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The development of a preliminary rock reef fish multimetric index for assessing thermal and urban impacts in a tropical bay

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ABSTRACT

We developed a multimetric index for assessing ecological conditions in rocky reefs areas to evaluate thermal and urban influences on fish community. Eight metrics were selected to assess thermal influence: (1) total number of species; (2) number of water column species; (3) number of transient species; (4) density of individuals with low resilience; (5) density of omnivores; (6) density of carnivores; (7) number of cryptic species; (8) density of herbivores. For urban influence, six metrics were selected: (1) total density; (2) ratio between the number of rare species and the total number of species; (3) density of individuals with heavy fishing pressure; (4) number of resident species; (5) number of cryptic species; (6) density of herbivores. This preliminary index succeed in discriminating control/impacted sites and proved to be an important tool to assess impacts that alter fish community and have potential to be used in tropical rock reef coastal areas.

1. Introduction

The human development with growth of urban centers and demand for natural resources is increasingly impacting coastal environments. Anthropogenic activities in coastal areas are directly or indirectly associated with tourism, oil and gas industries, fisheries and services that cater to the dynamic economy generated by these activities. Impacts of pollution on coastal ecosystems have the potential to alter the patterns of natural variability at various scales of organization within the community (Guidetti et al., 2002; Azzurro et al., 2010). Knowledge of these processes is an important step to establish an effective policy of management and conservation of the coastal biodiversity (Islam and Tanaka, 2004; Begossi et al., 2011). This is of special importance in tropical areas, where assessment and permanent monitoring programs are scarce.

The use of multimetric indices (MMI) for international agencies has been employed for monitoring and assessing the impacts in different ecosystems. MMIs use biological or ecological measurements of communities, compiled into metrics, to quantify the degree of human disturbance on biological communities (Coates et al., 2007; Delpech et al., 2010; Schoolmaster-Jr et al., 2013). In this sense, metrics are sensible indicators of a given type of environmental disturbance and are useful tools to be used in multimetric indices to assess the level of ecosystem integrity (Harrison and Whitfield, 2004; Henriques et al., 2008a).

The closest studies related to marine environments were developed for estuarine systems (e.g., Cooper et al., 1994; Deegan et al., 1997; Quinn et al., 1999; Harrison and Whitfield, 2004; Coates et al., 2007; Henriques et al., 2008b; Delpech et al., 2010; Cabral et al., 2012). To our knowledge, only Henriques et al. (2013) has selected metrics of rocky-shore fish assemblages to evaluate the impacts of different human disturbances (e.g. fishing, sewage discharges, port activities and thermal effluent). However, all these studies were developed for temperate regions and there is a lack of information for tropical rocky shores. Differences between temperate and tropical ecosystems in species richness, habitat diversity and ecological/biological characteristics such as growth rate, early age-at-maturity, r-selected species (Fromentin and Fonteneau, 2001), range of size (Fulton and Bellwood, 2004) may influence metric selections and index sensitivity.

In this study, we considered two types of impacts: 1) the influence of a nuclear power plant's cooling water (thermal influence), and, 2) the influence of coastal urban areas as diffuse impact; mainly the constant access of tourists, proximity of marinas, harbors, fishing activities (urban influence).

Temperature is a very important ecological parameter that affects almost every aspect of aquatic life (Krishnakumar et al., 1991; Rajadurai et al., 2005). Heated effluents introduced into the marine environment may induce dramatic and unpredictable effects such as temperature-induced changes in energy utilization (Finn et al., 2002), effects on sex determination (Brown et al., 2014), metabolic organization and scope for growth (Hanel et al., 1996). Heat from the cooling water of nuclear power plants changes environmental conditions and consequently ecological components of tropical coastal systems with influence on fish

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assemblage, reduction of benthic cover, thus limiting resources availability (see Teixeira et al., 2009, 2012). Thus, species more sensible to changes in temperature may be potentially used as indicators of thermal influences in coastal areas.

Indices can also be applied to evaluate urban pollution caused by different human activities (urban influence). The Ilha Grande Bay suffers from anthropogenic impacts such as recreational fisheries targeting rocky reef fishes, tourism, high population, many marinas, sewage discharges, port activities and thermal effluent. These impacts can change characteristics of fish community, such as diversity, biomass and abundance, and may cause massive fish death, reproductive inhibition or failure, and unbalanced trophic structure (Guidetti et al., 2002; Smith et al., 1999, Islam and Tanaka, 2004).

