisheries in the region according to the literature (BEGOSSI; RICHERSON, 1993; CARVALHO-FILHO, 1999; FLOETER et al., 2007a; ROLIM; RODRIGUES; GADIG, 2017). Four families (Epinephelidae, Kyphosidae, Scaridae and Carangidae) identified as abundant or frequent and also targeted by fisheries were selected for analysis.

#### **b.** Habitat characteristics

Habitat classification and complexity (mean relief) were analysed using a single high definition image of each stereo-BRUV deployment and three single frames of each stereo-DOV transect separated by approximately 8 m. This method is shown to be effective to determine reefs structural complexity (BENNETT et al., 2016; CAPPO et al., 2011; WILSON et al., 2012). Images were analyzed in TransectMeasure software (www.seagis.com.au) using a standardised broad habitat classification scheme based on CATAMI (ALTHAUS et al., 2015) to classify benthic composition and based on Wilson et al. (2007) to classify relief characteristics (Table 2). Each image was divided into a 5 x 4 grid and the dominant habitat type of each square was recorded. The proportion of the total number of grid squares that fell on each category was used to estimate percent cover by sample. For stereo-BRUVs, this estimate was based on a single frame per deployment; and for stereo-DOVs it was based on the average of the three replicates per transect. An additional category, 'reef', was formed at the end of the image analysis by pooling macroalgae,

stony coral, rock and zoanthids, and is based on the similar broad structure these environments present.

Table 1. Habitat classification based on broad CATAMI Classification scheme (ALTHAUS et al., 2015) and on (WILSON; GRAHAM; POLUNIN, 2007), used in Baited Remote Underwater stereo-Videos and Diver Operated stereo-Videos images.

Criteria	Description										
Relief	<ul> <li>0 - Flat substrate, sandy, rubble with few features. ~0 substrate slope</li> <li>1 - Some relief features amongst mostly flat substrate/sand/rubble. &lt;45 degree substrate slope</li> </ul>										
	2 - Mostly relief fea	atures amongst sor	ne flat substrate o	r rubble. ~45 substra	ate slope						
	3 - Good relief stru	cture with some ov	erhangs. >45 sub	strate slope							
	4 - High structural	complexity, fissure	s and caves. Verti	cal wall. ~90 substra	ite slope						
	5 - Exceptional s	tructural complexit	y, numerous larg	e holes and caves.	. Vertical wall. ~90						
	substrate slope	·	-								
	Unknown										
Field of view	Facing up	Limited									
	Facing down	Open									
Broad/Benthos	Ascidians	Consolidated	Open water	Stony corals	Unknown						
	Bryozoa	Macroalgae	Sponges	Unconsolidated	Zoanthids						

#### c. Environmental variables

Environmental variables were recorded at each sampling event. Temperature and salinity were measured using a Castaway CTD (Conductivity, Temperature and Depth) and an average temperature and salinity value was calculated from values recorded at the BRUV or dive depth, and 1 m above and below this. Visibility was estimated using a Secchi disk.

#### Data analysis

The influence of habitat characteristics and environmental variables on fish assemblage richness, abundance and biomass was investigated using Generalized Additive Mixed Models (GAMM) (HASTIE; TIBSHIRANI, 1986; LIN; ZHANG, 1999) and a full-subsets multiple regression approach based on the function described by Fisher et al. (2018). GAMMs use smoothing splines to estimate non-parametric additive functions, allowing for overdispersion and correlation in the data (LIN; ZHANG, 1999), which may arise in studies like this.

Models were fitted to untransformed overall abundance, richness and biomass data, as well as to abundance by functional group and by families. Models for

biomass by functional group and by family were also determined, however, as the same trends were found, we decided to report results on abundance only. A prior selection of the predictor variables was made based on their coverage and on the high collinearity between them (Pearson correlation coefficient r > 0.8). As a result, Reef, Rock and Mean relief remained as continuous variables for the analysis. Null variables of the random model included Month, Method, Depth and Visibility, and fixed factors included Distance to shore (two levels: inshore and offshore) and Protection (two levels: no-take and open). Continuous predictor variables were square root transformed to reduce dispersion of data.

Model selection for each response variable was based on the second-order variant of Akaike's Information Criterion suited for small samples (AIC<sub>c</sub>) (AKAIKE, 1973) and on AIC<sub>c</sub> weights ( $\omega$ AIC<sub>c</sub>). The best model was the most parsimonious one (with the fewest variables) within two AIC<sub>c</sub> units of the lowest AIC<sub>c</sub> value ( $\Delta$ AIC<sub>c</sub><2) (BURNHAM; ANDERSON, 2003). Because the effect of protection status, and any interactions, were relevant to the primary hypothesis of this study, models that were within two AICc units of the model with the lowest AIC<sub>c</sub> and included protection status, were therefore preferentially investigated ('hypothesis model'). Selected models had their shape and effective degrees of freedom (EDF) examined to ensure they did not overfit the data.

The distributions of fish lengths for key families were compared inside and outside NTRs using Mann-Whitney *U* test, considering a significant difference as p-values below 0.05. All analyses were performed using R Language for Statistical Computing (R CORE TEAM, 2018), with the packages gamm4 (WOOD; SCHEIPL, 2017), mgcv (WOOD, 2006), MuMIn (BARTON, 2018), doParallel (MICROSOFT-CORPORATION; WESTON, 2017) and dplyr (WICKHAM et al., 2018).

#### Results

A total of 23,505 individuals were observed belonging to 126 species of 44 families (list in S1 Table). Large schools (>100) of sardines (Clupeidae), mullets (*Mugil* spp.), young scads (*Decapterus* spp.), young vermilion snapper (*Rhomboplites aurorubens*) and young grunts (Haemulidae) were excluded from statistical analysis in order to reduce dispersion of data and highlight effects. Not

considering these schools, the most abundant and frequent families were grunts (Haemulidae), damselfishes (Pomacentridae), jacks (Carangidae) and snappers (Lutjanidae). The most abundant species were tomtate grunt (*Haemulon aurolineatum*) (28.1%), sergeant major (*Abudefduf saxatilis*) (10.1%), Brazilian damsel (*Stegastes fuscus*) (4.7%), gobies (*Coryphopterus* spp.) (2.3%) and squirrelfish (*Holocentrus adscensionis*) (1.3%). And the most frequent species were tomtate grunt (64.2%), Brazilian damsel (54.2%), sergeant major (49.6%), porkfish (*Anisotremus virginicus*) (38.9%) and dusky grouper (*Mycteroperca marginatus*) (35.1%).

Tomtate grunt was listed as a highly common and abundant species in inshore and offshore areas, and in both no-take and fished areas (Table 2). Brazilian damsel and sergeant major were also highly recorded as abundant and frequent, except for the abundance in offshore no-take areas, which was mainly represented by schools of grunts, scads and vermilion snappers (Table 2).

Table 2. Top five most abundant and common species (% of samples a species was observed) in notake and open to fisheries areas in inshore and offshore regions.

		ie .	Open					
	Abundance (n)		Frequency (%)		Abundance (n)		Frequency (%)	
	Haemulon aurolineatum	873	Haemulon aurolineatum	73	Haemulon spp.	651	Abudefduf saxatilis	48
ø	Abudefduf saxatilis	604	Mycteroperca marginatus	62	Abudefduf saxatilis	415	Stegastes fuscus	46
ρď	Decapterus spp.	500	Stegastes fuscus	53	Stegastes fuscus	335	Mycteroperca acutirostris	35
nsl	Coryphopterus spp.	289	Anisotremus virginicus	50	Haemulon aurolineatum	168	Haemulon aurolineatum	26
_	Stegastes fuscus	265	Abudefduf saxatilis	43	Caranx latus	96	Anisotremus virginicus	22
	Haemulon aurolineatum	4336	Haemulon aurolineatum	90	Haemulon aurolineatum	1231	Haemulon aurolineatum	50
ē	Decapterus punctatus	2304	Pomacanthus paru	75	Abudefduf saxatilis	939	Abudefduf saxatilis	56
ᅙ	Rhomboplites aurorubens	1807	Holocentrus adscensionis	s71	Haemulon spp.	500	Halichoeres poeyi	33
)ffs	Haemulon spp.	630	Kyphosus spp.	63	Stegastes fuscus	285	Stegastes fuscus	53
O	Decapterus spp.	504	Stegastes fuscus	60	Mugil spp.	264	Chaetodon striatus	32

Nineteen species recorded are endemic to the Brazilian Province (FLOETER; GASPARINI, 2000; GASPARINI; JOYEUX; FLOETER, 2003; JOYEUX et al., 2001) and fourteen species are considered threatened (vulnerable/endangered) or near threatened, by the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2018) and the Brazilian legislation (MMA, 2014) (detailed list in S1 Table).

The most parsimonious model for total richness included distance to shore and mean relief, whereas for both total abundance and biomass the selected models included protection status and distance to shore (Table 3, Figures 2 and 3). The model for overall abundance was selected based on the primary hypothesis of

interest, and was within 2AIC of the top model, but it is interesting to note that mean relief was highly important (Figure 2) and present in the most parsimonious model.

Table 3. Top generalised additive mixed models (GAMMs) to predict different aspects of fish assemblage.  $\Delta AICc=$  Difference between lowest reported corrected Akaike Information Criterion;  $\Delta BIC=$  Bayesian Information Criterion;  $\omega AICc=$  AICc weights;  $\omega BIC=$  BIC weights; R2= variance explained; EDF= effective degrees of freedom. Model selection was based on the most parsimonious model within two units of the lowest AICc which has the fewest variables.

Dependent variables	Best models	ΔΑΙС	ΔΒΙΟ	ωΑΙС	ωΒΙΟ	$\mathbb{R}^2$	EDF
Overall							
Richness	Distance to shore + Mean relief.by.Distance to shore	0.00	0.00	1.00	0.89	0.57	18.75
Abundance	Mean relief + Distance to shore	0.49	0.00	0.32	0.68	0.30	8.53
	Protection + Distance to shore	0.00	1.57	0.41	0.31	0.34	8.76
	Distance to shore + Mean relief.by.Distance to shore	0.89	7.52	0.26	0.02	0.30	10.33
Biomass	Protection + Distance to shore	0.00	0.00	1.00	1.00	0.27	16.50
Importance to fisheries							
Non-target species richness	Distance to shore + Mean relief.by.Distance to shore	0.00	0.00	1.00	1.00	0.47	14.53
Non-target species abundance	$Distance\ to\ shore+Mean\ relief. by. Distance\ to\ shore$	0.00	4.83	0.90	0.08	0.39	8.54
Target species richness	Mean relief + Distance to shore	0.00	0.00	0.61	0.89	0.56	18.42
Target species abundance	Protection + Distance to shore	0.00	0.00	1.00	1.00	0.25	13.55
Abundance by functional gro	ир						
Carnivores/Piscivores	Protection + Mean relief.by.Protection	0.00	6.50	0.86	0.02	0.30	17.28
Mobile invertebrate feeders	Protection + Distance to shore	0.00	0.00	1.00	1.00	0.23	10.21
Sessile invertebrate feeders	Protection	1.43	0.00	0.15	0.40	0.13	7.31
	Protection + Reef.by.Protection	0.17	4.73	0.28	0.04	0.16	9.16
	Protection + Mean relief.by.Protection	0.00	12.54	0.31	0.00	0.11	10.92
Omnivores	Mean relief + Distance to shore	0.00	0.00	0.90	0.96	0.31	13.38
Planktivores	Protection + Mean relief.by.Protection	0.00	3.54	0.92	0.14	0.03	8.81
Roving herbivores	Mean relief + Distance to shore	0.00	0.00	0.44	0.48	0.17	6.95
	Distance to shore + Mean relief.by.Distance to shore	0.47	17.10	0.35	0.00	0.18	9.22
Territorial herbivores	Protection + Mean relief.by.Protection	0.00	0.00	0.89	0.93	0.40	14.66
Abundance by family							
Epinephelidae	Protection + Reef.by.Protection	0.00	0.00	1.00	1.00	0.27	19.49
Kyphosidae	Distance to shore + Reef.by.Distance to shore	0.00	0.00	0.53	0.42	0.15	17.92
	Distance to shore + Rock.by.Distance to shore	0.30	0.58	0.46	0.31	0.16	17.94
Scaridae	Protection + Distance to shore	0.00	0.00	1.00	1.00	0.10	15.14
Carangidae	Protection + Reef.by.Protection	0.00	0.00	1.00	1.00	0.22	20.22

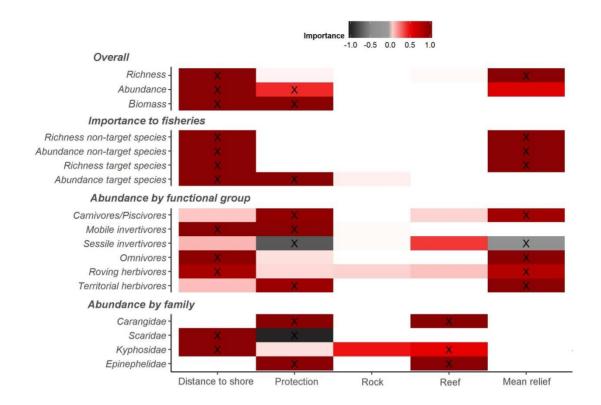


Figure 2. Variable importance scores from full-subset generalised additive mixed models analysis, with >10% variance explained shown. X= Predictor variables within the most parsimonious model for each response variable (see Table 1).

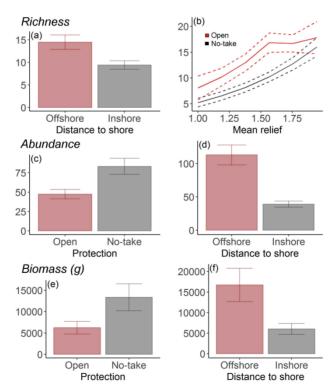


Figure 3. Plots of the most parsimonious models, with >10% variance explained shown. (a,b) species richness, (c,d) total abundance and (e,f) total biomass. The dotted line represents 95% confidence interval.

Higher richness of target and non-target species and greater abundance of non-target species were best predicted by increased distance to shore and mean relief, whilst the most parsimonious models for the abundance of target species indicated they were likely to increase with protection and distance to shore (Figure 4). Concerning abundance by functional groups, the most parsimonious models for carnivores/piscivores, planktivores and territorial herbivores all included a positive relationship with protection and mean relief. However, as the variance explained by the model for planktivores was very low (R²<10) (Table 2), it was not represented graphically in Figures 2 and 5. Contrary to the trend found for the other functional groups, the abundance of sessile invertebrate feeders was found to be negatively correlated with protection status and mean relief. For mobile invertebrate feeders, the abundance is likely to increase with protection and distance to shore. The number of herbivores and omnivores was higher in areas further from shore and also on structurally complex reefs (Figure 5).

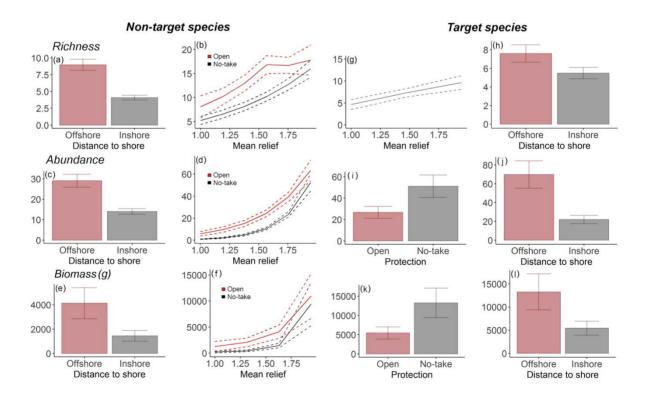


Figure 4. Plots of the most parsimonious models for target and non-target fish assemblage overall characteristics, with >10% variance explained shown. on-target species (a,b) richness, (c,d) abundance, (e,f) biomass. And for target species (g,h) richness, (i,j) abundance, (k,l) biomass. The dotted line represents 95% confidence interval.

