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Are Abrolhos no-take area sites of naïve fish? An evaluation using flight initiation distance of labrids

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Fishing pressure affects the behavior of reef fish, especially of fishery-targeted species. In this context, it is critical to understand if fish behavior is preserved in no-take areas (NTAs), which are considered the best instrument for the recovery of fish stocks. Comparing the flight initiation distances (FIDs) of fish inhabiting multiple-use areas (MUAs), where fishing is allowed (including spearfishing), and NTAs is a useful approach to test whether NTAs can be effective as fish refuges. Here, we compared whether the FIDs of two target (*Sparisoma axillare* and *Scarus trispinosus*) and one non-target (*Halichoeres poeyi*) labrids are greater in the MUAs than in the NTAs. We also investigated whether group size (GS) and body size (BS) exert any effect on the FID. We sampled four MUAs and four NTAs in the Abrolhos Bank (Brazil). We found that only for the targeted species FID was shorter inside the NTAs and that the BS had a positive effect on the FID of all species. The GS and BS of the fish are greater in the NTAs than in the multiple-use areas only for *S. trispinosus*. Our study shows that fish, especially those species that are fishery targets, display an avoidance behavior against spearfishermen likely as a consequence of fishing pressure. We suggest that the NTAs in Abrolhos are not only important sites for the recovery of fish stocks but also possibly act as a repository area of naïve fish (fish that allow human approach), for reefs open for fishing.

A pressão pesqueira afeta o comportamento de peixes recifais, especialmente nas espécies alvo de pesca. Nesse contexto é importante entender se áreas fechadas a pesca ("no-take areas": NTAs), consideradas como melhor instrumento para recuperação dos estoques pesqueiros, garantem a integridade dos comportamentos dos peixes. Comparar distâncias iniciais de fuga ("fligth initiation distance": FIDs) de espécies entre recifes de uso múltiplos ("multiple-use areas": MUAs), onde a pesca é permitida (incluindo a pesca submarina), e as NTAs é uma abordagem útil para testar se as NTAs podem ser efetivas como refúgios para peixes recifais. Nesse trabalho FIDs de duas espécies alvo (*Sparisoma axillare* e *Scarus trispinosus*) e uma não alvo (*Halichoeres poeyi*) foram comparadas entre MUAs e NTAs. A possível influência do tamanho do grupo ("group size": GS) e o tamanho do corpo ("body size": BS) sobre FID também foram investigados. Foram amostradas quatro MUAs e quatro áreas NTAs no banco dos Abrolhos (Brasil). Nossos resultados mostraram que apenas FID das espécies alvo de pesca foi menor dentro de NTAs e que o BS teve um efeito positivo no FID de todas as espécies. Dentro da NTA, GS e BS foram maiores do que em recifes abertos à pesca apenas para *S. trispinosus*. Finalmente, sugerimos que NTAs em Abrolhos não são apenas importantes como recuperação dos estoques pesqueiros, mas estão possivelmente atuando como uma área repositória de peixes "ingênuos" (peixes que permitem aproximação humana) para os recifes onde a pesca é permitida.

Keywords: Parrotfish, Reef fish behavior, Refuges, Southwest Atlantic, Spearfishing impacts.

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Introduction

Among the anthropogenic damages on coral reefs, fishing activities stand out as one of the main drivers causing ecosystem degradation (Jackson *et al.*, 2001). Thus, tackling overexploitation is mandatory to ensure that all goods and services provided by reefs will remain, specifically (1) the aesthetic natural beauty sought for tourism, (2) the megadiversity developed during the Earth's evolutionary processes, (3) the protection of the coastline from wave action, (4) the provision of food and (5) the biogenic carbonate content the reefs store (approximately 50% of global production) (McClanahan *et al.*, 1999). Thus, understanding the consequences of fishing for reef communities, especially for reef fishes, is of utmost importance.

Fishing has clear direct effects on harvested species, but its cascading, indirect effects are relatively less understood (Madin *et al.*, 2010). Research has shown that fish behavior can be strongly influenced by fishing pressure (Feary *et al.*, 2011; Januchowski-Hartley *et al.*, 2013). Fishing can remove larger and predatory fishes from marine food webs, consequently, their prey may not only increase in number but also alter its behavior (Madin *et al.*, 2010). When behavioral responses impact the food resources of prey species, behaviorally mediated trophic cascades can dramatically shape seascapes (Madin *et al.*, 2010; 2011).