Teixeira et al. (2009), studying the effects of a nuclear power plant thermal discharge on fish community structure in Ilha Grande Bay found that thermal pollution alters benthic cover and influences fish assemblages by altering composition and decreasing richness. Moreover, Teixeira-Neves et al. (2015) found that coastal reef fish species richness in Ilha Grande Bay was positively associated with deeper areas and greater distance from the coast, thus being less accessible to human influence, and that richness and density increased with the physical complexity indicated by the physical structure index, suggesting that the presence of a variety of refuges enhances the availability of shelter. The main goals of this study were (1) select fish metrics that best distinguish impacted from control sites for both thermal and urban influences and, (2) develop a preliminary multimetric index to assess ecological condition based on the rocky reef fish assemblage.

2. Materials and methods

2.1. Study area and sampling

This study was conducted along coastal and insulars rocky shores in the Ilha Grande Bay (23°02′-23°14′S, 44°07′-44°42′W), a tropical region in the Brazilian southeastern coast (Fig. 1). Ilha Grande Bay covers an area of about 650 km^2 and contains roughly 350 islands surrounded by shallow water (typically no more than 8 m in depth) (Ignacio et al., 2010). Physical structure of the study sites is characterized by rocky shores covered by granite boulders, ending in a sand bottom.

Sites to assess thermal influence (both impacts and controls) had predominant small boulders and slight slope, whereas the sites to assess urban impact had predominant larger boulders, and a more steep slope. The benthic cover in the control sites (for both impacts) was characterized by the dominance of fleshy algae, turf, soft coral and filamentous