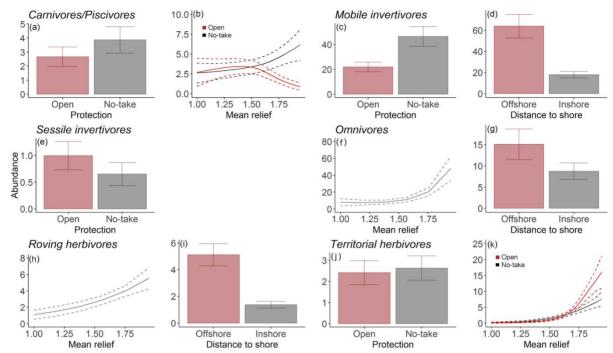


Figure 5. Plots of the most parsimonious model for abundance by functional group, with >10% variance explained shown. (a,b) Carnivores/piscivores, (c,d) Mobile invertebrate feeders, (e) Sessile invertebrate feeders, (f,g) Omnivores, (h,i) Roving herbivores, (j,k) Territorial herbivores. The dotted line represents 95% confidence interval.

Targeted families Carangidae and Epinephelidae increased with protection and presence of reef, whilst kyphosids were found in greater abundance in areas with more reef and greater distance from shore. Scarid abundance showed a negative correlation with protection and a positive correlation with distance to shore (Figure 6). In terms of body size of these families, the largest individuals were found inside the NTRs, with significant differences (Carangidae: U = 38283, p-value<0.001; Scaridae: U = 4462.0, p-value<0.001; Kyphosidae: U = 6450.5, p-value<0.001; Epinephelidae: U = 9341.5, p-value = 0.013) (Figures 6C, F and I).

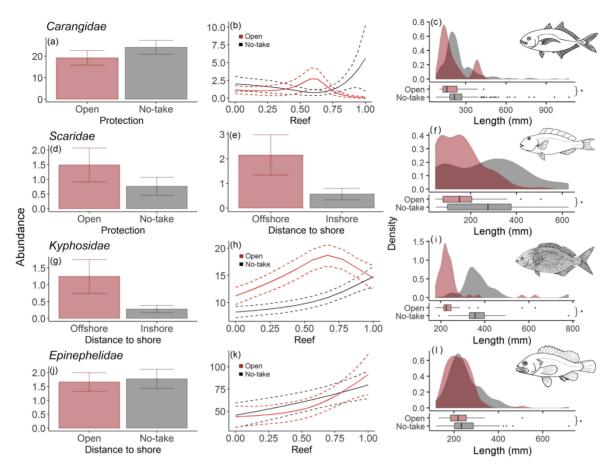


Figure 6. Plots of the most parsimonious models for abundance, Kernel density plots and boxplots for fork length (mm) for important fishing target families. (a,b,c) Carangidae, (d,e,f) Scaridae, (g,h,i) Kyphosidae and (j,k,l) Epinephelidae. The dotted line represents 95% confidence interval. \* Significant difference. Fish drawings were based on Carvalho-Filho (1999).

#### **Discussion**

This study is the first to generate fisheries independent data using non-destructive stereo-video methods in the Southwestern Atlantic. Besides that, the approach adopted here made it possible to distinguish the effect of fishing from habitat variables on different components of the fish assemblage, demonstrating how NTRs can be used as benchmarks to contribute to resource management and marine conservation.

#### The role of no-take marine reserves

Broadly, total abundance and biomass were greater inside no-take areas, a pattern also registered in previous studies (HALPERN, 2003; MICHELI et al., 2004; SOLER et al., 2015). The assessment of biomass in the marine environment is important and can reveal the health status of an environment especially because it can be used to represent the energy flux, as well as the potential of the ecosystem to provide goods and services (CARDINALE et al., 2006; HOOPER et al., 2005). Based on this, the results indicate that the NTR in question is protecting natural processes and resources, which are being effectively converted into biomass. Conversely, the opposite was found in areas open to fishing, presenting a decreased ecosystem functioning driven by the selective removal of large individuals (CROWDER et al., 2008; MORA et al., 2011). Higher overall abundance and biomass within NTRs indicates the significant removal of fish by fisheries in the open access areas in the region.

Distance from the coast was an important factor, explaining the higher richness, abundance and biomass recorded in islands further from the coast. This factor has been demonstrated to influence fish assemblages' structure in several coral and rocky reefs around the world (LECCHINI et al., 2003; MALCOLM; JORDAN; SMITH, 2010; SCHULTZ et al., 2014; VAN NGUYEN; KIM PHAN, 2007) and also in the Brazilian Province (FLOETER et al., 2001; GIBRAN; MOURA, 2012; MORAIS; FERREIRA; FLOETER, 2017; TEIXEIRA-NEVES; NEVES; ARAÚJO, 2015). The first hypothesis we raise to explain the higher richness and abundance in offshore islands may be related to the total area of rocky reefs. In the region, offshore reefs are typically deeper and form a larger continuous extension when compared to inshore reefs that are often interspersed with sandy beaches, probably leading a smaller surface area available for reef fishes. Surface area of reef has been directly attributed to fish assemblage structure in some studies. For example, Francini-Filho and Moura (2008b) found a more pronounced increase of overall biomass over time in areas adjacent to coral reefs that reach deeper water. Furthermore, Roberts and Ormond (1987) registered higher species richness with depth, and Gibran and Moura (2012) also detected this tendency for rocky reefs in the same region of the present study. These findings might be due to higher availability of resources and a possible lower competition in offshore islands, especially for space (SALE, 1977).

The second hypothesis to explain the higher values of ecological metrics is related to the proximity of anthropogenic activities. The close proximity of human populations to a fish assemblage causes negative effects and is demonstrated worldwide (BABCOCK et al., 2010; EDGAR et al., 2014, 2017; LANGLOIS; HARVEY; MEEUWIG, 2012; MORA et al., 2011). Areas close to the mainland are easier to access and tend to have more fishing activities. Nearshore waters (<50 m water depth) of the São Paulo state coast, are highly explored by both artisanal and industrial fishing fleets, with artisanal, low mobility fleets most dominant in water depth <20 m (IMOTO; CARNEIRO; ÁVILA-DA-SILVA, 2016). Coastal regions with high population densities, such as São Paulo, are more exposed to human activities causing disturbances and changes in coastal dynamics, especially concerning the high input of nutrients and pollution through air deposition, river discharges, urban and industrial wastewater effluents, groundwater and surface runoff (VON GLASOW et al., 2013). These potentially harmful components cause environmental stress and may damage coastal biota directly or indirectly (ADAMS, 2005). In addition, areas near the coast also face greater exposure to major developments, such as harbors and marinas, which can also significantly change the coastal landscape, causing degradation of habitats and consequently affecting fish assemblage. Further studies in the region are needed to test these hypotheses in order to determine whether or how much of this pattern is explained by biogeography or anthropogenic activities.

#### Target and non-target species

Higher abundance of target species was observed within NTRs, but protection status did not correlate with any differences in the abundance of non-target species. Indeed, studies have shown increased abundance of highly targeted fishes inside notake NTRs, with lower influence on non-target (EDGAR et al., 2014; EVANS; RUSS, 2004; FLOETER; HALPERN; FERREIRA, 2005; MALCOLM et al., 2018; MICHELI et al., 2004), reinforcing evidence of the direct effects of fishing. Abundance of target species also increased with greater distance from the shore, which can be related to

the increased fisheries activity close to the shore as described above (IMOTO; CARNEIRO; ÁVILA-DA-SILVA, 2016).

Conversely, species richness and richness of target and non-target fish, was not related to protection status, being mostly explained by relief. Higher species diversity in more complex environments has been described in the literature (EDGAR et al., 2014; NEVES et al., 2016; PINHEIRO; MARTINS; JOYEUX, 2013), and is likely related to increased availability of food, decreased competition, and lower probability of predator-prey encounters (EKLÖV; DIEHL, 1994; HAUZY et al., 2010; SALE, 1977). Structurally complex environments have higher availability and diversity of niches, accommodating a higher number of species in a small area.

## Fish functional groups

Although functional groups responded differently to fishing pressure, we found evidence that protection status affected the trophic structure of the fish assemblage, since carnivores/piscivores, mobile invertebrate feeders, and territorial herbivores were more abundant within the NTR, whilst sessile invertebrate feeders were less abundant. However, protection was not relevant for omnivores and roving herbivores. The abundance of the carnivores/piscivores functional group, which is comprised of species targeted by fisheries in the region (BEGOSSI; RICHERSON, 1993; CARVALHO-FILHO, 1999; FLOETER et al., 2007a; ROLIM; RODRIGUES; GADIG, 2017), was higher within NTRs. Even though relief was important, it was relevant only when combined with protection. These results suggest that the NTR is facilitating the recovery of high trophic level organisms, which are usually the first group depleted by fisheries (CHRISTENSEN et al., 2003; CROWDER et al., 2008; PAULY et al., 1998).

Some mobile invertebrate feeder species are targeted by fisheries, but are not considered as important to fisheries as carnivores because of their smaller body size, such as haemulids, labrids and small carangids. Nevertheless, protection was still an important factor to predict abundance of this group, suggesting some fishing pressure, albeit less than highly targeted carnivores/piscivores. This might be related to a depletion of top predators, leading to an exploration of lower trophic levels, as already described worldwide (PAULY et al., 1998), including Brazilian coast (Freire

and Pauly, 2010). The other factor strongly affecting abundance of this group is distance to shore, which may be related to the larger rocky reef surface, offering more resources and, consequently, less competition (SALE, 1977). This is especially important for small and benthic mobile invertebrate feeders of the families Blenniidae and Serranidae, which live closely associated with the substrate (CARVALHO-FILHO, 1999). Similar results were found for omnivores, in which higher abundance is more likely to occur in high complex habitats in offshore islands, probably for the same reasons, since this group encompasses blennies, pomacentrids, pomacanthids and species of the order Tetraodontiformes. Although some species within this category are targeted by fisheries (Mugilidae, Sparidae, Ephippidae), protection was not an important factor to determine abundance. This is probably related to the plasticity of the omnivorous diet, which can enable greater resistance to environmental changes (e.g. Bellwood et al., (2006) and Pratchett et al. (2011)).

As the abundance of sessile invertebrate feeders was very low in samples, the model was not robust. However, lower abundance found within NTRs and in more complex reefs indicated by the model may be related to the elusive behaviour of these species, which usually hide from divers and may not be recorded. Since these species feed on benthic invertebrates generally associated with hard substrate, we would expect a higher abundance in more topographic complex environments. For planktivores, models did not predict the abundance well, most likely because species in this group show highly variable body sizes, occupying very different niches. For example, fish from Echeneidae and Carangidae families are mobile and large-bodied species, occupying the pelagic environment, whilst the species from Pomacentridae and Pempheridae families are small-bodied species that live associated with burrows and crevices on the rocky reef (CARVALHO-FILHO, 1999). Therefore, it was not possible to determine a single robust model to explain abundance of this functional group with the predictor variables used.

Abundance of roving herbivores was related to distance from shore and topographic complexity, which is expected considering its diet, algae and detritus, are mostly found in reef environments (FRANCINI-FILHO et al., 2010), which are more likely abundant in larger rocky reefs of offshore islands. This is similar with the results for territorial herbivores, in which protection was only important when combined with topographic complexity. This is also likely, since territorial herbivores, such as damselfishes (*Stegastes* spp.), are found in complex regions of the reef protecting

colonies of the major components of their diet, primarily fast growing red and green filamentous algae (FERREIRA et al., 1998, 2015). As habitat characteristics were more influential in herbivores abundance than protection status, fisheries effects were not evident for these groups, even though some of them are targeted in the region.

## **Targeted fish families**

One of the consequences of large removal of individuals by fisheries activities is represented by a rapid decrease in abundance and richness, especially of large bodied target species (CROWDER et al., 2008). Indeed, the effects of fishing on the size of individuals is well described, in which target species reach larger sizes within NTRs (DEMARTINI et al., 2008; EDGAR et al., 2017; FLOETER; HALPERN; FERREIRA, 2005; MALCOLM et al., 2018; WATSON et al., 2009). The present study corroborates these findings, showing a significantly higher density of larger individuals of target species of the families Epinephelidae, Kyphosidae, Carangidae and Scaridae within protected areas. This also represents an increase in reproduction capacity of these groups in protected areas as larger individuals usually present much higher fecundity (JENNINGS; KAISER; REYNOLDS, 2009). This increases the probability of exporting larvae from NTRs to adjacent areas (FRANCINI-FILHO; MOURA, 2008b; PALUMBI, 2004) repopulating fished reefs and helping to restock targeted species in fished areas.

Networks of moderate size (10-100 km²) NTRs have demonstrated to be more effective in resource management and conservation when compared to smaller protected areas (HALPERN; WARNER, 2003). However, small (1-5 km²) and very small (<1 km²) areas have been widely implemented and shown to have some advantages, specifically for small bodied and sedentary species with smaller home ranges (AFONSO; FONTES; SANTOS, 2011; BONALDO et al., 2017; DI FRANCO et al., 2018; MCLAREN et al., 2015). In particular, individuals of the Epinephelidae family presented a higher abundance with protection and also in complex environments within the very small NTR in question. These species live associated with burrows within rocky reefs (GIBRAN, 2007) and are highly targeted by fisheries,

indicating that they may be the group benefiting most from protection, as seen in this study.

The abundance of kyphosids was not related to protection and was more abundant in regions offshore with the greater presence of reef. As this species is considered herbivorous, grazing predominately on macroalgae (*Sargassum* spp.) associated with rocks (SILVANO; GÜTH, 2006), we expect to record higher numbers at locations with greater food availability, including offshore areas with more rocky reef. However, larger individuals could be targeted by fishers, resulting in their higher abundance recorded within NTRs. This indicates that the NTRs allow the growth of individuals, and therefore provide greater reproductive capacity for the species.

For the Carangidae family, an effect of protection in abundance was evident, suggesting a high removal, especially of large individuals, in areas open to fisheries. Besides, regardless of being a mobile species, they are frequently found associated with hard structures (BOND et al., 2018) and even following other species (SAZIMA et al., 2007), and probably for this reason, individuals of this family have shown to benefit from NTRs in reefs (EDGAR et al., 2014; SANTANA-GARCON et al., 2014).

Fish of the Scaridae family showed a higher abundance in fished areas, likely due to the absence of top predators (carnivores/piscivores), since species of this family have been registered to be preyed upon by epinephelids, carangids and muraenids (RANDALL, 1967). Even though they were more abundant in fished areas, fish size was smaller, representing a fishing pressure in larger sizes, as also described by Floeter et al. (2005). Also, the abundance of these roving herbivores was higher with distance from the coast, what could be related to the availability of food and lower competition in larger and continuous reefs offshore.

### **Conclusions**

Brazil shelters the second richest reefs in the Atlantic Ocean (PINHEIRO et al., 2018), and also stands out for the proportion of endemic and endangered species concentrated in small areas (FLOETER et al., 2007b; MOURA, 2000; PINHEIRO et al., 2018). Therefore, it is crucial to generate information about the role NTRs can play in fish assemblages of this region. In addition, a better understanding of patterns in the effects of fishing on a fish assemblage provides robust metrics for conservation

and fisheries management, whilst also providing information on focal species and biological variables most relevant to monitor the effectiveness of NTRs to protect fish assemblages.

The present study presents evidence that very small NTRs (<1km²) can protect fish assemblages from the direct effects of fishing, increasing abundance and biomass, especially of targeted species, therefore contributing to the management of fisheries resources at a local and regional scale. Some functional groups showed a higher benefit from protection, such as carnivores/piscivores and mobile invertebrate feeders, while others decreased in abundance, such as the Scaridae family. Another outstanding difference is concerning body size, mostly for target species, in which NTRs allow target species to reach larger sizes. The Epinephelidae family showed greater evidence to benefit from these very small NTRs, especially due to its high importance to fisheries and its small home range. However, we recommend that networks of larger NTRs (>10Km²) should be established in the region, which would provide a more robust framework for investigating and managing the effects of fishing and informing conservation and fisheries management more broadly.