Increasing the knowledge on behavioral aspects of ecosystem change will greatly improve our ability to predict the cascading consequences of conservation tools aimed at ecological restoration, such as marine reserves (Madin *et al.*, 2012).

To tackle the damages caused by fishing pressure (including spearfishing practices), the implementation of no-take areas (NTAs) is considered one of most powerful instruments to promote the recovery of stocks and ecosystem levels (Hoegh-Guldberg, 2006). Prohibiting fishing usually promotes the stabilization of populations, the increment of biomass and recovery of neighboring areas through spillover and the migration of individuals (McClanahan *et al.*, 2006).

The flight initiation distance (FID) is the shortest distance between an animal and what threatens it before it flees (Blumstein, 2003; Blumstein *et al.*, 2003). The study of Kelley & Magurran (2003) reported that the FID of fishes is influenced by previous experiences with predators (risk of predation). Moreover, other variables could be influencing a prey's escape decisions such as structural complexity, group size and body size (Gotanda *et al.*, 2009; Januchowski-Hartley *et al.*, 2011; Nunes *et al.*, 2015). Thus, it is expected that in marine NTAs, where fishes are not familiar with the presence of spear fishers, the FIDs would be shorter than those in multiple-use areas (MUAs), where spearfishing (and other human activities) is frequent (Feary *et al.*, 2011; Januchowski-Hartley *et al.*, 2012), even when NTAs and MUAs are close to each other (Gotanda *et al.*, 2009).

In this context, we studied the FID of three labrid species, two being a fishing target and the other a non-target species, in both NTAs and MUAs of the Abrolhos reef complex (Brazil). The hypotheses were (1) that the FID would be greater for the fish inside MUAs than for the fish in NTAs and (2) that fish would be smaller and form smaller groups in the MUAs than in the NTAs. Through scuba dives we reproduced the spear fisherman behavior for estimulating of flight. Compering the FID in neighboring protected and unprotected reefs is necessary to assess whether NTAs are being effective as refuges for fish (especially in sites where management strategies are inefficient), and large FIDs can indicate overexploitation (Gotanda *et al.*, 2009; Januchowski-Hartley *et al.*, 2011; Feary *et al.*, 2011).

Materials and Methods

Study area. Abrolhos Bank (17°20', 18°00'S and 38°30', 39°30'W) consists of a 200 km-wide expansion of the continental shelf and constitutes the largest and richest reef complex of the South Atlantic Ocean. It is situated in a large shallow area (45,000 km²) (Amado-Filho et al., 2012; Francini-Filho & Moura, 2008) where depths rarely exceed 30 m (Francini-Filho et al., 2013) over the Abrolhos Bank, on the eastern Brazilian continental shelf. In this region, the most prominent ecosystems are coral reefs and rhodolith beds (Amado-Filho et al., 2012; Leão et al., 2003). The reefs are composed of a greater nearshore reef arc and a shorter offshore reef arc (Leão et al., 2003; Francini-Filho et al., 2013), 60 km away from the mainland. In both arcs, the reefs show mushroom-shaped pinnacles called chapeirões, which can be up to 25 m in height. Frequently, the tops of the pinnacles coalesce, forming wide flat areas that can extend for miles (Leão & Kikuchi, 2005). Abrolhos reefs are habitats for many fish, approximately 270 species according to Moura & Francini-Filho (2005), representing the richest fish fauna of the Southwestern Atlantic Ocean (Moura, 2000).

The Abrolhos Reef Complex encompasses areas with different management strategies, where most parts of the reefs are multiple-use areas (MUAs) (e.g. with no fishing and/or anchorage restrictions and uncontrolled tourism). Two distinct polygons are, however, "no-take areas" (NTAs) and are part of the National Marine Park of Abrolhos (the first NTA of Brazil, established in 1983 by the Brazilian government with 882 km²). The Abrolhos Archipelago and Parcel dos Abrolhos Reefs constitute one area, distant from the coast, and its protection is enforced by the Brazilian Federal agency ICMBio (Chico Mendes Institute for Biodiversity Conservation) (Francini-Filho & Moura, 2008). We sampled in four protected stations (NTAs) of Parcel dos Abrolhos and in other four stations located in Parcel das Paredes (MUAs) (Fig. 1). The depth and the visibility varied between 6-12 m and 6-10 m, respectively. These two sets of reefs have some similarities in terms of their physical structure (chapeirões), the coral community structure (Leão et al., 2003) and the composition of fish fauna (Moura & Francini-Filho, 2005).