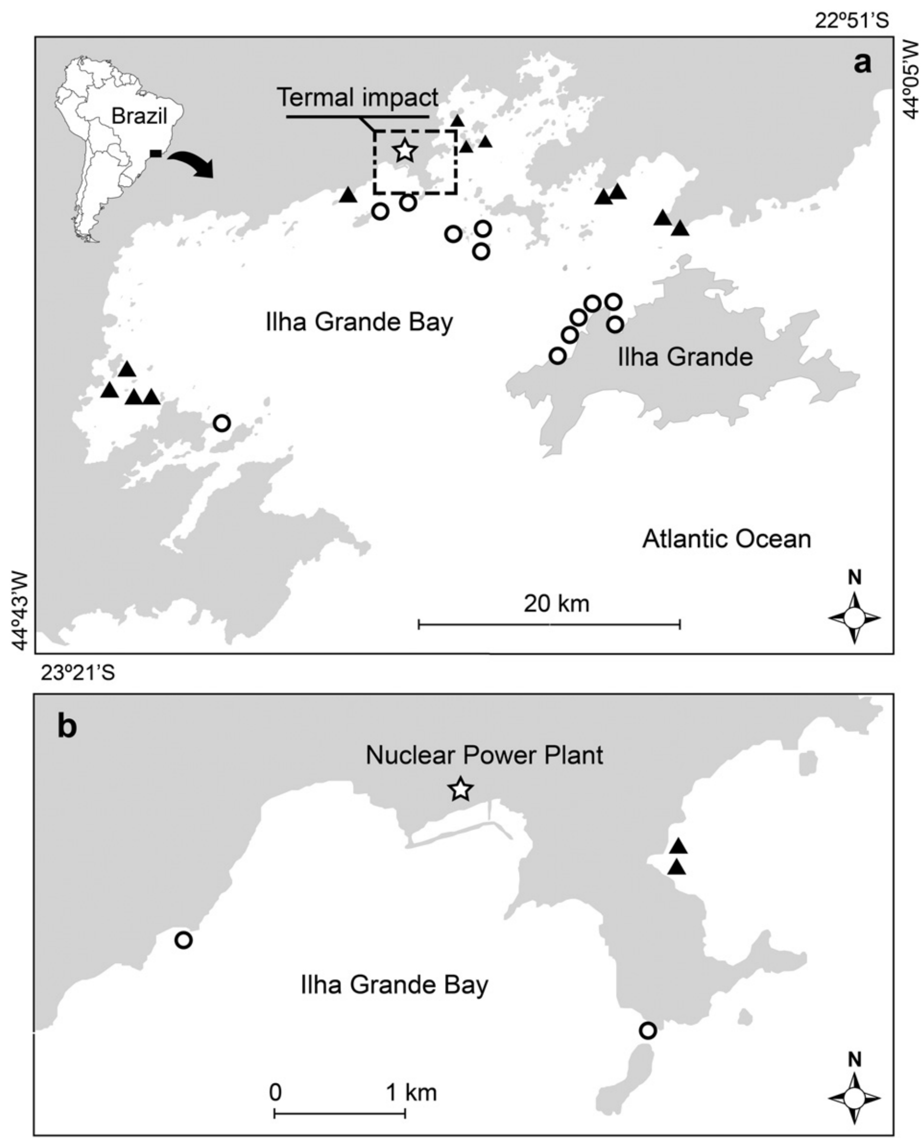

Fig. 1. a. Map of the study area with indications of the sampling sites. Circles indicate the control sites under urban influence and triangles the impacted sites (above). The inserted rectangle indicates the position of the thermal sites. b. Sites impacted by thermal influence, with circles corresponding to the control sites and triangles the impacted sites.

algae. Bottom-dwelling species present in these sites included urchins, mollusks, tunicates, sponges and corals. The benthic cover in the impacted sites to assess thermal influences was characterized by the dominance of turf - composed of a matrix of small macroalgae mainly belonging to the orders Corallinales, Ceramiales and other green and red filamentous algae (Thrush et al., 2011), *Petaloconchus* sp. vermitid, filamentous algae and branched calcareous algae from the *Jania* genus. The average depth of control and impacted sites for assessing thermal influences were 2.1 ± 0.8 m and 1.8 ± 0.6 m respectively, whereas the control and impacted sites for assessing urban influence had average depth of 4.2 ± 1.0 m and 2.3 ± 0.9 m, respectively.

Underwater visual censuses were performed by SCUBA diving and snorkeling along transects 20 m long and 2 m wide (40 m^2) (Ferreira et al., 2001). Transects were performed twice to record fish species, the number of individuals of each species and to estimate size or range of size for each specimen or group of a given species. The first time, the observer recorded the conspicuous species, and the second time, the observer focused the search beneath rocks and in all crevices to observe the more cryptic species. The sampled areas for habitat characterization were the same as for fish sampling. The sampling unit was defined as the pooled number of conspicuous and cryptic species per 40 m^2. Samples were performed in good weather and stable

Table 1
List of the 35 candidate metrics in seven categories of structural/functional traits to assess thermal and urban influences, their respective descriptions and references.

Metrics	Description	References
Diversity/composition		
Total number of species	Measure of species richness	
Total density	Measure of abundance	Guidetti et al., 2002
Density of individuals of rare species	Measure of conservation value of the system	
Number of rare species	(Species with <1% of total abundance)	
Number of rare species/total number of species	(Species with <1% of total abundance)/number of species	
Dominance	Number of species that make up 90% of the total density	
Fishing pressure		
Density of individuals with light fishing pressure	Measure the effects of fishing pressure	
Density of individuals with heavy fishing pressure	Species with high commercial value	
Commercial (heavy and light fishing pressure)/non-commercial Ratio (in density)		Gasparini et al., 2005;
Number of species with non-fishing pressure	Number of no commercial value species	Floeter et al., 2006;
Number of species with heavy fishing pressure	Number of highly commercial value species	Tubino et al., 2007;
Number of ornamental species	Species important for aquarium market	Begossi et al., 2011
Trophic structure		
Density of omnivores	Species that feed on a variety of organisms including invertebrates, fish and algae.	Ferreira et al., 2004 Floeter et al., 2007
Density of invertivores	Feed on mobile and sessile invertebrates.	Luiz et al., 2008
Density of carnivores	Feed on fish and a variety of benthic organisms.	
Density of planktivores	Feed primarily on macro and micro-zooplankton.	
Density of herbivores	Feed on turf and macroalgae.	
Mobility/behaviour		
Density of territorial species	Limited movements and territorial behavior	Ferreira et al., 1998
Number of solitary species		Froese and Pauly, 2014
Number of gregarious species	Species living in shoals	Magurran and Henderson, 2003;
Number of transient species	Rare species that appear unpredictably, less closely associated with a particular habitat and their presence more likely to be governed by stochastic events	Ulrich and Ollik, 2004; Belmaker, 2009
Number of resident species	Species are usually abundant, occur predictably and are biologically associated with the sampled habitat, adapted to their habitat and show high affinity to it.	
Habitat association		
Density of individuals that use water column and holes	Species using mainly the water column and holes.	Froese and Pauly, 2014
Density of individuals using mainly substrate covered	Species using mainly substrates covered by algae (rocky and flats covered by turf algae), these species feed and shelter on benthic encrusting organisms and are swimming and moving over the substrate.	
Number of cryptic species	Directly associated with rocky bottom	
Water column to cryptic ratio (in number of species)		
Density of water column species	Species using mainly the water column	
Number of water column species		
Density of rock hole (>20 cm) specialists	Rock hole specialists – mainly used large holes	
Density of rock hole (<10 cm) specialists	Rock hole specialists – mainly used small holes	
Resilience		
Density of individuals with "low" and "very low" resilience	Capacity to recover from changes in the environment; minimum population doubling time: high (up to 1.4 years), medium (1.4–4.4 years), low (4.5–14 years), very low (more than 14 years)	Froese and Pauly, 2014; Hollings, 1973
Density of individuals with "medium" resilience		
Density of individuals with "high" resilience		
Thermal tolerance[a]		
Number of species with Critical Thermal Maximum above 30 °C	In the dynamic method the Critical Thermal Maximum (CTMax) is quantified as the mean temperature at which individual fish reach such critical points.	Mora and Ospina, 2001;
Density of species with Critical Thermal Maximum above 30 °C		