As a concluding remark, our findings show strong influence of protection, distance from the shore and mean relief on fish assemblage characteristics, in which protected areas further from the human influence and with a higher topographic complexity tend to have greater abundance and biomass of fish. Our results highlight the crucial role these areas play in the conservation and recovery of highly valuable commercial stocks to the fishing activity of the region, displaying the importance of keeping and implementing more NTRs in the region. The use of stereo-videos in this study has shown to be effective and feasible in this region, providing valuable and robust information to aid conservation and fisheries management in Brazil.

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# Supplementary material

Table S1 - List of species found within no-take reserves (Tupinambás Ecological Station) and fished areas. \* Endemic from Brazilian biogeographic province (FLOETER; GASPARINI, 2000; JOYEUX et al. 2001; GASPARINI; JOYEUX; FLOETER, 2003; PINHEIRO et al. 2018);  $VU_I$ = Vulnerable by International Union for Nature Protection Red List (IUCN, 2018);  $VU_I$ = Near threatened by IUCN;  $VU_{Br}$ = Vulnerable by Brazilian legislation (MMA, 2014);  $VU_{Br}$ = Critically endangered by Brazilian Legislation; V= Target; V= Non-target; V= Abundance; V= Frequency.

		Francisco el		_		No-take					TOTAL	TOTAL				
Family	Species	Functional group	Target	Insl	nore	Offshore	Tota	Total	Ins	hore	Offsl	ore	Tota	Total	TOTAL N	F%
		group		N	F%	N F%	IN	F%	N	F%	N	F%	ΙN	F%	.,	1 /0
Dasyatidae	Hypanus spp.	Carnivore	Υ	39	5.2	3 4.2	42	4.9	5	3.1	1	2.1	6	2.8	48	3.82
Dasyatidae	Hypanus americanus	Carnivore	Υ	1	1.0	- 0.0	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Gymnuridae	Gymnura altavela (VUı;CR <sub>Br</sub> )	Piscivore	Υ	2	1.0	1 2.1	3	1.4	1	1.0	-	0.0	1	0.7	4	1.04
Myliobatidae	Aetobatus narinari	Mob. inv. feeder	Υ	-	0.0	- 0.0	-	0.0	1	1.0	3	2.1	4	1.4	4	0.69
Myliobatidae	Myliobatis spp.	Mob. inv. feeder	Υ	-	0.0	2 4.2	2	1.4	-	0.0	-	0.0	-	0.0	2	0.69
Rhinopteridae	Rhinoptera spp.	Mob. inv. feeder	Υ	3	3.1	- 0.0	3	2.1	2	1.0	-	0.0	2	0.7	5	1.39
Albulidae	Albula vulpes	Mob. inv. feeder	Υ	-	0.0	- 0.0	-	0.0	1	1.0	-	0.0	1	0.7	1	0.35
Muraenidae	Gymnothorax moringa	Carnivore	Υ	4	4.2	1018.8	14	9.0	1	1.0	5	10.4	6	4.2	20	6.60
Muraenidae	Gymnothorax funebris	Carnivore	Υ	4	4.2	3 6.3	7	4.9	1	1.0	3	6.3	4	2.8	11	3.82
Muraenidae	Gymnothorax vicinus	Carnivore	Υ	-	0.0	1 2.1	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Synodontidae	Synodus spp.	Piscivore	Ν	1	1.0	2 2.1	3	1.4	-	0.0	1	2.1	1	0.7	4	1.04
Synodontidae	Synodus intermedius	Piscivore	Ν	1	1.0	2 4.2	3	2.1	-	0.0	2	2.1	2	0.7	5	1.39
Ogcocephalidae	Ogcocephalus vespertilio	Mob. inv. feeder	Ν	2	2.1	1 2.1	3	2.1	-	0.0	1	2.1	1	0.7	4	1.39
Holocentridae	Holocentrus adscensionis	Mob. inv. feeder	Ν	63	38.5	21570.8	278	49.3	9	2.1	40	43.8	49	16.0	327	32.64
Fistulariidae	Fistularia tabacaria	Piscivore	Υ	-	0.0	- 0.0	-	0.0	-	0.0	2	4.2	2	1.4	2	0.69
Fistulariidae	Fistularia petimba	Piscivore	Υ	-	0.0	- 0.0	-	0.0	-	0.0	2	4.2	2	1.4	2	0.69
Dactylopteridae	Dactylopterus volitans	Mob. inv. feeder	Υ	-	0.0	- 0.0	-	0.0	-	0.0	9	10.4	9	3.5	9	1.74
Serranidae	Serranus spp.	Mob. inv. feeder	Ν	1	1.0	3 6.3	4	2.8	-	0.0	1	2.1	1	0.7	5	1.74
Serranidae	Serranus flaviventris	Mob. inv. feeder	Ν	8	6.3	- 0.0	8	4.2	2	2.1	-	0.0	2	1.4	10	2.78
Serranidae	Serranus baldwini	Mob. inv. feeder	Ν	12	9.4	16 18.8	28	12.5	-	0.0	20	22.9	20	7.6	48	10.07
Serranidae	Serranus atrobranchus	Mob. inv. feeder	Ν	2	2.1	- 0.0	2	1.4	-	0.0	-	0.0	-	0.0	2	0.69
Serranidae	Diplectrum radiale	Carnivore	Ν	27	6.3	7 8.3	34	6.9	-	0.0	10	6.3	10	2.1	44	4.51
Epinephelidae	Mycteroperca acutirostris	Carnivore	Υ	35	31.3	812.5	43	25.0	49	35.4	20	31.3	69	34.0	112	29.51
Epinephelidae	<i>Mycteroperca marginatus</i> (EN <sub>I</sub> ;VU <sub>Br</sub> )	Carnivore	Υ	133	61.5	44 52.1	177	58.3	9	8.3	15	18.	8 2	24 11.8	3 201	35.07
Epinephelidae	Mycteroperca bonaci (NT <sub>I</sub> ;VU <sub>Br</sub> )	Carnivore	Υ	3	3.1	- 0.0	3	2.1	1	1.0	2	2.1	3	1.4	6	1.74

	Species	Francisco d				No-	-take		·	·		Op	en		TOTAL :	TOTAL	
Family		Functional group	Target	Insl	nore	Offsl	hore	Tota	Total	Insl	nore	Offs	hore	Tota	Tota	TOTAL T N	F%
		9.04		N	F%	N	F%	IN	F%	N	F%	N	F%	IN	F%		. ,0
Epinephelidae	Epinephelus morio (NT <sub>I</sub> ;VU <sub>Br</sub> )	Carnivore	Υ	7	7.3		2.1	8	5.6	3	3.1	1	2.1	4	2.8	12	4.17
Epinephelidae	Cephalopholis spp.	Carnivore	Υ	-	0.0		2.1	4	0.7	-	0.0	-	0.0	-	0.0	4	0.35
Epinephelidae	Cephalopholis fulva	Carnivore	Υ	1	1.0	1	2.1	2	1.4	-	0.0	-	0.0	-	0.0	2	0.69
Priacanthidae	Heteropriacanthus cruentatus	Mob. inv. feeder	Υ	14	4.2	1	2.1	15	3.5	-	0.0	38	8.3	38	2.8	53	3.13
Echeneidae	Echeneis naucrates	Planktivore	Ν	-	0.0	-	0.0	-	0.0	-	0.0	1	2.1	1	0.7	1	0.35
Malacanthidae	Malacanthus plumieri	Carnivore	Ν	-	0.0	16	20.8	16	6.9	-	0.0	1	2.1	1	0.7	17	3.82
Rachycentridae	Rachycentron canadum	Carnivore	Υ	-	0.0	1	2.1	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Carangidae	Caranx bartholomaei	Carnivore	Υ	1	1.0	2	4.2	3	2.1	2	1.0	1	2.1	3	1.4	6	1.74
Carangidae	Caranx crysos	Carnivore	Υ	4	4.2	20	10.4	24	6.3	1	1.0	39	4.2	40	2.1	64	4.17
Carangidae	Caranx hippos	Carnivore	Υ	2	1.0	1	2.1	3	1.4	-	0.0	-	0.0	-	0.0	3	0.69
Carangidae	Caranx latus	Carnivore	Υ	114	20.8	46	27.1	160	22.9	96	18.8	40	31.3	136	22.9	296	22.92
Carangidae	Pseudocaranx dentex	Mob. inv. feeder	Υ	42	6.3	66	16.7	108	9.7	8	1.0	5	4.2	13	2.1	121	5.90
Carangidae	Seriola spp.	Carnivore	Υ	1	1.0	12	2.1	13	1.4	-	0.0	-	0.0	-	0.0	13	0.69
Carangidae	Seriola dumerili	Carnivore	Υ	3	1.0	3	6.3	6	2.8	-	0.0	-	0.0	-	0.0	6	1.39
Carangidae	Seriola lalandi	Carnivore	Υ	-	0.0	5	4.2	5	1.4	-	0.0	-	0.0	-	0.0	5	0.69
Carangidae	Seriola rivoliana	Carnivore	Υ	3	2.1	4	8.3	7	4.2	-	0.0	-	0.0	-	0.0	7	2.08
Carangidae	Selene vomer	Carnivore	Υ	2	1.0	-	0.0	2	0.7	3	2.1	-	0.0	3	1.4	5	1.04
Carangidae	Decapterus spp.	Planktivore	Υ	500	1.0	504	2.1	1004	1.4	-	0.0	-	0.0	-	0.0	1004	0.69
Carangidae	Decapterus macarellus	Planktivore	Υ	2	1.0	-	0.0	2	0.7	-	0.0	1	2.1	1	0.7	3	0.69
Carangidae	Decapterus punctatus	Planktivore	Υ	-	0.0	2304	4.2	2304	1.4	-	0.0	-	0.0	-	0.0	2304	0.69
Carangidae	Chloroscombrus chrysurus	Carnivore	Υ	-	0.0	-	0.0	-	0.0	2	1.0	-	0.0	2	0.7	2	0.35
Carangidae	Trachinotus carolinus	Carnivore	Υ	1	1.0	-	0.0	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Carangidae	Trachinotus goodei	Carnivore	Υ	1	1.0	-	0.0	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Carangidae	Elagatis bipinnulata	Planktivore	Υ	-	0.0	-	0.0	-	0.0	-	0.0	1	2.1	1	0.7	1	0.35
Lutjanidae	Lutjanus spp.	Carnivore	Υ	1	1.0	-	0.0	1	0.7	-	0.0	1	2.1	1	0.7	2	0.69
Lutjanidae	Lutjanus analis (NT₁)	Carnivore	Υ	4	3.1	4	8.3	8	4.9	2	2.1	-	0.0	2	1.4	10	3.13
Lutjanidae	Lutjanus jocu	Carnivore	Υ	1	1.0	-	0.0	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Lutjanidae	Lutjanus synagris	Carnivore	Υ	4	3.1	-	0.0	4	2.1	-	0.0	-	0.0	-	0.0	4	1.04
Lutjanidae	Lutjanus chrysurus	Carnivore	Υ	5	4.2	1	2.1	6	3.5	2	1.0	1	2.1	3	1.4	9	2.43
Lutjanidae	Rhomboplites aurorubens (VU <sub>I</sub> )	Carnivore	Υ	-	0.0	1807	22.9	1807	7.6	20	1.0	1	2.1	21	1.4	1828	4.51
Gerreidae	Eugerres brasilianus	Sess. inv. feeder	Υ		0.0		0.0		0.0	1	1.0		0.0	1	0.7	1	0.35

	Species	Functional group	·			No-take		·			TOTAL:	TOTAL				
Family			Target	Insl	nore	Offshore	Tota	Total	Insl	hore	Offshore		Tota Tota		TOTAL <sup>*</sup> N	F%
		group		N	F%	N F%	IN	F%	N	F%	N	F%		F%		1 /0
Haemulidae	Haemulon spp.	Mob. inv. feeder	Υ	240	3.1	630 8.3	870	4.9	651	4.2	500	2.1	1151	3.5	2021	4.17
Haemulidae	Haemulon parra	Mob. inv. feeder	Υ	161	15.6	218 18.8	379	16.7	76	19.8	4	4.2	80	14.6	459	15.63
Haemulidae	Haemulon plumieri	Mob. inv. feeder	Υ	1	1.0	4 8.3	5	3.5	-	0.0	2	4.2	2	1.4	7	2.43
Haemulidae	Haemulon aurolineatum	Mob. inv. feeder	Υ	873	72.9	433689.6	5209	78.5	168	26.0	1231	97.9	1399	50.0	6608	64.24
Haemulidae	Haemulon steindachneri	Mob. inv. feeder	Υ	27	10.4	16 16.7	43	12.5	29	13.5	2	4.2	31	10.4	74	11.46
Haemulidae	Anisotremus spp.	Mob. inv. feeder	Υ	-	0.0	9 4.2	9	1.4	10	1.0	-	0.0	10	0.7	19	1.04
Haemulidae	Anisotremus surinamensis	Mob. inv. feeder	Υ	28	19.8	9 8.3	37	16.0	10	8.3	1	2.1	11	6.3	48	11.11
Haemulidae	Anisotremus virginicus	Mob. inv. feeder	Υ	96	50.0	121 58.3	217	52.8	67	21.9	17	31.3	84	25.0	301	38.89
Sparidae	Calamus spp.	Omnivore	Υ	-	0.0	1 2.1	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Sparidae	Calamus pennatula	Omnivore	Υ	1	1.0	9 8.3	10	3.5	3	1.0	1	2.1	4	1.4	14	2.43
Sparidae	Calamus bajonado	Omnivore	Υ	-	0.0	1 2.1	1	0.7	-	0.0	_	0.0	-	0.0	1	0.35
Sparidae	Calamus penna	Omnivore	Υ	-	0.0	2 4.2	2	1.4	-	0.0	_	0.0	-	0.0	2	0.69
Sparidae	Diplodus argenteus	Omnivore	Υ	83	28.1	4443.8	127	33.3	9	8.3	61	39.6	70	18.8	197	26.04
Sciaenidae	Odontoscion dentex	Carnivore	Υ	14	11.5	1314.6	27	12.5	17	4.2	2	4.2	19	4.2	46	8.33
Sciaenidae	Pareques acuminatus	Mob. inv. feeder	Ν	19	10.4	2 4.2	21	8.3	10	7.3	4	6.3	14	6.9	35	7.64
Mullidae	Pseudupeneus maculatus	Mob. inv. feeder	Υ	17	12.5	5 8.3	22	11.1	8	7.3	21	33.3	29	16.0	51	13.54
Mullidae	Mullus argentinae	Carnivore	Υ	-	0.0	- 0.0	-	0.0	-	0.0	1	2.1	1	0.7	1	0.35
Pempheridae	Pempheris schomburgkii	Planktivore	Ν	61	2.1	2 2.1	63	2.1	20	1.0	-	0.0	20	0.7	83	1.39
Kyphosidae	Kyphosus spp.	Roving herbivore	Υ	60	21.9	96 62.5	156	35.4	12	1.0	36	31.3	48	11.1	204	23.26
Kyphosidae	Kyphosus sectatrix	Roving herbivore	Υ	-	0.0	1 2.1	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Kyphosidae	Kyphosus incisor	Roving herbivore	Υ	-	0.0	4 4.2	4	1.4	-	0.0	3	2.1	3	0.7	7	1.04
Chaetodontidae	Chaetodon sedentarius	Sess. inv. feeder	Ν	4	1.0	- 0.0	4	0.7	-	0.0	_	0.0	-	0.0	4	0.35
Chaetodontidae	Chaetodon striatus	Sess. inv. feeder	Ν	47	26.0	2429.2	71	27.1	41	18.8	50	58.3	91	31.9	162	29.51
Pomacanthidae	Pomacanthus paru	Omnivore	Ν	59	34.4	9475.0	153	47.9	10	6.3	31	43.8	41	18.8	194	33.33
Pomacanthidae	Holacanthus tricolor	Sess. inv. feeder	Ν	2	2.1	- 0.0	2	1.4	-	0.0	2	4.2	2	1.4	4	1.39
Pomacentridae	Abudefduf saxatilis	Omnivore	N	604	42.7	424 45.8	1028	43.8	415	47.9	939	70.8	1354	55.6	2382	49.65
Pomacentridae	Chromis multilineata	Planktivore	N	50	21.9	9654.2	146	32.6	18	2.1	40	33.3	58	12.5	204	22.57
Pomacentridae	Chromis jubauna*	Planktivore	N	-	0.0	11 12.5	11	4.2	-	0.0	7	6.3	7	2.1	18	3.13
Pomacentridae	Chromis enchrysura	Planktivore	N	-	0.0	3 2.1	3	0.7	-	0.0	-	0.0	-	0.0	3	0.35
Pomacentridae	Stegastes fuscus*	Territorial herbivore	N	265	53.1	226 60.4	491	55.6	335	45.8	285	66.7	620	52.8	1111	54.17
Pomacentridae	Stegastes pictus*	Territorial herbivore	N	3	2.1	9 8.3	12	4.2	-	0.0	-	0.0	-	0.0	12	2.08