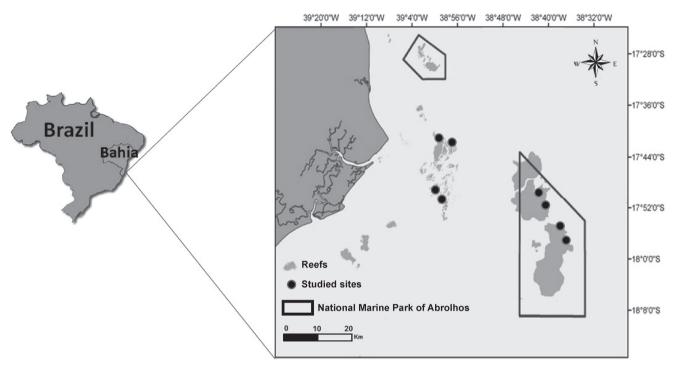


Fig. 1. Map of study area showing sampled sites (MUAs: Multiple-use areas; NTAs: No-take areas) in the Abrolhos bank.

Species studied. We used three distinct species of labrids in our investigations - the two Brazilian endemic parrotfish (see Moura et al., 2001), Sparisoma axillare (Steindachner, 1878) and Scarus trispinosus (Valenciennes, 1840), both are spearfishing targets in the region, and a non-targeted wrasse, Halichoeres poeyi (Steindachner, 1867).

Sparisoma axillare are preferentially associated with sites with intermediate to high levels of coral cover, and their biomass is relatively higher in the MUAs (Francini-Filho & Moura, 2008). This species consumes large amounts of epilithic algae matrix, fleshy algae and detritus (Bonaldo et al., 2006; Ferreira & Gonçalves, 2006; Francini-Filho et al., 2010). They are commonly captured by spearfishermen in many reefs of Bahia state (Nunes et al., 2012).

Scarus trispinosus contributes approximately 77% of the total parrotfish biomass in the Abrolhos Bank and has become one of the most important fishery resources in this region (Francini-Filho & Moura, 2008). This species forages mainly on crustose calcareous algae (Francini-Filho et al., 2010).

Halichoeres poeyi can be found in several reef habitats (Rocha et al., 2005), mainly feeding on mobile invertebrates, such as bivalves and crustaceans (Nunes et al., 2013), and it is not a target for spearfishing.

Flight initiation distance. We estimated fish FID through scuba dives. The diver would reproduce the spearfisherman behavior (modified from Januchowski-Hartley *et al.*, 2011, 2012, 2013; Nunes *et al.*, 2015). Although Januchowski-Hartley *et al.* (2011, 2013) and

Nunes *et al.* (2015) did not used scuba equipment, we decided to use scuba because it facilitates to spot the fish and kept the dives safer. The diver swam directly toward the fish at a constant speed (~0.5 m/s, measured using portable GPS at the sea surface, according to Nunes *et al.*, 2015) using a wooden handle to simulate the spearfishing gun (Fig. 2). When the fish fled, the distance between the end of the fake speargun and the fish's location prior to fleeing (FID) was measured with a scale tape and recorded. Fishes were targeted only if they were feeding or swimming normally (DeLoach & Humann, 1999; Nunes *et al.*, 2015) and flight were considered only when the fish increased its swim speed to greater than the approach speed of the diver (Januchowski-Hartley *et al.*, 2011, 2012, 2013).



Fig. 2. Scuba diver using a wooden handle to simulate the spearfisher behavior.

Fish body size (BS) and group size (GS). All sampled fish, *i.e.* FID replicates, body sizes (cm) were visually estimated and, when applicable, the number of fish forming its mono or heterospecific group counted. We considered as group members all the individuals positioned within a 1-meter maximum distance from the focal animal (Nunes *et al.*, 2013). The observations were made between 09:00 a.m. and 16:00 p.m., which is the main activity period for labrids (Martha & Jones, 2002).