[a] Thermal tolerance only tested for thermal influence.

oceanographic conditions, typical of the winter, between 9:00 and 14:00 h, during neap tide, near quarter moon.

2.2. Thermal influence

The evaluation of the disturbance from thermal effluent was conducted along a rocky shore close to the water discharge of the Brazilian Nuclear Power Plant (BNPP), which is comprised of two plant units (Fig. 1). Samplings were performed between 2007 and 2008 during the dry season (winter) to minimize the effects of seasonal changes in the temperature. Six repeated censuses were performed at four sites (two impacted and two controls) during four field trips, yielding a total of 96 transects (4 field trips × 4 sites × 6 replicates). Few sites as necessary to assess thermal impact because this type of impact is very punctual. Replicates were performed to assess eventual changes in cooling water discharges during the studied period. Thermal discharge in this area is a local anomaly that can reach some square kilometers (≈2 km^2) from the outfall (Lucca et al., 2005). The disturbed sites were located near the cooling water outfalls (<200 m) of the power station, and the control sites were ca. 4 and 9 km far from the outfall of the cooling water discharges. The average surface temperature ranged from 30.3 ± 0.28 to 31.0 ± 0.61 °C in the impacted sites, and from to 25.42 ± 0.22 to 28.3 ± 0.62 °C in the control sites. For more detailed information on temperature and benthic cover in the area see Teixeira et al. (2012). There are no tourism activities (e.g., diving, sport fishing, etc.) in both impacted and control sites with thermal influence.

2.3. Urban influence

Sites under urban influence were sampled between 2010 and 2011 during the dry season (winter). Six repeated censuses were performed in 24 sites (12 impacts and 12 controls) yielding a total of 144 transects (24 sites × 6 transects per site). The impacted sites with differing intensity of alteration were located along the coast or in closest islands with constant access of tourists, close to the marinas, harbors and urban areas. These areas also included impacts as fishing activity (including destructive bottom trawling) and recreational fisheries targeting rocky reef fishes (harpoon fishery and hook-and-line). Conversely, areas inside a marine protected area (Ecological Station of Tamoios) and sites with difficult access for tourists (absence of ramps and beaches) were selected as control sites. Benthic cover did not change significantly among the sites according to Teixeira-Neves et al. (2015). More detailed informations on anthropogenic influences and other features to characterize the sites as either control or impacted by urban alterations are provide in Table suppl. 1.

2.4. Candidate metrics

The list of candidate metrics was based on: 1) existing studies on rocky reef fish to assess ecological condition in temperate water (Henriques et al., 2008a, 2008b, 2013) and, 2) studies on fish response to anthropogenic disturbances and description of rocky fish assemblages in rocky reef areas. Data were organized as densities of species-by-site arrays that were transformed into metric level-by-samples arrays.

A total of 33 candidate metrics for assessing both thermal and urban influence were selected in 6 common categories. The 6 functional/structural categories are (Table 1): (1) diversity/composition; (2) fishing pressure; (3) trophic structure; (4) mobility/behavior; (5) habitat association; and, (6) resilience. For thermal influence, two additional metrics related with Critical Thermal Maximum (CTM) category (see Mora and Ospina, 2001) were also considered and grouped as (7) thermal tolerance category (Table 1). Functional attributes associated to habitat use and mobility/behaviour for the ichthyofauna were shown in Table suppl. 2.