		Fthanal	•	-		No-take		·			Op		FOTAL :	TOTAL		
Family	Species	Functional group	Target	Insl	hore	Offshore	Tota	Total	Insl	hore	Offs	hore	Tota	Tota	ΓΟΤΑL <sup>·</sup> N	F%
		9.000		N	F%	N F%	IN	F%	N	F%	N	F%	IN	IF%		1 /6
Pomacentridae	Stegastes variabilis*	Territorial herbivore	Ν	-	0.0	3 4.2	3	1.4	1	1.0	-	0.0		0.7	4	1.04
Sphyraenidae	Sphyraena guachancho	Carnivore	Υ	1	1.0	- 0.0	1	0.7	-	0.0	-	0.0		0.0	1	0.35
Labridae	Bodianus pulchellus	Mob. inv. feeder	Υ	6	6.3	3752.1	43	21.5	2	1.0	39	52.1		18.1	84	19.79
Labridae	Bodianus rufus	Mob. inv. feeder	Υ	21	16.7	2435.4	45	22.9	2	1.0	22	37.5	24	13.2	69	18.06
Labridae	Halichoeres spp.	Mob. inv. feeder	Υ	1	1.0	- 0.0	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Labridae	Halichoeres brasiliensis*	Mob. inv. feeder	Υ	2	2.1	1020.8	12	8.3	-	0.0	10	20.8	10	6.9	22	7.64
Labridae	Halichoeres poeyi	Mob. inv. feeder	Ν	20	14.6	22 27.1	42	18.8	24	15.6	121	68.8	145	33.3	187	26.04
Labridae	Halichoeres sazimai*	Mob. inv. feeder	N	-	0.0		-	0.0	-	0.0	1	2.1	1	0.7	1	0.35
Labridae	Halichoeres dimidiatus*	Mob. inv. feeder	Ν	-	0.0	3 4.2	3	1.4	-	0.0	-	0.0	-	0.0	3	0.69
Labridae	Halichoeres bivittatus	Mob. inv. feeder	Ν	-	0.0	8 4.2	8	1.4	-	0.0	-	0.0	-	0.0	8	0.69
Labridae	Halichoeres penrosei*	Mob. inv. feeder	Ν	-	0.0	- 0.0	-	0.0	-	0.0	1	2.1	1	0.7	1	0.35
Labridae	Clepticus brasiliensis*	Planktivore	Ν	1	1.0	1 2.1	2	1.4	-	0.0	-	0.0	-	0.0	2	0.69
Labridae	Sparisoma spp.	Roving herbivore	Υ	1	1.0	2 2.1	3	1.4	-	0.0	-	0.0	-	0.0	3	0.69
Labridae	Sparisoma amplum*	Roving herbivore	Υ	5	4.2		5	2.8	-	0.0	-	0.0	-	0.0	5	1.39
Labridae	Sparisoma axillare* (VU <sub>Br</sub> )	Roving herbivore	Υ	14	10.4	23 25.0	37	15.3	1	1.0	53	33.3	54	11.8	91	13.54
Labridae	Sparisoma frondosum* (VU <sub>Br</sub> )	Roving herbivore	Υ	14	8.3	11 12.5	25	9.7	12	1.0	22	20.8	34	7.6	59	8.68
Labridae	Sparisoma tuiupiranga*	Roving herbivore	Υ	1	1.0	1014.6	11	5.6	1	1.0	20	10.4	21	4.2	32	4.86
Labridae	Sparisoma radians	Roving herbivore	Υ	-	0.0	- 0.0	-	0.0	-	0.0	4	4.2	4	1.4	4	0.69
Labridae	Scarus trispinosus* (ENI;ENBR)	Roving herbivore	Υ	-	0.0	- 0.0	-	0.0	-	0.0	1	2.1	1	0.7	1	0.35
Labridae	Scarus zelindae* (VU <sub>Br</sub> )	Roving herbivore	Υ	1	1.0	- 0.0	1	0.7	-	0.0	5	8.3	5	2.8	6	1.74
Labridae	Cryptotomus roseus	Roving herbivore	Ν	-	0.0	1 2.1	1	0.7	-	0.0	26	6.3	26	2.1	27	1.39
Labrisomidae	Labrisomus nuchipinnis	Mob. inv. feeder	Ν	-	0.0	- 0.0	-	0.0	8	8.3	-	0.0	8	5.6	8	2.78
Labrisomidae	Malacoctenus delalandii	Mob. inv. feeder	Ν	3	3.1	- 0.0	3	2.1	7	7.3	1	2.1	8	5.6	11	3.82
Chaenopsidae	Emblemariopsis signifer*	Mob. inv. feeder	Ν	46	16.7	1012.5	56	15.3	1	1.0	7	8.3	8	3.5	64	9.38
Blenniidae	Parablennius spp.	Omnivore	Ν	39	13.5	1818.8	57	15.3	8	6.3	3	4.2	11	5.6	68	10.42
Blenniidae	Parablennius marmoreus	Omnivore	Ν	-	0.0	3 6.3	3	2.1	-	0.0	-	0.0	-	0.0	3	1.04
Blenniidae	Parablennius pilicornis	Omnivore	Ν	-	0.0	1 2.1	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Blenniidae	Scartella cristata	Territorial herbivore	Ν	2	2.1	- 0.0	2	1.4	-	0.0	-	0.0	-	0.0	2	0.69
Gobiidae	Coryphopterus spp.	Mob. inv. feeder	Ν	289	32.3	22737.5	516	34.0	3	2.1	27	20.8	30	8.3	546	21.18
Gobiidae	Coryphopterus glaucofraenum	Mob. inv. feeder	Ν	-	0.0	6 4.2	6	1.4	-	0.0	1	2.1	1	0.7	7	1.04
Gobiidae	Coryphopterus thrix (VUi)	Mob. inv. feeder	N	2	1.0	- 0.0	2	0.7	-	0.0	-	0.0	-	0.0	2	0.35

	Species	Functional group	Target	•		No-take		•		Open						TOTAL
Family				Insl	nore	Offshore	Tota	Total	Inshore		Offshore		Tota Tota		N	F%
		<b>0</b> 1		N	F%	N F%	IN	F%	N	F%	N	F%	IN	IF%		
Gobiidae	Elacatinus Figurearo* (VU <sub>Br</sub> )	Mob. inv. feeder	Ν	7	5.2	19 8.3	26	6.3	2	1.0	-	0.0	2	0.7	28	3.47
Ephippidae	Chaetodipterus faber	Omnivore	Υ	57	6.3	91 14.6	148	9.0	6	2.1	4	4.2	10	2.8	158	5.90
Acanthuridae	Acanthurus spp.	Roving herbivore	Ν	3	3.1	25 8.3	28	4.9	2	1.0	12	14.6	14	5.6	42	5.21
Acanthuridae	Acanthurus bahianus*	Roving herbivore	Ν	4	4.2	33 22.9	37	10.4	-	0.0	45	20.8	45	6.9	82	8.68
Acanthuridae	Acanthurus chirurgus	Roving herbivore	Ν	44	14.6	2612.5	70	13.9	4	1.0	40	22.9	44	8.3	114	11.11
Mugilidae	Mugil spp.	Omnivore	Υ	239	7.3	- 0.0	239	4.9	34	8.3	264	14.6	298	10.4	537	7.64
Mugilidae	Mugil liza	Omnivore	Υ	7	2.1	- 0.0	7	1.4	-	0.0	-	0.0	-	0.0	7	0.69
Mugilidae	Mugil curema	Omnivore	Υ	104	2.1	- 0.0	104	1.4	25	3.1	5	2.1	30	2.8	134	2.08
Bothidae	Bothus ocellatus	Carnivore	Ν	1	1.0	3 2.1	4	1.4	-	0.0	5	6.3	5	2.1	9	1.74
Bothidae	Bothus spp.	Carnivore	Ν	-	0.0	- 0.0	-	0.0	-	0.0	4	4.2	4	1.4	4	0.69
Balistidae	Balistes vetula (NT <sub>I</sub> )	Omnivore	Υ	1	1.0	- 0.0	1	0.7	2	1.0	7	12.5	9	4.9	10	2.78
Balistidae	Balistes capriscus (VU <sub>I</sub> )	Omnivore	Υ	-	0.0	- 0.0	-	0.0	2	1.0	1	2.1	3	1.4	3	0.69
Monacanthidae	Cantherhines pullus	Omnivore	Ν	1	1.0	912.5	10	4.9	8	1.0	9	16.7	17	6.3	27	5.56
Monacanthidae	Cantherhines macrocerus	Omnivore	Ν	-	0.0	914.6	9	4.9	-	0.0	3	4.2	3	1.4	12	3.13
Monacanthidae	Aluterus monoceros	Omnivore	Ν	7	2.1	- 0.0	7	1.4	-	0.0	20	2.1	20	0.7	27	1.04
Monacanthidae	Aluterus scriptus	Omnivore	Ν	-	0.0	3 4.2	3	1.4	-	0.0	-	0.0	-	0.0	3	0.69
Monacanthidae	Stephanolepis hispidus	Mob. inv. feeder	Ν	-	0.0	- 0.0	-	0.0	-	0.0	3	6.3	3	2.1	3	1.04
Ostraciidae	Acanthostracion polygonius	Omnivore	Ν	-	0.0	1 2.1	1	0.7	-	0.0	-	0.0	-	0.0	1	0.35
Tetraodontidae	Canthigaster figueiredoi*	Omnivore	Ν	3	3.1	710.4	10	5.6	-	0.0	5	8.3	5	2.8	15	4.17
Tetraodontidae	Sphoeroides spengleri	Mob. inv. feeder	Ν	2	2.1	1 2.1	3	2.1	3	3.1	9	14.6	12	6.9	15	4.51
Tetraodontidae	Sphoeroides greeleyi	Mob. inv. feeder	N	-	0.0	- 0.0	-	0.0	11	10.4	-	0.0	11	6.9	11	3.47

# Chapter 3

Level of compliance dictates response of target fisheries species to no-take marine reserve status in the largest coral reef of the South Atlantic



Abstract. Negative fisheries effects have frequently been documented across coral reefs worldwide, and no-take marine reserves (NTRs) are being established as a core component to reverse these effects and achieve biodiversity conservation. NTRs with good levels of compliance can allow the direct and indirect effects of fishing to be investigated. The Abrolhos Marine National Park, off the coast of Brazil, encloses the largest coral reef in the South Atlantic. To investigate the effects of fishing on the abundance, richness and biomass of the fish assemblage, we contrasted fish assemblages at sites across three protection levels, high and low levels of compliance within NTR and areas open to fisheries, using baited remote underwater stereo-video systems (stereo-BRUVs). Previous studies within these locations have found positive effects inside the NTRs but used underwater visual sampling methods that were suspected to be constrained by diver avoidance behaviour of large mobile predators. Protection level was found to be an important predictor within parsimonious models for total biomass, and biomass of target species, carnivores combined and specifically for the Carcharhinidae (sharks) and Epinephelidae (groupers) families, with greater values at sites with a high level of protection indicating direct fisheries effects on these groups. In contrast, the most parsimonious models explaining non-target fish distribution were characterized by habitat complexity, including mean relief and variance of relief. For roving herbivores and specifically for Scaridae family (parrot fishes), also highly targeted in the region, a combination of macroalgae coverage, hardcorals and relief variation explained the distribution of biomass. The presence of highly mobile predators and the overall higher biomass of carnivores inside the NTR, with high compliance, indicates the effect of fishing and importance of good compliance. Conversely, the higher abundance of omnivores in fished areas may indicate an indirect effect of fishing whereby species with a more plastic diet are selected, as well as large-scale changes in the environment. Our results confirm the major ecological value of the NTR, and the importance of using remote sampling methods to assess large mobile predators. Due to the critical state of coralline reefs - in Abrolhos and globally - we suggest that additional NTRs are established and, more importantly, high levels of compliance are encouraged.

**Key-words:** Marine Protected Area, stereo-BRUVs, stereo-videos, South Atlantic, Chondrichthyes, Actinopterygii, reef ecology, reef fish.

### Introduction

Fisheries activities have been documented to play an important role in different aspects of fish assemblages structure through both direct and indirect effects (BABCOCK et al., 2010; BIANCHI et al., 2000; BORNT et al., 2015; CROWDER et al., 2008; DEMARTINI et al., 2008; WATSON et al., 2009). Direct effects include the removal of biomass of economically important species, decreasing abundance, richness and body size generally of top predators and larger species of other functional groups (CROWDER et al., 2008). Due to cascading effects through the food web, this removal has the potential to indirectly affect non-target species, prey populations and benthic composition. In addition, areas of intense fishing can decrease genetic diversity, and also present alterations in life-history traits, such as growth rates and size of maturation (FIDLER et al., 2018).

No-take marine reserves (NTRs) have been adopted globally to conserve biodiversity in the marine environment and also in an attempt to revert the negative effects of fisheries (SALA; GIAKOUMI, 2018, LESTER et al., 2009). By preserving the ecosystem as a whole, NTRs promote the protection of populations and habitats, increasing resilience and preserving areas for spawning and refuge of several species (LEENHARDT et al., 2015). In general, it aims to manage human activities and marine resources, promote the recovery of exploited marine populations, conserve or restore biodiversity habitats and food webs, as well as manage and improve ecosystem services, such as food production, water and recreational activities (CLAUDET, 2011; LEENHARDT et al., 2015).

For these reasons, NTRs have been widely implemented in sensitive biodiversity hotspots with high fisheries pressure, such as coral reefs worldwide. Within these protected areas, increased abundance, richness and biomass of fish have been observed, indicating that NTRs can reverse fisheries effects and provide the recovering of the area and even the surroundings, mainly by spillover of biomass and larvae (ABESAMIS; RUSS, 2005; CLAUDET, 2011; HALPERN; LESTER; KELLNER, 12/2009; LEENHARDT et al., 2015; LESTER et al., 2009). The South Atlantic has a few isolated coral reefs but with high species richness and rates of endemism (CASTRO; PIRES, 2001; FRANCINI-FILHO et al., 2018; LEÃO; KIKUCHI; TESTA, 2003; MOURA, 1999; MOURA, 2000; PINHEIRO et al., 2018; ROCHA, 2003), however the high fishing pressure and low levels of regulations compliance

and enforcement have been constantly threatening it (FRANCINI-FILHO; MOURA, 2008a; FREITAS et al., 2011).

The largest and richest coral reef area in the South Atlantic is located in the Abrolhos Bank off the central coast of Brazil, approximately from 17 to 20°S (LEÃO; KIKUCHI; TESTA, 2003). The bank covers an area of 46,000 km² and is comprised of contrasting habitats dominated by seagrasses, mangroves, algae bottoms, rhodolith beds and corals reefs surrounding a group of small emergent islands that comprises the Abrolhos Archipelago. The emergents and quasi-emergent reefs and banks with high coral cover make up approximately 18% (8800km²) of the Abrolhos Bank and present different morphological formations, including a set of world unique emerging pinnacle mushroom formations (LEÃO; KIKUCHI; TESTA, 2003; MOURA et al., 2013). This formation is defined by isolated reef columns that can reach 25 m in height, surrounded by unconsolidated sediments (LEÃO; KIKUCHI, 2001; LEÃO; KIKUCHI; TESTA, 2003; MOURA et al., 2013). A small fraction of the bank, i.e less than 0.2% of each benthic megahabitat area, is protected by the NTR of the Abrolhos Marine National Park (MOURA et al., 2013). Created in 1983, it is the first marine park of Brazil with 882km² of protected area (BRAZIL, 1983).