Structural complexity. Structural complexity can negatively influence FID of labrids, probably because sites with higher structural complexity have more shelter and can provide more possibilities to short escapes than areas with lower structural complexity (Nunes et al., 2015). Structural complexity was estimated using the rugosity index (RI) (Graham & Nash, 2013). To measure rugosity, a 1 m chain was placed over the substrate so that it conformed as closely as possible to all of the contours and crevices, and a measure of the actual surface distance in relation to the linear distance was obtained using a scale tape (Luckhurst & Luckhurst, 1978). A total of 120 samples were obtained, 60 in the NTAs (15 per reef) and 60 in the MUAS (15 for each sampled station). The rugosity index (RI) was calculated as RI = linear/surface. "Linear" refers to the distance covered when the scale tape is pulled taut (1m) and "surface" refers to the distance between the start and the end of the chain when it was fitting the contours of the sea bottom.

Data analysis. We used two-way analysis of covariance (ANCOVA) to investigate whether the FID (response variable) of each labrid species differed between the areas (fixed factor with two levels: NTAs and MUAs), in addition to testing whether the group size and body size (continuous covariates: random factors) influenced the FID of those labrids. ANCOVA is a hybrid of regression and analysis of variance and predicts that the covariate also contributes to variation in the response variable (Gotelli & Ellison, 2004). Normality and homoscedasticity were assessed through quantile-quantile (Q-Q) plots and Levene's test, respectively. The FID was $\log (x + 1)$ transformed. We used Student's t-Test to test possible differences in the structural complexity (estimated by the Rugosity index), group size and fish size between the sampled NTAs and MUAs.

We used Software R version 2.12.1 for Windows R 40 (R Development Core Team, 2012) for all statistical procedures and adopted an α -value of 0.05 for all tests.

Results

We estimated the FID of individuals from three species: *H. poeyi* (n=26 in NTAs; n=27 in MUAs), *S. trispinosus* (n=51 in NTAs; n=50 in MUAs) and *S. axillare* (n=44 in NTAs; n=48 in MUAs). The ANCOVA analysis revealed

significant differences in the FID of the two parrotfishes studied, *S. trispinosus* (F=81.13; p<0.001) and *S. axillare* (F= 104.44; p<0.001) between the sampled MUAs and NTAs, with the FID in the NTAs being significantly shorter (see Fig. 3). There was no difference for the FID of the *H. poeyi* (Table 1 and Fig. 3). One of the two continuous covariates used, GS, did not influence the FID for any species. In contrast, the BS had a positive effect on the FID of all the labrid species studied in either the NTAs or MUAs (Table 1; Fig. 4).

Table 1. Analysis of covariance (ANCOVA) output showing the influence of fishery protection (categorical factor) on the flight initiation distances (FID) of three species of labrids. The table also shows the relationship between FID and group and fish sizes (continuous covariates). ns indicate non-significance, while * (p < 0.05) and ** (p < 0.001) represent significant differences.

	SS	DF	MS	F	P
Scarus trispinosus					
Protection	1.349	1	1.349	81.130	<0.001 **
Group	0.017	1	0.017	1.066	0.304 ns
Size	0.170	1	0.170	10.256	<0.001 **
Error	1.613	97	0.016		
Sparisoma axillare					
Protection	6.200	1	6.200	104.440	<0.001**
Group	0.077	1	0.077	1.310	0.255 ns
Size	2.223	1	2.223	37.445	<0.001**
Error	4.690	79	0.593		
Halichoeres poeyi					
Protection	0.004	1	0.004	0.140	0.709 ns
Group	0.0004	1	0.0004	0.014	0.904 ns
Size	0.177	1	0.177	5.582	0.022*
Error	1.560	49	0.031		

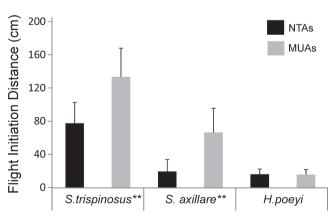


Fig. 3. Mean of flight initiation distances for the three labrid species studied in MUAs (Multiple-use areas) and NTAs (No-take areas) in Abrolhos. The upper limits of lines indicate standard deviation; *symbols indicate significant differences according to ANCOVA; **(p < 0.001).