The category *Diversity/composition* was related to biodiversity and is linked to species richness. Disturbances on coastal habitats (e.g., sewage discharge, fishing, dredging and port activity) may affect directly local richness (Cabral et al., 2012) and diversity. Sewage for instance, may affect fish diversity, abundance, mortality and fecundity, and makes some fishes more susceptible to infections and parasite infestation (Islam and Tanaka, 2004; Azzurro et al., 2010). The metric Number of rare species/total number of species was assessed considering that rare species were those that had relative numerical abundance accounting for lower than 1% of the total number of fishes. The record of rare species is extremely dependent on sampling effort (Keough and Quinn, 1991) and its evaluation is assessing through a ratio that ponders its presence (in number of species) in reference to the total number of species captured to minimize the effect of sampling (Henriques et al., 2013). The metric total density provides an efficient indicator of biotic integrity (Harrison and Whitfield, 2004; Coates et al., 2007). Fish density has been considered as a good indicator of the integrity of the community because the increased anthropic pressure mainly produces a decline in fish abundance. However, abundance of certain species can actually increase in the face of anthropogenic impact (e.g. organic enrichment, artificial increase of habitat complexity, disappearance of a predator). Therefore, this attribute should be considered with care because it can change in either way (Teixeira et al., 2009).

Fishing pressure has increased importance and extractive exploration have raised some concerns in the last years. Research suggests that both commercial and aquarium fisheries are taking large numbers of fish from Brazilian reefs, leading to significant changes in community structure (Costa et al., 2003; Gasparini et al., 2005; Floeter et al., 2006). Both artisanal and commercial fisheries appear to be affecting the population size and size structure of fish populations (Ferreira and Gonçalves, 1999; Gasparini et al., 2005).

Trophic structure relates to energy flows and matter cycles within the community or imported/exported by the community from/to the neighboring areas. The different trophic levels are important to the equilibrium and stability of the communities. According to Khalaf and Kochzius (2002), a stable environment should have species in all trophic guilds. Trophic guild metrics are important because they reflect the impact generated by feeding resources of different origins, from the organisms of benthic cover (invertebrates and algae) up to the fish species.

Metrics included in the category *Mobility/Behaviour* refer to movements of different species within and outside the rocky reefs as part of their life cycle and associated to their behavior. Resident species (restricted mobility), were usually abundant and frequent, and close associated with the microhabitat, whereas the transients were rare and appeared unpredictably (extensive mobility) (Magurran and Henderson, 2003; Ulrich and Ollik, 2004). Such metrics are important because emphasize the importance of the habitat by discriminating species that use this habitat throughout the whole life from those that depend on the rocky reef resources during part of life cycle only.

The category *Habitat association* is directly related to microhabitat (e.g., benthic cover and topographic complexity). There are many species closely related to benthic coverage, mainly small-sized species that have close association to the substrate (e.g., Blenniidae, Labrisomidae and Gobiidae) (La Mesa et al., 2006). This metric reflects disturbances that affect benthic organisms that provide habitat structure for fish species and consequently, for fish assemblages. Another group typical of rock reef fish is formed by fish species present in the water column as small or large schools. Acanthuridae, Haemulidae, Pomacentridae and Sparidae are important families in the characterization of the rocky shores because they are very frequent and abundant in tropical coastal areas.

Resilience category refers to the capacity of species to recover from changes in the environment (Hollings, 1973; Musik 1999). Degraded systems are generally expected to yield fewer species with low or very-low resilience than less impacted systems.

Table 2
Score system of the multimetric index and respective values for the thermal and urban influences.

Metrics	Scores				
Thermal influence	1	2	3	4	5
Total number of species	0–2	3–5	6–8	9–11	≥12
Number of cryptic species	0	1	2	3–5	≥6
Number of water column species	0–1	2–3	4–6	7–8	≥9
Number of transient species	0–2	3–5	6–8	9–11	≥12
Density of individuals with “low” and “very low” resilience	0	1	2	3	≥4
Density of omnivores	0–38	39–76	77–114	115–152	≥53
Density of herbivores	0–3	4–9	10–15	16–21	≥22
Density of carnivores	0	1	2	3–4	≥5
Urban influence	1	2	3	4	5
Total density	0–70	71–144	145–217	218–290	≥291
Number of rare species/total number of species	0–0.17	0.18–0.30	0.31–0.48	0.49–0.66	≥0.67
Density of individuals with heavy fishing pressure	0–18	19–38	39–57	58–76	≥77
Number of cryptic species	0–1	2	3	4	≥5
Number of resident species	0–1	2–3	4–5	6–7	≥8
Density of herbivores	0–4	5–9	10–14	15–19	≥20