Although considered a biodiversity hotspot with high conservation priority in the Southwestern Atlantic (MOURA, 2000), the Abrolhos Bank, especially the shallow reefs, has been negatively affected by overfishing (FRANCINI-FILHO; MOURA, 2008a; FREITAS et al., 2011), coastal development (LEÃO; KIKUCHI, 2005), low overall water quality (BRUCE et al., 2012) and consequential coral diseases (FRANCINI-FILHO et al., 2008; GARCIA et al., 2013).

Despite of demonstrating positive effects of protection from fisheries, previous studies in the region were performed using visual census, and it is suspected that dive-avoidant behaviour could have lead to the underrepresentation of some groups, such as large mobile carnivores, or even to the attraction and overestimation of some species (CHAPMAN et al., 1974; WATSON; HARVEY, 2007; GOETZE et al., 2015).

The present study, therefore, intends to complement previous studies by using baited remote underwater stereo-video systems (stereo-BRUVs), comparing fish assemblage structure, abundance, richness, biomass and body size distribution among different areas subjected to different enforcement regimes (low enforcement, high enforcement and open access areas). We hypothesize that 1) Abundance,

biomass and body-size of target species will be greater in NTR with high compliance; 2) Non-target species distribution will be related to habitat complexity.

### Material and methods

The Abrolhos Marine National Park (AMNP) is composed of two areas in which the largest, southwards, covers the Abrolhos Archipelago and the Parcels of Abrolhos (PAB); while further north the marine park encompasses the Timbebas reef (Figure 1). The Abrolhos Archipelago is composed by shallow coral formations and rocky reefs surrounding the islands, while the PAB region, known as external arc, is characterized by mushroom shaped coral reefs (pinnacles). The open-access areas used to quantify the effect of the NTR were the reefs in the internal arc, Pedra de Leste reef, which is a portion of a larger reef formation named Paredes Reef, and Sebastião Gomes reef (Figure 1). Both open-access areas present emerging coral reef formations in which Pedra de Leste also features mushroom shaped reefs and Sebastião Gomes is a continuous plateau of shallow reef. Fishing activity, mainly large and small scale artisanal fisheries, such as hook-and-line (hand lines and longlines), gillnets and spearfishing, are frequent in these areas, supplying the local and regional market (FERREIRA, 2005; FRANCINI-FILHO; MOURA, 2008b; FREITAS, 2009; GIGLIO; LUIZ; GERHARDINGER, 2015; PREVIERO; GASALLA, 2018).

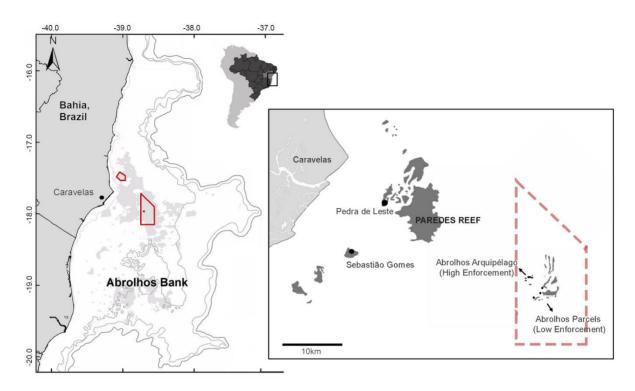


Figure 1. The study area with the Abrolhos Bank, the Abrolhos Marine National Park (no-take marine reserve) in red, the main coral reefs in the area in dark grey and the black dots are the sample sites inside (Low and High Enforcement) and outside the no-take reserve.

The National Park category prohibits any type of direct exploitation of its resources and it has as one of its objectives to develop activities of education and tourism. Although it is a frequent destination for recreational diving, the main focus is on the main island of the archipelago (Santa Bárbara Island) (KIKUCHI et al., 2003) and its major impact is on boat anchorage (CREED; AMADO-FILHO, 1999). Also, the Abrolhos main island is a Brazilian Navy's property, and it is inhabited by military and government officials all year round. For being frequently visited by tourists and officials, the archipelago presents higher enforcement when compared to other regions of the park. The PAB region is not as well enforced, especially due to the large area and lack of management resources to cover it. Therefore, in this study, we considered the Abrolhos Archipelago as an area with "High Enforcement", Parcels of Abrolhos (external arc) with "Low Enforcement" and the internal arc (Sebastião Gomes and Pedra de Leste) as "Open-access".

Sampling was conducted in March/2017 using Baited Remote Underwater stereo-Videos (stereo-BRUVs). The stereo equipment was comprised of two cameras (GoPro Hero3+) that converge approximately 8 degrees inwardly, with a bait arm placed 1.5 m from the cameras to attract fish to the field of view. The

calibration process and equipment is described in detail in Harvey and Shortis (1996, 1998). The stereo-BRUVs were deployed for 30 minutes, during daylight (between 7:00AM and 4:00PM), using 800 g of a mix of fresh and salted sardines (*Sardinella brasiliensis*) as bait. Deployments with less than 30 minutes, with very low visibility (<2 m) and limited field of view were excluded from the analysis, resulting in a total of 73 deployments, in which 16 were in the High Enforcement area (Archipelago), 30 at the Low Enforcement area (two different locations in the Parcels of Abrolhos), and 27 in Open-access (10 Pedra de Leste reef and 17 at Sebastião Gomes reef). The equipment was positioned above the reef or in the interface with the sandy bottom, to avoid large variations in the deployment depths, which ranged from 2 to 14 m.

The abundance of each species in the footages was estimated by the MaxN, defined as the maximum number of individuals appearing in the video in the same frame within 7m from the cameras. The fork length of individuals was measured only at the time of MaxN. Biomass was calculated using the allometric length-weight conversion W= aTLb, in which where parameters a and b are species-specific constants, TL is the estimated total length in cm, and W is weight in grams. Length-weight parameters (a and b) were obtained for each species from FishBase (FROESE; PAULY, 2018). When the parameters for fork length were not available, length/length conversions were made from total and standard length.

Fish were classified in functional groups based on the main diet components according to the literature (FERREIRA; GONÇALVES; COUTINHO, 2001; GIBRAN; MOURA, 2012; FROESE; PAULY, 2018), resulting in carnivores, mobile invertebrate feeders, sessile invertebrate feeders, omnivores, roving herbivores, territorial herbivores and planktivores. Fish were also divided in fisheries target and non-target according to the economic value in the region (CARVALHO-FILHO, 1999; FRANCINI-FILHO; MOURA, 2008b).

Habitat complexity in the present study was estimated based on regional, local and microhabitat scales, considering components and reef structure using the stereo-BRUV footage. The benthic composition was based on CATAMI (ALTHAUS et al., 2015) and topographic complexity on Wilson et al. (2007). Resulting in the following relief levels: 0-Flat substrate, sandy, rubble with few features, ~0 substrate slope; 1-Some relief features amongst mostly flat substrate/sand/rubble, <45 degree substrate slope; 2-Mostly relief features amongst some flat substrate or rubble, ~45 substrate slope; 3-Good relief structure with some overhangs, >45 substrate slope; 4-High

structural complexity, fissures and caves, vertical wall, ~90 substrate slope; 5-Exceptional structural complexity, numerous large holes and caves, vertical wall, ~90 substrate slope and Unknown. Benthic community was classified in 10 categories: Ascidians, Consolidated, Open water, Stony corals, Bryozoa, Macroalgae, Sponges, Unconsolidated, Zoanthids and Unknown. Following methods from Collins et al. (2017), the frame was divided into a 5X4 grid and the dominant habitat and level of complexity of each square is registered, using the software TransectMeasure. The percentage cover by sample is calculated based on the proportion of the squares in each category. Visibility and broad field of view (open, facing down, facing up or intermediate) were also classified based on stereo-BRUV footages.

Generalized Additive Mixed Models (GAMMs) with a full-subsets multiple regression approach (FISHER et al., 2018) were fitted to predict fish overall abundance, richness and biomass, as well as by target and non-target species. functional groups and the most conspicuous economically important families (Carcharhinidae, Carangidae, Haemulidae, Epinephelidae, and Labridae). Exploratory analyses were performed to select the continuous predictor variables, including the level of correlation among them (Pearson correlation coefficient r<0.7) and distribution of the variables, resulting in mean relief, relief variation, hardcorals, sand, macroalgae. Null variables of the random model included water transparency and field of view, and fixed factor was Protection (three levels: High Enforcement, Low Enforcement and Open-access).

Model selection was based on the second-order variant of Akaike's Information Criterion suited for small samples (AICc) (AKAIKE, 1973) and on AICc weights ( $\omega$ AICc). The selected model was the most parsimonious within two AICc units of the model with the lowest AICc value ( $\Delta$ AICc<2) (BURNHAM; ANDERSON, 2003). The most parsimonious models with <10% variance explained from metrics relevant to analyze fisheries effects were chosen to be plotted.

The distributions of fish lengths for key families were compared among protection levels using Kruskal-Wallis test, and post-hoc test Multiple Comparisons Kruskal (KruskalMC), considering a significant difference as p-values below 0.05. All analyses were performed using R Language for Statistical Computing (R CORE TEAM, 2018), with the packages FSSgam (FISHER et al., 2018), gamm4 (WOOD; SCHEIPL, 2017), mgcv (WOOD, 2006), MuMIn (BARTON, 2018), doParallel

(MICROSOFT-CORPORATION; WESTON, 2017), dplyr (WICKHAM et al., 2018) for tidying data and ggplot2 (WICKHAM, 2016) for plotting.

### Results

A total of 3109 fish individuals belonging to 27 families and 74 species were identified in the stereo-BRUV footages (list in supplementary material). The most abundant species were, in this order, yellowtail snapper (*Lutjanus chrysurus* = 32.1% of the individuals registered), tomtate grunt (*Haemulon aurolineatum* = 14.1%), greenback parrotfish (*Scarus trispinosus* = 6.8%), sergeant-major (*Abudefduf saxatilis* = 6.3%), ocean surgeon (*Acanthurus bahianus* = 6.8%) and blue tang surgeon (*Acanthurus coeruleus* = 3.4%). The most conspicuous species were yellowtail snapper (*Lutjanus chrysurus*), occuring in 93.1% of the footages, white grunt (*Haemulon plumieri*, 47.9%), sergeant major (*Abudefduf saxatilis*, 46.6%), ocean surgeon (*Acanthurus bahianus*, 46.6%), greenback parrotfish (*Scarus trispinosus*, 46.8%) and black grouper (*Mycteroperca bonaci*, 43.8%). Carnivores were the most abundant functional group, representing 39.8% of the individuals sampled. Mobile invertebrate feeders were the second most abundant (22.2%), followed by roving herbivores (20.4%), omnivores (9.8%), planktivores (4.8%) and sessile invertebrate feeders (1%).

Protection levels were important to predict biomass of the total sample, as well as for biomass of target species, carnivores combined and specifically for the fish families Carcharhinidae and Epinephelidae (Table 1, Figure 2, 3f, 5a, 6ab). Carcharhinidae abundance, was higher with hardcorals coverage (Table 1, Figure 2). For omnivores abundance, protection was also in the most parsimonious model but with a negative correlation (Figure 3c).

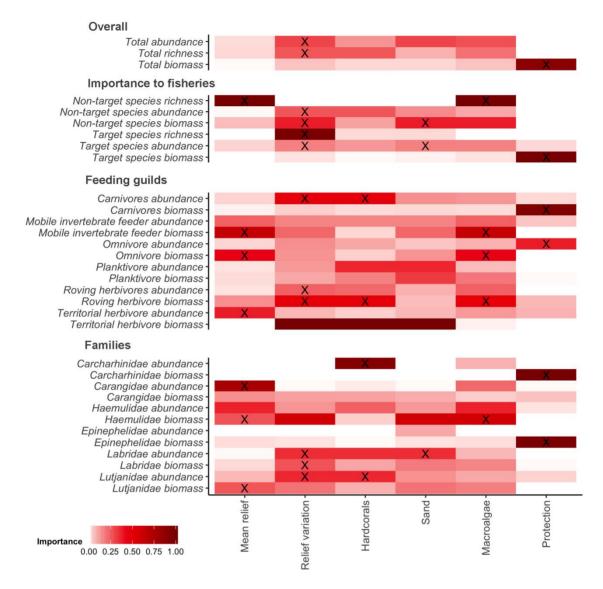


Figure 2. Variable importance scores from full-subset generalised additive mixed models analysis. X= Predictor variables within the most parsimonious model for each response variable within >10% variance explained.

Higher total abundance and richness was correlated with increased relief variation (Figure 3ab), as well as for non-target species abundance and biomass and target species abundance (Figure 2, 4a). Concerning richness, non-target species was correlated with mean relief and macroalgae, while target species was explained only by relief variation (Table 1, Figure 2, 3).

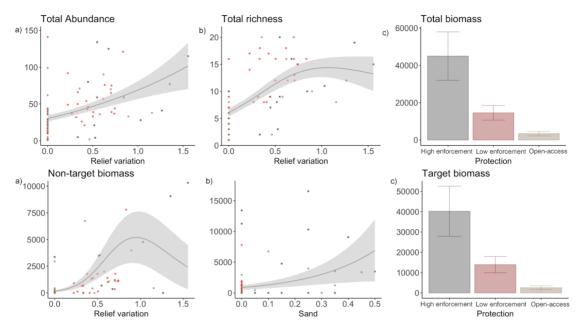


Figure 3. Plots of the most parsimonious models for the overall characteristics of the fish assemblage, total abundance (a), total richness (b) and total biomass (grams) (c). Plots of the most parsimonious models selected for biomass (grams) of fisheries target (a and b) and non-target (c) fish species. Each dot is a sample unit and the shaded areas represent the confidence interval.

Carnivores and Lutjanidae family abundance was explained by relief variation and hardcorals coverage (Table 1, Figure 2). Roving herbivore abundance and biomass was also related to relief variation, but biomass was also explained by hardcorals and macroalgae (Figure 2, 5ghi). Mobile invertebrate feeder and omnivore biomass, as well as territorial herbivores abundance, was higher in complex habitats (mean relief) and with less macroalgae coverage (Figure 2, 5ef).

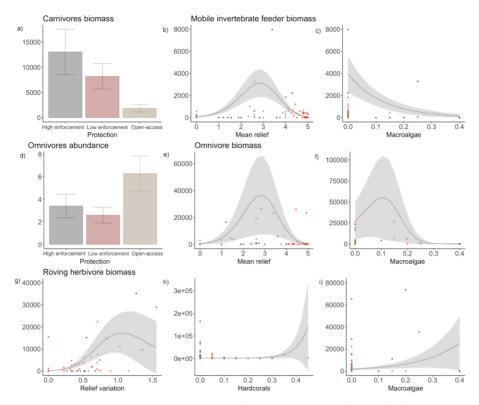


Figure 5. Plots of the most parsimonious models for the biomass (grams) of carnivores (a), mobile invertebrate feeders (b), omnivores (e and f) and roving herbivores (g, h and i) and abundance of omnivores (d). Each dot is a sample unit and the shaded areas represent the confidence interval.

Haemulidae and Lutjanidae biomass presented a correlation with mean relief, with Haemulidae showing larger biomass with macroalgae (Table 1, Figure 2, 6d). Labridae abundance and biomass was higher in deployments with larger variation in relief, with abundance being also explained by sandy bottom (Table 1, Figure 2, 6c).

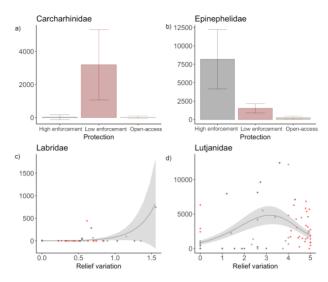


Figure 6. Plots of the most parsimonious models for the biomass (grams) of fish families with high economic value in the region. Each dot is a sample unit and the shaded areas represent the confidence interval.