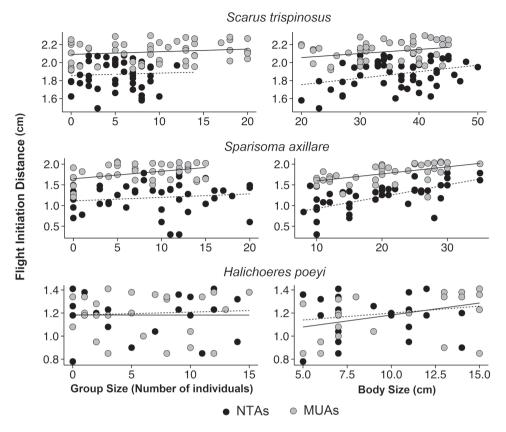


Fig. 4. Relationship between flight initiation distances (FID), group size and body size (continuous covariates) inside NTAs and MUAs (categorical factor). *Black dots* represent samples from no-take areas (NTAs); *white dots* represent samples from multiple-use areas (MUAs). The *continuous line* represents the best fit for MUAs data and the *dotted line* that for NTAs data.

Student's t-Test showed differences between the MUAs and NTAs for only one species, *S. trispinosus*, regarding both GS (t =-3.8; df = 99; p < 0.001; Fig. 5a) and BS (t = 3.6; df = 99; p = 0.03; Fig. 5b). In the NTAs, the GS (9.1 \pm 0.12, mean \pm SE) and BS (36 \pm 0.14) were significantly greater than those estimated in the MUAs, which were 5.3 \pm 0.06 and 28 \pm 0.14, respectively.

However, for the other two species considered, *S. axillare* (fishery target) and *H. poeyi* (non-target), we did not find any differences in GS (t=1.3; df=81; p=0.2 for *S. axillare* and t=-0.01; df=51; p=0.99 for *H. poeyi*), with an average of 8.8 ± 0.14 and 7.3 ± 0.12 for *S. axillare* and 5.8 ± 0.19 and 5.7 ± 0.17 for *H. poeyi* in the NTAs and MUAs, respectively (Fig. 5a). Student's t-Test also did not reveal differences in the BS for these two species when comparing the NTAs and MUAs (t=-1.112; df=81; p=0.27 for *S. axillare* and t=-0.20; df=51; df=81; df=81

Additionally, Student's t-Test revealed that the structural complexity was similar between the NTAs and MUAs (t = 1.54; df = 119; p = 0.173); in the NTAs, the mean of the Rugosity Index (RI) was equivalent to 2.8 ± 0.2 , while in MUAs, the estimated mean RI was 2.6 ± 0.4 .

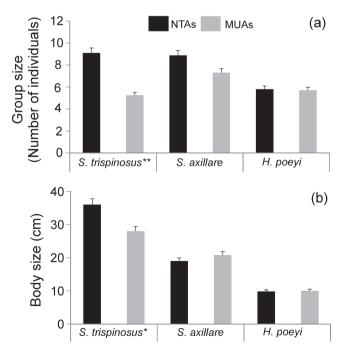


Fig. 5. Average group size (a) and body size (b) estimated for the three labrids studied in both no-takes (NTAs) and multiple-use areas (MUAs) sampled in the Abrolhos Bank. The upper limits of lines indicate standard error.

Discussion

Our study indicates that the FID was different for the two harvested species, more specifically the two endemic parrotfishes *S. trispinosus* and *S. axillare*. These results corroborate other studies developed in the Caribbean (Gotanda *et al.*, 2009) and the Pacific Ocean (Januchowski-Hartley *et al.*, 2012, 2013), which showed that fish display avoidance behaviors against spearfishermen as a consequence of fishing pressure, especially those species that are fishery targets. There is no doubt that studies involving FID can be used to assess the impact of spearfishing on reef fish behavior.

Januchowski-Hartley *et al.* (2013) investigated whether no-take areas would produce naïve fish in response to the human presence. These authors showed that FID of the target species varied significantly and marine protected areas, not only enhance the recovery of fish stocks, both inside the reserves and outside through the 'spillover' process, but also generate fish that are easier to catch. Thus, fish learn to adjust their FID according to the level of risk, where risk is low FIDs are shorter (*i.e.* within reserves) and fish that spill over into the fished area will need time to learn and increase their FIDs (Sale, 2013).