Thermal tolerance category has an important metric because of the increased use of the coastal waters for cooling industrial machinery and power plants. Increased water temperature influences directly fish and components of benthic cover affecting their relationship (Lardicci et al., 1999; Teixeira et al., 2012), since species respond differentially to thermal impact. Therefore, alterations in species composition and abundance in areas of artificially increased temperature are expected.

2.5. Metric selection

The metric selection method followed well-defined principles (Roset et al., 2007) which consider that metrics should be biologically meaningful, reliable and easily to quantify, sensitive to human disturbance, and not redundant with other metrics. We adopted a metric selection procedure originally employed by Henriques et al. (2013). Differences in the structure of fish metrics (considering all candidate metrics) between impacted and control sites of both thermal and urban influences were assessed using Permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001). As impacted sites by urban disturbances were generally closer to the coast (Fig. 1), we account for the potential spatial influence on the variability of fish metrics using spatial covariates in the PERMANOVA design. The study area was subdivided into grid cells of 800 × 800 m and sequential values were attributed for each row (east–west axis, X covariate) and column (north–south axis, Y covariate). Hence, the routine PERMDISP (a test of homogeneity of multivariate dispersion) was used to check if statistically significant differences between impacted and control sites detected with PERMANOVA were not influenced by differences in dispersion among groups (Anderson et al., 2008). To visualize multivariate patterns revealed by PERMANOVA, we performed a Principal Coordinates Analysis (PCO) on Bray-Curtis dissimilarities (Anderson et al., 2008). To identify metrics discriminating impacted vs. control sites, we used Canonical Analyses of Principal Coordinates (CAP) (Anderson and Willis, 2003). Fish-based metrics Spearman correlations with the first CAP axis were examined to identify the metrics contributing most to distinguish control from impacted sites (correlation coefficients r > |0.5|). The selected metric values were then compared between control and impacted sites for each type of influence, using Mann–Whitney U-tests. Finally, redundancy among metrics was assessed by Spearman correlations. Pairs of metrics with r > |0.85| were considered redundant. The choice

Table 3
Ecological condition scale for the multimetric index for both impacts (thermal influence and urban influence).

Ecological condition	Min. value	Max. value
Bad	0.2	0.36
Poor	0.36	0.52
Moderate	0.52	0.68
Acceptable	0.68	0.84
Good	0.84	1

Adapted from Coates et al. (2007).