The economically important species greenback parrotfish (Scarus trispinosus) presented the higher total biomass registered (25.8%), followed by black grouper (M. bonaci, 11.9%), Caribbean reef shark (Carcharhinus perezi, 11.4%), yellowtail snapper (Lutjanus chrysurus, 10.1%), queen triggerfish (Balistes vetula, 7.8%) and sea chub (Kyphosus spp., 6.6%). The functional groups that presented higher biomass were carnivores (43%), followed by roving herbivores (36.7%), omnivores (15.1%), mobile invertebrate feeders (4.5%), planktivores (0.4%), sessile invertebrate feeders (0.1%) and territorial herbivores (0.01%) (Figure 7). Within the carnivores functional group, the Carcharhinidae family presented the higher biomass and was only found within the Low Enforcement area, whilst Dasyatidae family was found only in the High Enforcement area. Families Epinephelidae, Lutjanidae and Carangidae also presented high biomass values within the group. Amongst the roving herbivores, the families registered were Acanthuridae, Kyphosidae and Labridae. Concerning omnivores, Balistidae Pomacanthidae, Pomacentridae and Sparidae were most representative families. And families Haemulidae, Labridae and Mullidae presented the higher biomass values within the mobile invertebrate feeders group.

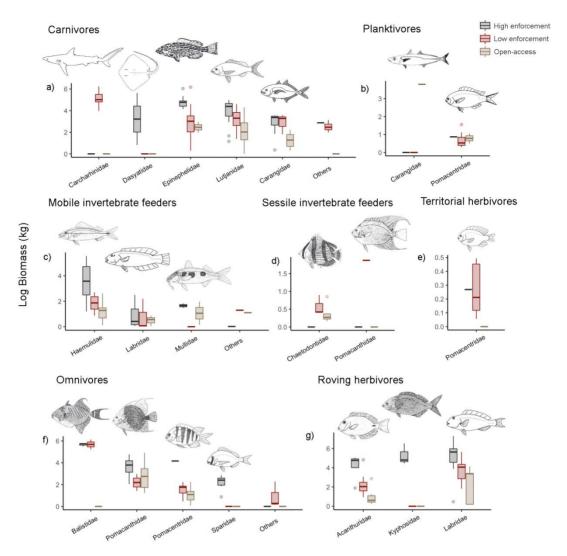


Figure 7. Logarithmized biomass (grams) (log<sub>1+x</sub>) of fish families and functional groups by the different management regimes. Fish drawings based on Carvalho-filho (1999).

Significant higher proportion of larger-bodied individuals of the economically important families Lutjanidae (p<0.001, H=190.19) and Carangidae (p<0.001, H=48.24) were found within both NTRs (Low and High Enforcement) when compared to open-access areas. However, Epinephelidae (p<0.001, H=19.31), Labridae (p=0.001, H=13.53) and Haemulidae (p<0.001, H=27.79) presented larger sizes only for the High Enforcement NTR (Figure 8).

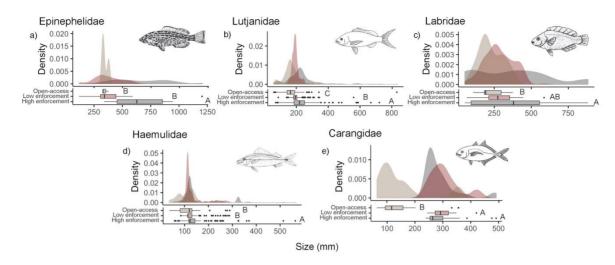


Figure 8. Kernel density plots and boxplots for fork length (mm) for important fisheries target families. Fish drawings based on Carvalho-filho (1999).

Table 1. Top generalised additive mixed models (GAMMs) to predict different aspects of fish assemblage.  $\Delta AIC_c$ : Adjusted Akaike Information Criteria of alternative model –  $AIC_c$  of best model;  $\Delta BIC$ : Bayesian Information Criterion of alternative model – BIC of best model;  $wAIC_c$ :  $AIC_c$  weight; wBIC: BIC weight;  $R^2$ : variance explained; EDF: Effective degrees of freedom. \*most parsimonious models selected within  $\Delta AIC_c>2$  and  $R^2>0.1$ 

Dependent variables	Best models	ΔAIC <sub>c</sub>	ΔΒΙC	wAIC <sub>c</sub>	wBIC	R <sup>2</sup>	EDF
Overall							
Total abundance	Relief variation *	2.00	0.68	0.11	0.29	0.22	4.2
	Relief variation+Sand	0.00	0.00	0.30	0.40	0.24	5.0
	Relief variation+Sand+Macroalgae	0.17	2.01	0.27	0.15	0.26	6.0
	Relief variation+Hardcorals+Sand	1.56	4.98	0.14	0.03	0.27	6.7
Total richness	Relief variation *	0.88	0.00	0.17	0.34	0.52	6.2
	Relief variation+Hardcorals	0.00	0.41	0.26	0.28	0.54	7.0
	Relief variation+Macroalgae	0.50	1.32	0.20	0.18	0.54	7.3
	Relief variation+Hardcorals+Macroalgae	0.68	2.81	0.19	0.08	0.55	8.1
Total biomass	Protection *	0.00	0.00	0.90	0.98	0.25	5.4
Importance to fisheries							
Non-target species richness	Mean relief+Macroalgae *	0.00	0.00	1.00	1.00	0.40	7.0
Non-target species abundance	Relief variation *	0.87	0.00	0.17	0.44	0.35	4.8
	Relief variation+Hardcorals	0.00	0.93	0.26	0.28	0.37	5.8
	Relief variation+Hardcorals+Sand	1.08	4.58	0.15	0.04	0.38	7.0
	Relief variation+Sand	1.10	3.17	0.15	0.09	0.37	6.1
	Relief variation+Hardcorals+Macroalgae	1.96	4.67	0.10	0.04	0.38	6.8
Non-target species biomass	Relief variation+Sand *	0.31	0.00	0.33	0.41	0.27	8.0
	Relief variation+Sand+Macroalgae	0.00	2.45	0.38	0.12	0.25	9.9
Target species richness	Relief variation *	0.00	0.00	0.98	0.99	0.54	6.4
Target species abundance	Relief variation+Sand *	0.00	1.47	0.18	0.17	0.13	5.0
	Relief variation+Sand+Macroalgae	0.04	3.35	0.17	0.06	0.15	6.0
	Relief variation+Macroalgae	0.43	1.89	0.14	0.13	0.14	5.0
	Relief variation	0.45	0.00	0.14	0.34	0.11	4.0
	Relief variation+Hardcorals+Sand	0.59	5.57	0.13	0.02	0.17	6.8
	Relief variation+Hardcorals+Macroalgae	1.65	6.59	0.08	0.01	0.18	6.7
	Relief variation+Hardcorals	2.05	5.14	0.06	0.03	0.15	5.7
Target species biomass	Protection *	0.00	0.00	0.99	1.00	0.22	5.4
Functional group							
Carnivores abundance	Relief variation+Hardcorals *	0.00	0.00	0.49	0.51	0.29	5.1
Carnivores biomass	Protection *	0.00	0.00	0.94	0.96	0.25	5.8
Mobile invertebrate feeder abundance	Mean relief+Macroalgae	0.00	0.56	0.24	0.15	0.09	5.9
	Relief variation+Hardcorals+Sand	0.67	2.93	0.17	0.05	0.08	6.8
	Hardcorals	1.47	0.00	0.11	0.20	0.04	4.7
	Mean relief	1.61	0.31	0.11	0.17	0.06	4.9

	Relief variation+Hardcorals	1.79	2.22	0.10	0.07		5.7
Mobile invertebrate feeder biomass Mean relief+Macroalgae *		0.00	0.00	0.65	0.88	0.16	
Omnivore abundance	Protection *	0.00	0.00	0.38	0.31	0.13	
	Relief variation	1.95	1.04	0.15	0.19	0.10	
Omnivore biomass	Mean relief+Macroalgae *	0.00	0.00	0.45	0.25	0.21	
Planktivore abundance	Hardcorals+Sand	0.00	1.22	0.37	0.30	0.04	
	Sand	0.69	0.00	0.26	0.54	0.03	
Planktivore biomass	Sand	0.00	0.00	0.32	0.59	0.00	
	Sand+Macroalgae	1.04	2.94	0.19	0.13	0.01	
	Hardcorals+Sand	1.21	3.18	0.18	0.12	0.00	5.9
	Mean relief+Macroalgae	0.00	0.00	0.54	0.50	0.42	6.8
Roving herbivores abundance	Relief variation *	0.12	0.85	0.23	0.28	0.31	5.8
	Relief variation+Macroalgae	0.00	0.00	0.24	0.43	0.31	6.8
	Relief variation+Hardcorals	0.15	1.63	0.22	0.19	0.31	7.4
	Relief variation+Hardcorals+Macroalgae	1.06	4.22	0.14	0.05	0.32	8.5
Roving herbivore biomass	Relief variation+Hardcorals+Macroalgae *	0.00	5.01	0.49	0.05	0.13	8.9
Sessile invertebrate feeder abundance	null	0.00	0.00	0.69	0.88	0.00	3.0
Sessile invertebrate feeder biomass	Mean relief	0.00	3.36	0.17	0.10	0.03	4.9
	Relief variation	0.04	3.33	0.17	0.10	0.06	
	null	0.52	0.00	0.13	0.54	0.02	
	Mean relief+Macroalgae	1.16	6.39	0.10	0.02	0.01	
	Hardcorals	1.17	4.49	0.10	0.06	0.04	
Territorial herbivore abundance	Mean relief *	0.00	0.00	0.39	0.41	0.11	
Territorial herbivore biomass	Relief variation+Hardcorals+Sand	0.00	0.00	1.00	0.84	0.03	10.1
Targeted families	Trener variation in landorate realia	0.00	0.00	1.00	0.01	0.00	10.1
Carcharhinidae abundance	Hardcorals *	0.00	0.00	0.92	0.96	0.11	6.4
Carcharhinidae biomass	Protection *	0.00	0.00	1.00	1.00	0.11	
Carangidae abundance	Mean relief *	0.00	0.00	0.76	0.90	0.09	
	null	0.00	0.00	0.76	0.90	0.11	
Carangidae biomass	Mean relief	0.00	3.23	0.16	0.42	0.03	
	Relief variation		2.42	0.14	0.08		
		0.67				0.05	
	Hardcorals Sand	0.69 1.12	2.38	0.11	0.13	0.04	
			2.86	0.09			
He and dide a phone de a ca	Relief variation+Hardcorals	1.38	4.75	0.08	0.04	0.05	
Haemulidae abundance	Mean relief+Macroalgae	0.00	0.00	0.38	0.26	0.05	
Hanny didag hisasaa	Hardcorals+Macroalgae	0.90	0.77	0.24	0.18	0.04	
Haemulidae biomass	Mean relief+Macroalgae *	1.57	0.00	0.27	0.60	0.15	
	Relief variation+Sand+Macroalgae	0.00	3.07	0.58	0.13	0.20	
Epinephelidae abundance	null	0.00	0.00	0.90	0.96	0.17	
Epinephelidae biomass	Protection *	0.00	0.00	0.98	0.99	0.09	
Labridae abundance	Relief variation+Sand *	0.71	5.23	0.24	0.05	0.31	
	Relief variation+Hardcorals+Sand	0.00	4.94	0.35	0.06	0.32	
	Relief variation	1.51	0.00	0.16	0.70	0.25	
Labridae biomass	Relief variation *	0.00	0.00	0.27	0.39	0.16	
	Relief variation+Sand	0.73	1.45	0.18	0.19	0.15	
	Relief variation+Macroalgae	0.99	2.07	0.16	0.14	0.14	-
	Relief variation+Sand+Macroalgae	1.08	3.03	0.15	0.09	0.13	
	Relief variation+Hardcorals	1.88	4.38	0.10	0.04	0.16	
Lutjanidae abundance	Relief variation+Hardcorals *	0.00	0.00	0.36	0.33	0.26	
	Relief variation+Hardcorals+Sand	1.88	4.04	0.14	0.04	0.27	
	Hardcorals	1.97	0.05	0.14	0.32	0.22	
Lutjanidae biomass	Mean relief *	0.00	0.00	0.26	0.32	0.22	
	Relief variation+Sand	0.52	0.53	0.20	0.24		5.9
	Mean relief+Macroalgae	0.80	4.11	0.18	0.04	0.25	7.5
	ivicali relici+iviacioalyae	0.00	7.11	0	0.04	0.20	

## **Discussion**

One of the consequences of large removal of individuals by fisheries activities is represented by a significant and rapid decrease in abundance and biomass, specially of large bodied target species (CROWDER et al., 2008). Worldwide, NTRs have been reported to be effective in preventing or recovering from fisheries effects,

showing increased richness, abundance, biomass and size of individuals within their boundaries (LESTER et al., 2009). The results here indeed showed larger total and target fish biomass with protection, particularly for economically important carnivores such as Carcharhinidae and Epinephelidae, indicating that even the NTR with low enforcement is attenuating the effect of fisheries in the region and allowing the growth of target species, presenting larger body sizes within their boundaries.

Omnivores abundance was the only characteristic negatively affected by protection, presenting larger numbers in areas open to fisheries. The role of omnivory in the food web within coral reefs is not a consensus in the literature. Some studies have indicated that this trophic group is important to reduce the likelihood of trophic cascades, once top predators are removed (BASCOMPTE; MELIÁN; SALA, 2005). This could also indicate a buffer reaction of highly fished reefs. On the other hand, the higher abundance of omnivores in fished areas could indicate a more degraded environment, once it has selected individuals with a more plastic diet, i.e low specialization, therefore, more likely resistant to pressures [e.g., Bellwood et al. (2006) and Pratchett et al. (2011)]. Besides that, the species belonging to this functional group are not as target in the region as carnivores and roving herbivores, explaining the higher abundance in fished area.

Apart from omnivores, most abundance and richness estimates of the fish assemblage explored here were not influenced by protection, indicating that the assemblage general structure is more related to habitat complexity. However, the great variation in the habitat characteristics in the region, as well as the difficulty in establishing control areas for the inside-outside comparison, can mask the fishing/protection effects, making it difficult to quantify the influence of this factor on the fish assemblage. On the other hand, even with broad habitat variation being taken into account in our analysis, effects of fisheries were strongly detected, specially for some biomass estimates. This indicates that the effect of fisheries in the region is mostly manifesting through decrease of biomass by direct removal of large bodied individuals targeted by fisheries, and possibly not strongly altering the composition of the assemblage.

Indeed, habitat complexity have been shown to play an important role influencing different aspects of coral reef fish assemblage (FRANCINI-FILHO; MOURA, 2008b; FRIEDLANDER et al., 2003; JENNINGS; BOULLÉ; POLUNIN, 1996; MCCLANAHAN; ARTHUR, 2001). In the present study, mean relief and relief

variation explained most of the abundance, richness and biomass estimates, especially for groups not targeted by fisheries. Indeed, in general, the presence of different microhabitats, i.e higher topographic complexity, is expected to harbour higher richness and abundance by reducing competition (food and space) and predation (predator-prey encounters and availability of refugees) (HIXON; MENGE, 1991; HOLT, 1987; MACARTHUR; LEVINS, 1964). It is indeed expected that, as fisheries effects are not significant for these groups, the main factors influencing the assemblage is related to habitat characteristics, not evidencing indirect fisheries effects by top down control through the food web.

However, for some important target groups, habitat was more determinant than protection. This was the case for Haemulidae and Lutjanidae biomass. As fisheries effects are very likely to influence for these groups, we could attribute this result to two reasons: 1) the high variation in the habitat could be suppressing protection effect; 2) not detection of the effect due to low sample size and/or 3) the absence of top predators in open access areas allowing prey population to increase. Nevertheless, our results indicate that the fisheries effects is probably manifesting through the higher densities of large individuals only inside the NTRs, but still needs more investigation to have a more concrete conclusion for these families.