As expected, no-take areas had no influence on the avoidance behavior of the non-targeted species studied here, the wrasse *Halichoeres poeyi*. Possibly because they are not caught by spearfishermen outside of the NTAs, *H. poeyi* individuals did not learn to defend themselves from the possible catching threats. The study by Januchowski-Hartley *et al.* (2013) used Chaetodontidae fish as the control species (non-targeted by spearfishing) and also showed that the FID did not vary significantly over a gradient from the inside to the outside of NTAs.

The group size (GS) did not influence the FID in any of the species studied here. According to Ydenberg & Dill (1986) larger groups lead to the identification of predators at greater distances and can result in increased FID; however, the risk dilution associated with larger groups tends to reduce the FID. From six reef fish families studied by Januchowski-Hartley *et al.* (2012), only Acanthuridae showed increasing FID with increasing GS. This phenomenon only occurred in an area with a high fishing pressure, and according to the authors, it could indicate an independent anti-predation response to an increased fishing pressure. Gotanda *et al.* (2009), studying parrotfishes inside and outside NTAs in Barbados, concluded that the GS effect was relatively weak compared with BS and the NTA's protection against fishing activities.

Body size (BS) influenced the FID in all species, and some variables can explain the relationship between the FID and BS. The eco-morphology of the predator/prey relationship is one such variable (smaller prey are more cryptic, harder to identify, and metabolically less profitable to target than larger sized prey; Gill & Hart, 1994). The locomotive ability and visual acuity of prey fishes may also affect FID and are expected to increase with BS (McGill & Mittelbach, 2006).

Our findings corroborate the optimal fitness theory, which predicts an increase in a fish's FID with an increase in BS (Cooper Jr. & Frederick, 2007). According to Januchowski-Hartley *et al.* (2012), the evidence for applying this theory to coral reef fish remains conflicting: BS in the Caribbean parrotfish was the largest single determinant of increases in FID (Gotanda *et al.*, 2009), while in Indo-Pacific reef fish, the BS was not significant in determining FID (Feary *et al.*, 2011).

Our results indicate that the relationship between the BS and FID does vary according to fishing pressure. Due to the depletion of reef sharks, Januchowski-Hartley *et al.* (2012) hypothesized that predation escape via increased BS in coral reef fishes may be increasingly common or may be occurring for lower prey body sizes. They did not expect a significant impact of BS on the FID in NTAs, a hypothesis that was supported in our study. A similar result was also found by Feary *et al.* (2011) studying the effects of BS and protection against fishing in Papua New Guinea reefs. The FID would likely increase with BS, as reported here and for the Caribbean (Gotanda *et al.*, 2009).

Our results regarding the target-species S. trispinosus found estimates of larger GS and longer fish length inside the NTAs. Similarly, Floeter et al. (2006) showed that NTAs exert positive effects on the size and abundance of reef fish compared with multiple-use areas along the Brazilian reef. Top predators and roving herbivores clearly presented higher abundance and size in areas with fishing closures. Francini-Filho & Moura (2008) evaluated fish biomass in Abrolhos reefs subject to different management strategies. They indicate that despite some positive signs at a local scale, the effective use of NTAs as fishery management tools in the Abrolhos Bank is still largely dependent on an effective network of enforced NTAs, encompassing several critical habitats that are still unprotected (e.g. deep reefs, rhodolith beds and mangroves). A reduction in the biomass of herbivorous species and increased cover of macroalgae, which are the main competitors of reef-building corals and therefore a threat for reef construction (McCook et al., 2001; Padovani-Ferreira et al., 2012) in the Abrolhos Bank, highlighted the poor enforcement of Brazilian marine NTAs (Bruce et al., 2012).

Our study shows that labrid fish, especially those species that are fishery targets, display an avoidance behavior against spearfishermen as a consequence of fishing pressure. New approaches are helping in assessing the health of the National Marine Park of Abrolhos (Bruce *et al.*, 2012; Moura *et al.*, 2013). We believe that the use of multiple approaches, including studying the behavioral responses to fear, such as the flight initiation distance, will increase our knowledge not only of the ecology of the greatest and richest coral reef complex of the South Atlantic Ocean but also of how to evaluate the effectiveness of no-take areas. If the Abrolhos region continues without appropriate surveillance, the recognition by fish of humans as predators and consequently the seascape of fear will expand inside Brazil's oldest marine park.

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