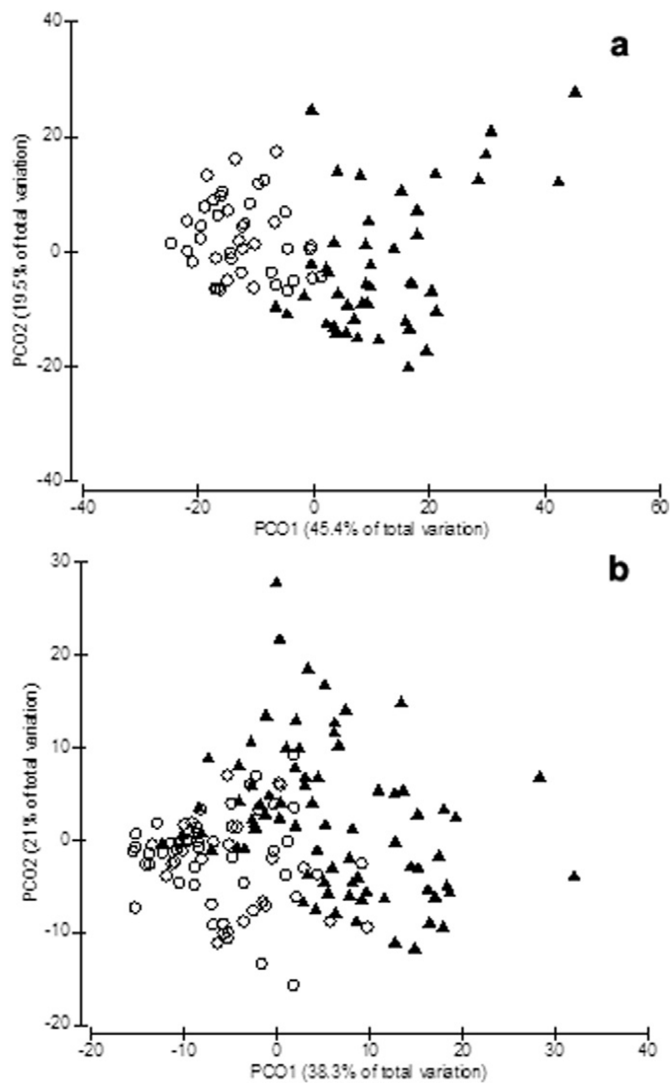

Fig. 2. Principal Coordinates Analysis (PCO) comparing fish metrics at control (circles) and impacted (triangles) sites for the impacts of thermal influence (a) and urban influence (b).

Table 4
Results of selection steps of the metrics for the thermal and urban impacts: 1. Canonical analysis of Principal Coordinates (CAP) with correlation coefficient >|0.5|; 2. Significant differences ($p < 0.01$) of Mann-Whitney test; 3. Redundancy of Spearman coefficient ($r_S < 0.85$). 4. Selected metric. Bold = >0.5.

Categories/metrics	Thermal influence				Urban influence			
	1	2	3	4	1	2	3	4
Diversity/composition								
Total number of species	**−0.77**	C > I	x	✓	**−0.67**	C > I	x	
Total density	−0.30				**−0.87**	C > I		✓
Density of individuals of rare species	**−0.55**	C > I	x		**−0.62**	C > I	x	
Number of rare species	**−0.73**	C > I	x		**−0.65**	C > I	x	
Number of rare species/total number of species	−0.46				**−0.59**	C > I	x	✓
Dominance	−0.45				−0.14			
Fishing pressure								
Density of individuals with light fishing pressure	−0.28				−0.35			
Density of individuals with heavy fishing pressure	**−0.73**	n.s.			**−0.60**	C > I	x	✓
Commercial (heavy + light)/non-commercial ratio (in density)	−0.35				−0.05			
Number of species with non-fishing pressure	**−0.62**	C > I	x		0.45			
Number of species with heavy fishing pressure	**−0.70**	C > I	x		**−0.64**	C > I	x	
Number of ornamental species	**−0.81**	C > I	x		**−0.58**	C > I	x	
Trophic structure								
Density of omnivores	**−0.74**	C > I	x	✓	**−0.55**	C > I	x	
Density of invertivores	−0.07				−0.36			
Density of carnivores	**−0.61**	C > I	x	✓	−0.41			
Density of planktivores	−0.05				−0.28			
Density of herbivores	**−0.70**	C > I	x	✓	**−0.66**	C > I		✓
Mobility/behaviour								
Density of territorial species	**−0.66**	C > I	x		**−0.57**	C > I	x	
Number of solitary species	**−0.75**	C > I	x		−0.49			
Number of gregarious species	**−0.63**	C > I	x		**−0.63**	C > I	x	
Number of transient species	**−0.86**	C > I		✓	**−0.67**	C > I	x	
Number of resident species	**−0.87**	C > I	x		**−0.52**	C > I	x	✓
Habitat associate								
Density of individuals that use water column and holes	**−0.82**	C > I	x		**−0.59**	C > I	x	
Density of Individuals using mainly substrate covered	**−0.75**	C > I	x		−0.48			
Number of cryptic species	**−0.87**	C > I		✓	**−0.56**	C > I		✓
Water column to cryptic ratio (in number of species)	**−0.64**	C > I	x		−0.58			
Density of water column species	−0.01				**−0.56**	C > I	x	
Number of water column species	**−0.55**	C > I	x	✓	**−0.54**	C > I	x	
Density of rock hole (>20 cm) specialists	**−0.61**	C > I	x		−0.45			
Density of rock hole (<10 cm) specialists	**−0.70**	C > I	x		**−0.61**	C > I	x	
Resilience								
Density of individuals with “low” and “very low” resilience	**−0.52**	C > I	x	✓	−0.48			
Density of individuals with “medium” resilience	**−0.67**	C > I	x		**−0.79**	C > I	x	
Density of individuals with “high” resilience	0.07				**−0.59**	C > I	x	
Thermal influence								
Density of species with Critical Thermal Maximum above 30 °C	0.31				–	–	–	
Number of species with Critical Thermal Maximum above 30 °C	0.38				–	–	–	

between two highly-correlated metrics was made according to their biological significance and higher correlation with the CAP axis. These analyses were conducted using PRIMER 6.0 + Permanova software (Anderson et al., 2008), with exception of the Mann–Whitney U-tests that were performed using STATISTICA 8.0 (Statsoft, 2007).