Higher overall richness and abundance, as well as non-target species aspects and target species richness and abundance, were found in habitats with high variation, that means that the stereo-BRUV deployment was recording, at the same time, high and low complex habitats. Besides the higher diversity of resource availability in these environments, this could be related to areas where the complex parts of the reef encounter the sandy bottom or open water, increasing the chances of encompassing individuals of both habitats in the footage.

For herbivores in general, as well as specifically for the Labridae family, protection was also not important to predict biomass. Instead, a combination of relief variation, hardcorals and macroalgae coverage showed to be influencing. This was not our primary hypothesis for this functional group once most species within this group are highly targeted by fisheries (FERREIRA, 2005; FRANCINI-FILHO; MOURA, 2008b). However, this result is also highly likely since this group has been registered associated with areas with greater availability of refuge and food, which is mostly composed by macroalgae (FERREIRA; GONÇALVES, 2006). Besides, the open access areas assessed in this study are closer to mainland, showing also

increased influx of sediments and nutrients, which is probably related to a higher macroalgae cover in detriment of scleractinian corals coverage in the shallow coastal reefs (BRUCE et al., 2012; FRANCINI-FILHO et al., 2013; FRANCINI-FILHO; MOURA, 2008b). Other explanation could be also related to the lack of top predators in fished areas as well. As a high biomass of these species is frequently removed by fisheries in the region (FERREIRA, 2005; PREVIERO, 2014), we recommend a more refined analysis focused on this functional group to ensure that the fisheries effect is indeed not an issue for this group.

The high biomass of greenback parrotfish (*Scarus trispinosus*) was also reported for the region by Francini-Filho and Moura (2008b). In the present study, this species presented the higher biomass registered and was also highly abundant and frequent in the footages. *Scarus trispinosus* presents high economic value in the region (PREVIERO, 2014) and our results indicate that fisheries effects is probably manifesting through decrease in the size of the species in open access areas.

Carnivores abundance and biomass outstanded from the other functional group, in which this latter was strongly related to protection. Furthermore, the presence of top predators only within protected areas found in the present study, such as Caribbean reef shark (*Carcharhinus perezi*) and nurse shark (*Ginglymostoma cirratum*), indicates that both species are responding positively to the protection, as well as to a more complex food web. The dominance of carnivores was also registered in the region by Moura and Francini-Filho (2005), and points out to a healthier ecosystem, since top predators can both be benefitted by healthy ecosystems (ESPINOZA et al., 2014) as they can maintain it (RUPPERT et al., 2013; RUPPERT; FORTIN; MEEKAN, 2016). Besides, the present study allowed the analysis of the Carcharhinidae family by using sampling methods without the use of divers, not previously available (FERREIRA, 2005), probably due to the elusive nature of the species in the area, evidencing fisheries effects for this group as well.

Even though the High Enforcement area (Abrolhos Archipelago) is mainly composed by rocky reefs, protection level was more relevant to predict economically important fish estimates, such as Epinephelidae and Carcharhinidae biomass, than habitat characteristics. For relatively sedentary species with small home ranges, as Epinephelidae species, protection seems to play even a more important role (ANDERSON et al., 2014, 2018; FRANCINI-FILHO; MOURA, 2008b; ROLIM et al., 2019). Besides, this area presented significantly larger individuals, indicating that the

enforcement level is also having an effect in the assemblage, preventing direct fisheries effects.

The effect of fisheries in size of individuals indeed have been well documented, in which target species reach larger sizes within MPAs (BIANCHI et al., 2000; DEMARTINI et al., 2008; HARASTI et al., 2018; WATSON et al., 2009). In fact, the present study showed a higher density of larger individuals of target species, such as for the families Lutjanidae, Haemulidae, Labridae, Epinephelidae and Carangidae within protected areas. Similar results were also previously found for the same region (FERREIRA, 2005; FERREIRA; GONÇALVES, 1999) and worldwide (LESTER et al., 2009). This can also indicate a likely decrease in reproduction capacity in fished areas, once larger individuals usually present higher fecundity (JENNINGS; KAISER; REYNOLDS, 2009). Even in the case of sequential hermaphroditism, such as for groupers (Epinephelidae) and parrotfishes (Labridae), fisheries pressure can decrease the average size of individuals (ROBINSON et al., 2017) and also probably affecting reproductive capacity. This happens because the removal of larger individuals can change the dynamics of sex change, in which the switch can be antecipated to compensate the lack of the other sex represented by older individuals (ROBINSON et al., 2017). Therefore, in both ways, the presence of larger individuals of both sexes points out to a higher probability of exporting larvae to adjacent areas (FRANCINI-FILHO; MOURA, 2008b), repopulating fished reefs and consequently ensuring fishing activity in the area.

### **Conclusions**

Even though our results showed a strong effect of habitat variation in diverse aspects of fish assemblage, negative effects in response to fisheries activities were detected in the region, mainly regarding biomass and body size of commercially important species. This indicates a positive response to the protection, however, despite of being a no-take reserve, only this factor itself does not guarantee the achievement of full potential and total effectiveness of the area (COX et al., 2017; EDGAR et al., 2014; OLIVEIRA JÚNIOR et al., 2016). Therefore, although we support the expansion of the NTRs network in the region, the most urgent issue that needs to be addressed is regarding the compliance and enforcement of the existing protected areas, specially for the low enforced area (Abrolhos Parcels), to better achieve the parameters of biodiversity and conservation outcomes in the region.

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# Supplementary material

Table S1 - List of species found within no-take reserve (High and Low Enforcement in Abrolhos Marine National Park) and fished areas. \*Endemic from Brazilian biogeographic province (FLOETER; GASPARINI, 2000; JOYEUX et al. 2001; GASPARINI et al. 2003; PINHEIRO et al. 2018);  $VU_I$ = Vulnerable by International Union for Nature Protection Red List (IUCN, 2018);  $VU_I$ = Near threatened by IUCN;  $VU_{Br}$ = Vulnerable by Brazilian legislation (MMA, 2014);  $VU_{Br}$ = Critically endangered by Brazilian Legislation; V= Target; V= Non-target; V= Abundance; V= Frequency.

Family	Species	Functional group	Target	High Enforcement		Low Enforcement		Open		TOTAL
				N	F%	N	F%	N	F%	Abundance
Ginglymostomatidae	Ginglymostoma cirratum	Carnivore	Υ	1	6.3	1	3.3	-	0.0	2
Carcharhinidae	Carcharhinus perezi	Carnivore	Υ	-	0.0	11	30.0	-	0.0	11
Dasyatidae	Hypanus americanus	Carnivore	Υ	1	6.3	-	0.0	-	0.0	1
Dasyatidae	Hypanus marianae	Carnivore	Υ	1	6.3	-	0.0	-	0.0	1
Dasyatidae	Dasyatis spp.	Carnivore	Υ	2	12.5	-	0.0	-	0.0	2
Muraenidae	Gymnothorax funebris	Carnivore	Υ	1	6.3	3	10.0	2	7.4	6
Muraenidae	Gymnothorax moringa	Carnivore	Υ	1	6.3	12	40.0	3	11.1	16
Muraenidae	Gymnothorax vicinus	Carnivore	Υ	-	0.0	3	10.0	2	7.4	5
Holocentridae	Holocentrus adscensionis	Mob. inv. feeder	N	-	0.0	5	13.3	1	3.7	6
Serranidae	Serranus atrobranchus	Mob. inv. feeder	N	-	0.0	-	0.0	1	3.7	1
Epinephelidae	Epinephelus adscensionis	Carnivore	Υ	-	0.0	-	0.0	1	3.7	1
Epinephelidae	Mycteroperca marginatus (EN <sub>I</sub> ;VU <sub>Br</sub> )	Carnivore	Υ	-	0.0	-	0.0	1	3.7	1
Epinephelidae	Epinephelus morio (NT <sub>I</sub> ;VÙ <sub>Br</sub> )	Carnivore	Υ	-	0.0	4	13.3	5	14.8	9
Epinephelidae	Epinephelus spp.	Carnivore	Υ	2	6.3	-	0.0	-	0.0	2
Epinephelidae	Mycteroperca acutirostris	Carnivore	Υ	-	0.0	-	0.0	1	3.7	1
Epinephelidae	Mycteroperca bonaci (NTı;VU <sub>Br</sub> )	Carnivore	Υ	16	50.0	37	80.0	-	0.0	53
Epinephelidae	Rypticus saponaceus	Mob. inv. feeder	N	1	6.3	-	0.0	-	0.0	1
Apogonidae	Apogon spp.	Mob. inv. feeder	N	1	6.3	-	0.0	-	0.0	1
Carangidae	Carangoides bartholomaei	Carnivore	Υ	8	25.0	-	0.0	30	22.2	38
Carangidae	Carangoides ruber	Carnivore	Υ	6	18.8	-	0.0	-	0.0	6
Carangidae	Carangoides spp.	Carnivore	Υ	1	6.3	-	0.0	-	0.0	1
Carangidae	Caranx crysos	Carnivore	Υ	12	25.0	1	3.3	12	14.8	25
Carangidae	Caranx ruber	Carnivore	Υ	9	31.3	9	13.3	-	0.0	18
Carangidae	Decapterus macarellus	Planktivore	Υ	-	0.0	-	0.0	63	3.7	63
Carangidae	Hemicaranx amblyrhynchus	Planktivore	Υ	3	6.3	-	0.0	-	0.0	3
Carangidae	Pseudocaranx dentex	Mob. inv. feeder	Υ	-	0.0	-	0.0	1	3.7	1
Carangidae	Seriola spp.	Carnivore	Υ	-	0.0	1	3.3		0.0	1
Lutjanidae	Lutjanus analis (VU <sub>I</sub> )	Carnivore	Υ	-	0.0		0.0	2	7.4	2
Lutjanidae	Lutjanus jocu	Carnivore	Υ	10	50.0	3	10.0	8	14.8	21
Lutjanidae	Lutjanus spp.	Carnivore	Υ	-	0.0	-	0.0	1	3.7	1
Lutjanidae	Lutjanus synagris	Carnivore	Υ	-	0.0	-	0.0	5	3.7	5

Family	Species	Functional group	Target	High Enforcement		Low Enforcement		Open		TOTAL
				N	F%	N	F%	N	F%	Abundance
Lutjanidae	Lutjanus chrysurus	Carnivore	Υ	174	81.3	602	100.0	222	92.6	998
Gerreidae	Eucinostomus gula	Planktivore	Ν	-	0.0	-	0.0	1	3.7	1
Haemulidae	Anisotremus surinamensis	Mob. inv. feeder	Υ	1	6.3	-	0.0	-	0.0	1
Haemulidae	Anisotremus virginicus	Mob. inv. feeder	Υ	11	12.5	17	43.3	7	18.5	35
Haemulidae	Haemulon aurolineatum	Mob. inv. feeder	Υ	135	25.0	118	16.7	187	55.6	440
Haemulidae	Haemulon chrysargyreum	Mob. inv. feeder	Υ	2	6.3	-	0.0	-	0.0	2
Haemulidae	Haemulon parra	Mob. inv. feeder	Υ	24	12.5	-	0.0	-	0.0	24
Haemulidae	Haemulon plumieri	Mob. inv. feeder	Υ	31	25.0	22	63.3	25	44.4	78
Haemulidae	Haemulon spp.	Mob. inv. feeder	Υ	-	0.0	2	3.3	-	0.0	2
Sparidae	Calamus pennatula	Omnivore	Υ	9	37.5	-	0.0	1	3.7	10
Mullidae	Pseudupeneus maculatus	Mob. inv. feeder	Υ	10	25.0	-	0.0	11	14.8	21
Kyphosidae	Kyphosus spp.	Roving herbivore	Υ	28	18.8	-	0.0	-	0.0	28
Chaetodontidae	Chaetodon striatus	Sess. inv. feeder	N	4	18.8	11	26.7	12	33.3	27
Pomacanthidae	Holacanthus ciliaris	Sess, inv. feeder	N	-	0.0	3	10.0	-	0.0	3
Pomacanthidae	Pomacanthus arcuatus	Omnivore	Ν	-	0.0	10	30.0	6	18.5	16
Pomacanthidae	Pomacanthus paru	Omnivore	N	24	56.3	5	16.7	30	29.6	59
Pomacanthidae	Pomacanthus spp.	Omnivore	Ν	-	0.0	-	0.0	5	3.7	5
Pomacentridae	Abudefduf saxatilis	Omnivore	N	15	6.3	42	50.0	139	66.7	196
Pomacentridae	Chromis jubauna*	Planktivore	N	1	6.3	7	6.7	-	0.0	8
Pomacentridae	Microspathodon chrysurus	Planktivore	Ν	-	0.0	1	3.3	-	0.0	1
Pomacentridae	Stegastes fuscus*	Territorial herbivore	N	7	12.5	10	13.3	-	0.0	17
Pomacentridae	Stegastes pictus*	Territorial herbivore	Ν	5	6.3	35	26.7	-	0.0	40
Pomacentridae	Stegastes spp.	Planktivore	Ν	7	12.5	57	66.7	10	18.5	74
Pomacentridae	Stegastes variabilis	Territorial herbivore	Ν	3	6.3	2	6.7	3	11.1	8
Sphyraenidae	Sphyraena barracuda	Carnivore	Υ	-	0.0	1	3.3	-	0.0	1
Scombridae	Scomberomorus brasiliensis	Carnivore	Υ	-	0.0	2	6.7	-	0.0	2
Scombridae	Scomberomorus regalis	Carnivore	Υ	-	0.0	1	3.3	-	0.0	1
Scombridae	Scomberomorus spp.	Carnivore	Υ	1	6.3	-	0.0	5	7.4	6
Labridae	Bodianus rufus	Mob. inv. feeder	Υ	-	0.0	-	0.0	1	3.7	1
Labridae	Cryptotomus roseus	Roving herbivore	N	2	6.3	-	0.0	1	3.7	3
Labridae	Halichoeres bivittatus	Mob. inv. feeder	Ν	1	6.3	-	0.0	-	0.0	1
Labridae	Halichoeres brasiliensis*	Mob. inv. feeder	Υ	5	18.8	8	20.0	2	7.4	15
Labridae	Halichoeres dimidiatus	Mob. inv. feeder	N	1	6.3	-	0.0	-	0.0	1
Labridae	Halichoeres penrosei	Mob. inv. feeder	N	2	6.3	-	0.0	-	0.0	2
Labridae	Halichoeres poeyi	Mob. inv. feeder	N	27	43.8	-	0.0	7	14.8	34
Labridae	Halichoeres radiatus	Mob. inv. feeder	N	1	6.3	5	3.3	-	0.0	6
Labridae	Halichoeres spp.	Mob. inv. feeder	N	16	6.3	-	0.0	-	0.0	16
Labridae	Scarus spp.	Roving herbivore	Υ	1	6.3	4	3.3	-	0.0	5
Labridae	Scarus trispinosus* (EN;EN <sub>BR</sub> )	Roving herbivore	Υ	88	43.8	104	60.0	21	25.9	213

	Species		Target	High Enforcement		Low		Open		TOTAL
Family		Functional group				Enforcement		оро		Abundance
				N	F%	N	F%	N	F%	Abulluance
Labridae	Scarus zelindae* (VU <sub>Br</sub> )	Roving herbivore	Υ	1	6.3	46	56.7	1	3.7	48
Labridae	Sparisoma amplum*	Roving herbivore	Υ	-	0.0	6	16.7	1	3.7	7
Labridae	Sparisoma axillare* (VU <sub>Br</sub> )	Roving herbivore	Υ	9	18.8	23	40.0	13	14.8	45
Labridae	Sparisoma frondosum* (VU <sub>Br</sub> )	Roving herbivore	Υ	1	6.3	25	36.7	2	3.7	28
Labridae	Sparisoma radians	Roving herbivore	Υ	-	0.0	-	0.0	1	3.7	1
Labridae	Sparisoma spp.	Roving herbivore	Υ	1	6.3	1	3.3	-	0.0	2
Blenniidae	Parablennius spp.	Omnivore	N	1	6.3	4	10.0	-	0.0	5
Acanthuridae	Acanthurus bahianus*	Roving herbivore	N	24	37.5	78	66.7	15	29.6	117
Acanthuridae	Acanthurus chirurgus	Roving herbivore	N	-	0.0	2	6.7	-	0.0	2
Acanthuridae	Acanthurus coeruleus	Roving herbivore	N	46	18.8	55	66.7	4	7.4	105
Acanthuridae	Acanthurus spp.	Roving herbivore	N	27	12.5	-	0.0	3	7.4	30
Balistidae	Balistes vetula (NT <sub>I</sub> )	Omnivore	Υ	3	18.8	3	10.0	-	0.0	6
Monacanthidae	Cantherhines macrocerus	Omnivore	N	-	0.0	4	10.0	-	0.0	4
Ostraciidae	Acanthostracion spp.	Omnivore	N	-	0.0	1	3.3	-	0.0	1
Tetraodontidae	Canthigaster figueiredoi*	Omnivore	N	-	0.0	2	6.7	-	0.0	2

### General conclusion

In a reality where traditional fisheries regulations, such as gear and boat restrictions, fishing seasons, minimum legal sizes and catch limits, are not showing enough positive results in recovering fish populations, no-take marine reserves (NTRs) have complemented management through an ecosystem approach. NTRs are one of the pillars of the ecosystem based management, protecting a wide range of resources and can also address both conservation and fisheries goals. These areas have been demostrated worldwide to contribue to oceans management by showing higher fish richness, abundance and biomass, and even evidencing spillover and enhancing fisheries in the vicinities.

According to the literature, fisheries target species have shown signs of recovery within NTRs in about five years of creation, evidencing direct effect of protection. Indirect effects of NTRs takes longer, showing results at about 14 years. The NTRs analyzed in the present Thesis, Tupinambás Ecological Station and Abrolhos Marine National Park, are respectively 32 and 36 years old, and have demonstrated strong direct effects and not as evident indirect effects. Target species and carnivores' biomass and abundance have increased with protection, showing a positive effect of NTRs both in rocky and coral reefs in the Southwestern Atlantic. On the other hand, sessile invertebrate feeders (Tupinambás) and omnivores (Abrolhos) have shown indirect effects. However, the high enforcement areas showed stronger positive effects, both in Abrolhos and in Tupinambás, represented by the region offshore in this latter, where less fisheries effort and stronger enforcement is registered. Both also problably for being navy areas.

Given the results here presented, it is clear that the need for compliance is crucial if the objective is to ensure a true effectiveness of a NTR. The enforcement of this areas is indeed necessary, however, the understanding of the importance of the management measure by the stakeholders is imperative. As part of the project, printed copies of the book produced about the reef fish species in Tupinambás Ecological Station were distributed in schools in the region, and in those occasions, it was clear the lack of knowledge about the NTR. Therefore, what I truly expect from this Thesis is to provide empirical evidences and raise awareness about the importance of this areas, encouranging the approximation of the scientific academy

with society, specially in a country where science is considered an expense instead of an investment. This way of facing scientific knowledge by the government conveys to the general public a complete misunderstanding of the importance of science and a great ignorance of the role of this matter to the society. As scientists, we have the duty to try to revert this scenario by making our results achievable and understandable to non-scientists.

As a conclusive remark, the effects of small and large NTRs in the Southwestern Atlantic have been tested using innovative stereo-video methods in the region (stereo-BRUVs and stereo-DOVs), showing positive influence of protection, specially for highly target species. Compliance and enforcement are necessary for the maintanence of this status and the involvement of stakeholders is crucial to achieve a real effectiveness in the region.

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Supplementary material

Baited videos to assess semi-aquatic mammals: occurrence of the

Neotropical otter Lontra longicaudis (Carnivora: Mustelidae) in a

marine coastal island in São Paulo, Southeast Brazil

Abstract A new record of the Neotropical otter (Lontra longicaudis) in the marine

environment near the rocky reef of a coastal island in Brazil is described here, being

the first published report of a semi-aquatic mammal by baited remote underwater

video system. This species is not usually sighted in marine waters away from the

mainland coast, and it has never been reported in this environment in this region.

This individual may have increased its feeding grounds further offshore as food

resources close to the mainland are reduced. Baited videos present a relatively low

cost option to understand better these animals existing in different habitats,

improving the monitoring for these species and providing valuable information on

their distribution, habitat use and life history.

**Key-words:** BRUV, Mammalia, estuaries, Southwestern Atlantic.

### Introduction

The Neotropical otter *Lontra longicaudis* (OLFERS, 1818) is a widely distributed semi-aquatic carnivorous mammal of the family Mustelidae, distributed from Mexico to northern Argentina (FOSTER-TURLEY; MACDONALD; MASON, 1990; RHEINGANTZ; SANTIAGO-PLATA; TRINCA, 2017). The species can be found in rivers, estuaries and, more rarely, in marine coastal regions (BLACHER, 1987; CHEHÉBAR, 1990). Despite its wide distribution, few ecological studies have been done on the species, especially concerning habitat preferences, behavior, and the impacts of human disturbance and habitat degradation on populations.

Rheingantz et al. (2014) highlighted the difficulty in assessing the distribution of large aquatic mammals, such as the Neotropical otter, employing traditional methods. This is mainly due to high effort and cost of field work, and the elusive and often aggressive behavior of the species. This lack of information makes it difficult to accurately assess the conservation status of the species. Therefore, it is crucial to develop new methods and strategies to gather important information on this species, which go beyond the current approach.

Baited Remote Underwater Videos (BRUVs) comprises a steel frame with a camera system and a bait pack that attracts organisms to the field of view, without the presence of divers. It has been widely used to assess fish assemblages around the world, especially large and mobile species that usually avoid the human presence (CAPPO; HARVEY; SHORTIS, 2006; WHITMARSH; FAIRWEATHER; HUVENEERS, 2017). Whilst the potential to assess aquatic and semi-aquatic mammals using BRUVs is evident (WHITMARSH; FAIRWEATHER; HUVENEERS, 2017), it is not extensively employed.

The present study aims to report the occurrence of the Neotropical otter in a marine coastal island using BRUVs and presents the advantages in this technology and method to assess a wide range of aspects concerning to the behavior, distribution, life history and habitat use of semi-aquatic mammals.

### Material and Methods

Baited Remote Underwater Video surveys were performed between 2016 and 2017 in four coastal islands (Mar Virado, Tamanduá, Palmas and Cabras) of São Paulo State, southeast Brazil, southwestern Atlantic (Figure 1).

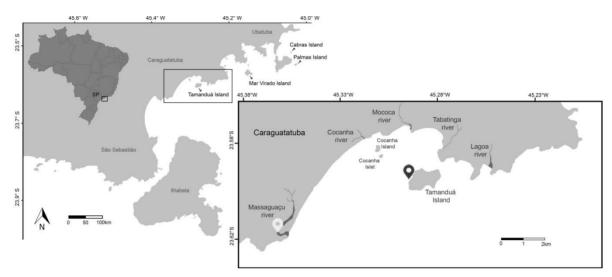


Figure 1 Coastal islands sampled during BRUV expeditions (Cabras, Palmas, Mar Virado and Tamanduá islands). In detail, Tamanduá island and the main estuaries in the region. The Neotropical otter occurrence in the present study was around Tamanduá (black spot) and the nearest occurrence registered in the literature was at the Massaguaçu river (grey spot) by Ribeiro and Miotto (2010).

A BRUV structure consists of a stainless steel frame that supports a camera system with a bait package on an arm 1.5 m in front of the camera (Figure 2). The bait package consisted of 800g of fresh sardines (*Sardinella brasiliensis*) which is used for its oily flesh and therefore, high odor plume dispersion.

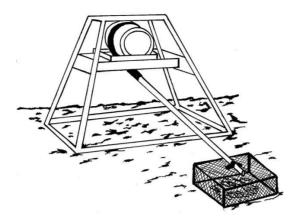


Figure 2 Schematic illustration of the Baited Remote Underwater Video (BRUV) equipment employed in the present study.

Expeditions aiming to assess fish assemblages were performed bi-monthly from March 2017 to January 2018 in all four islands (March, May, July, October, November and January), totaling 6 expeditions. In each expedition, two BRUVs were deployed for 90 minutes on the sandy bottom near the interface of rocky reefs, in water depths 5-10 m, on the leeward side of the islands. All expeditions combined, resulted in 12 BRUV deployments at each island, totaling 48 BRUVs (72 hours). Environmental parameters were taken at every deployment using a CTD (Conductivity, Temperature and Depth - Castaway®) throughout the entire water column and the visibility was estimated using a Secchi disk.

#### Results

Based on 72 hours of video, i.e. 18h hours in each island, one specimen of the Neotropical otter (*Lontra longicaudis*) was detected during the fourth BRUV expedition (Figure 3). The occurrence was at approximately 4:00 pm on the 20<sup>th</sup> October 2016, in Tamanduá island, northern coast of São Paulo state, Brazil (at 23.596°S 45.294°W) (Figure 1). The depth of the deployment was 2.5 m. The water temperature was 24.6°C, salinity of 31.99 and visibility was 6 m at this depth.

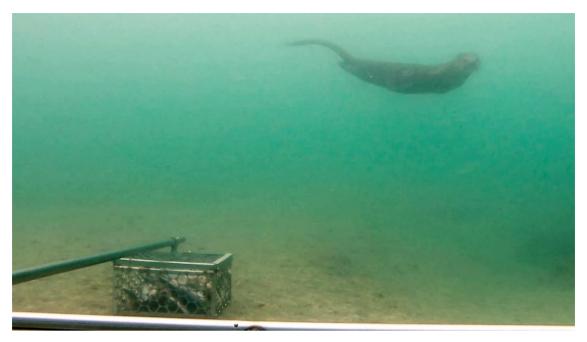


Figure 3 An individual of Neotropical Otter (*Lontra longicaudis*) recorded by Baited Remote Underwater Video (BRUV) deployment. Complete video in the supplementary material.

### **Discussion**

Currently, our global knowledge on otter biology is limited to their resource and habitat requirements, interspecific interactions and genetic aspects (ALMEIDA; PEREIRA, 2017; FOSTER-TURLEY; MACDONALD; MASON, 1990). Few available data exist regarding the Neotropical otter and is primarily focused on trophic ecology and biogeography, in which communication, behavior and demography are the less studied topics (ALMEIDA; PEREIRA, 2017). The present study aimed to contribute with the habitat uses of the species, as well as introducing a new and supplementary method to assist in monitoring this species.

The occurrence of the Neotropical otter in marine environment has been limited to nearshore coastal regions (ALARCON; SIMÕES-LOPES, 2003; BLACHER, 1987), in areas closely associated with the mainland or large islands with freshwater runoff. Although records from small coastal islands are unusual, and a few published articles are available. These studies are based on traces such as feces, urine, footprints, shelters and mucus in some islands of Santa Catarina state (Southern Brazil), up to 12.7km from the mainland coast (CARVALHO-JUNIOR; BANEVICIUS; MAFRA, 2006; CARVALHO-JUNIOR; FILLIPINI; SALVADOR, 2012). In the present

study, the video-recorded individual has moved at least 3 km, which is the distance between the nearest estuary on the mainland and the place of occurrence. The closest recorded occurrence of this species is at a southwest estuary located 6.8 km from where this study sighted the species (RIBEIRO; MIOTTO, 2010). These records might indicate a relatively high autonomy and capacity of the species to move away from the coast, with environmental conditions substantially different from local estuaries.

The marine environment is not preferred since this species needs land to breed and rest (FOSTER-TURLEY; MACDONALD; MASON, 1990; SANTOS; REIS, 2012), therefore, this occurrence might be related to feeding habits of the species, which is mainly composed by fish and crustaceans in coastal areas (ALARCON; SIMÕES-LOPES, 2004). However, habitat partitioning and preference for this otter species is still unknown and requires further detailed investigation.

The Neotropical otter is currently classified as near threatened by International Union of Conservation of Nature - IUCN (RHEINGANTZ; TRINCA, 2015), since its populations has been exposed to great habitat loss and fragmentation, poaching, conflict with fishermen, pollution and reduction of food availability (ALMEIDA; PEREIRA, 2017; BARBIERI et al., 2012; CHEHÉBAR, 1990; GARCÍA-HERNÁNDEZ; GALLO-REYNOSO, 2013). Concerning the Atlantic forests in Brazil, the species is considered vulnerable (RODRIGUES et al., 2013), especially as a consequence biome destruction. This reduction of resources may result in the species seeking alternate feeding grounds, increasing its home range and occurrence in the marine environment at greater distances from the shore.

The present record was made in the last hours of the day and was a single individual. These characteristics corroborate the behavior described for the species, in which individuals hunt alone or in small groups of two or three (CHEIDA et al., 2006), demonstrating increased activity during crepuscular hours (LARIVIERE, 1999; PARERA, 1993).

Whitmarsh et al. (2017) described the potential use of BRUVs to assess other mobile species, such as mammals, however, few official records exist. Some of these records were made by Whitmarsh et al. (2014), in which two mammals belonging to Ostariidae and Delphinidae families occurred in South Australia; however no studies were done specifically aiming to assess aquatic mammals using BRUVs. Based on the unexpected appearance of the otter during the sampling expeditions to assess

fish assemblages, a discussion to explore this technology and method to improve the biological information on the species is raised.

Smell is an important sense for otters, since they widely use chemical cues to communicate (KRUUK, 2006), and it may also play an important role finding food, including underwater, where they hunt fish and crustaceans in waters with variable visibility. The main mechanism of BRUVs is based on attracting animals to the field of view using a highly smell attractive bait, such as pilchards or sardines, something also attractive to otters. Although quite plastic, feeding habits of Neotropical otters in Brazil is mainly based on fish of the family Cichlidae and Loricariidae, as well as Palaemonidae crustaceans (RHEINGANTZ et al., 2017). Even though sardines have been effective to attract the individual encountered here, testing different bait compositions based on the diet of the species is highly recommended, in order to perfectly adapt the methodology to this group of animals.

Traditional methods to assess otters are mostly based on indirect evidences and traces such as footprints, feces, urine, smell, scat and scratches, as well as direct evidences using camera traps. They are useful tools in terrestrial environment, but these methods do not assess underwater habitats. Understanding the importance and habitat use of the different types of water bodies is crucial to assess valuable information on the species, such as habitat requirements, impact of anthropogenic actions and subaquatic behavior patterns.

BRUVs have been used to study general aspects of nektonic assemblages and behavior patterns (WHITMARSH; FAIRWEATHER; HUVENEERS, 2017). Additionally, the non-destructive nature of BRUVs is ideally suited to assess both endangered animals and fragile habitats, such as rocky reefs and estuaries. These characteristics makes the methodology appropriate for otter studies in freshwater, estuaries, and saltwater.

The present study required only 18 hours of baited videos around the island to record an otter in an unusual area, within a project aiming fish. Studies to assess otter populations, and other carnivores, usually requires greater effort, both time and spatially, to make direct encounters. Most of the studies are based on traces and do not cite the presence of individuals. For example, in Ribeiro and Miotto (2010) an encounter only occurred after four years of searching for traces; and Rheingantz et al. (2012) and Quadros and Monteiro-filho (2002) studied traces such as holts, sprainting sites and scats for approximately two years, and did not record a direct

sighing. Few studies, such as Leuchtenberger et al. (2014), register individuals using camera traps and telemetry; however, these studies require a high amount of time on the field, as well as large financial costs and time to process data. Furthermore, the majority of information generated from these studies concerns the land use by the species. The use of baited videos presents the potential to increase the records, giving more information about the species in the underwater environment.

Studies concerning the environmental degradation and population dynamics, as well as the long-term monitoring, behavior and ecological aspects of the species are valuable (ALMEIDA; PEREIRA, 2017; RHEINGANTZ; SANTIAGO-PLATA; TRINCA, 2017). For these topics, BRUVs can be also a useful tool. However, the visual aspect of this method limits its use to waters with adequate visibility, which in most cases, is 1.5 m or the distance from the camera to the bait package. This work aimed to describe and record a non-usual occurrence of the Neotropical otter (*Lontra longicaudis*) at a marine island using underwater baited videos, contributing to the distribution and habitat preferences of the species, as well as to suggest a new application for these methods to assess semi-aquatic mammals.

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