2.6. Metric and index scoring

There is no historical data available on the biological reef-fish communities of Ilha Grande Bay to a priori define less-disturbed sites that can be used as a reference condition in the scoring procedure. Considering that we sampled both disturbed and less-disturbed sites, the reference limits were calculated based on the fish metrics selected in this study. The highest value of each metric by type of impact was divided into quintiles, each interval corresponding to the range of the respective score (Table 2). We then attributed one score to each metric and calculated the observed scores as the sum of scores for all metrics selected by type of impact. The observed score was divided by the maximum attainable score for each impact to calculate the relative score. Finally, the relative score was used to assign the ecological condition of each site following the index application proposed by Coates et al. (2007) (Table 3). To ascertain the efficacy of the multimetric index, Mann–Whitney *U* test was used to compare the ecological condition (relative score) between control and impacted sites.

3. Results

3.1. Fish assemblage and metric selection

A total of 84 fish species were identified represented by the typical families of reef systems (such as, Pomacentridae, Sparidae and Haemulidae) and also estuarine families (Gerreidae and Mugilidae) (see Supplementary material; Table Suppl. 3). The sites affected by thermal influence had large numbers of Gerreidae (78.3% of the total number of fishes) and Haemulidae (9.6%), with the highest densities for *Eucinostomus argenteus* and *Haemulon steindachneri*. At the control sites, Pomacentridae (46.7%), Haemulidae (30.2%) and Sparidae (10.4%) were the dominant families, and *Abudefduf saxatilis*, *Haemulon aurolineatum*, *H. steindachneri* and *Diplodus argenteus* had the highest densities. Both, the sites impacted by urban influence and the control sites had large numbers of Pomacentridae (41.1%, impact; 52% control), Haemulidae (32.2%; 19%) and Sparidae (9.5%; 6.9%) with high densities

Table 5
Scores obtained with each metric of the multimetric index for the thermal influence.

Location	Sites[a]	Selected metrics								Observed score	Relative score	Ecological condition
		Total number of species	Number of cryptic species	Number of water column species	Number of transient species	Density of individuals with "low" and "very low" resilience	Density of omnivores	Density of herbivores	Density of carnivores			
Impact 1	1	2	2	3	2	1	1	1	2	14	0.35	Bad
	2	3	2	4	2	2	1	1	2	17	0.43	Poor
	1	3	3	4	3	2	1	1	2	22	0.48	Poor
	2	2	1	3	2	1	1	1	2	13	0.33	Bad
Impact 2	1	2	1	3	2	2	1	1	1	13	0.33	Bad
	2	3	3	3	2	1	1	1	1	15	0.38	Poor
	1	3	3	3	2	3	1	1	1	17	0.43	Poor
	2	3	2	3	2	1	1	1	1	14	0.35	Bad
Control 1	1	5	5	4	5	3	2	1	3	28	0.7	Acceptable
	2	4	3	5	4	3	3	1	2	25	0.63	Moderate
	1	4	3	5	4	4	3	1	5	29	0.73	Acceptable
	2	5	4	5	5	4	2	1	4	30	0.75	Acceptable
Control 2	1	5	5	5	4	3	5	4	4	35	0.88	Good
	2	5	4	5	5	4	5	5	4	37	0.93	Good
	1	5	4	5	5	4	4	5	4	36	0.90	Good
	2	5	5	4	5	5	5	5	3	37	0.93	Good

[a] Sites re-visited.

of *A. saxatilis* and *Haemulon aurolineatum* for the control sites, and *A. saxatilis* and *H. steindachneri* for the impacted sites (Supplementary information 1).

PERMANOVA results showed that there were significant differences in fish metrics between impacted and control sites for both thermal and urban influences: thermal (pseudo-$F = 19.32$, $p < 0.01$) and urban influence (pseudo-$F = 24.66$, $p < 0.01$). The covariates related to the spatial structure of sampling sites were significant ($p < 0{,}01$), however explained a smaller amount of the components of variance (X axis, 4% and Y axis, 5.5%) compared to the factor site (29.5%). The PCO explained 64.9% of the total variation in the fish metrics for thermal influence. The first PCO axis explained 45.4% of the total variation whereas the second PCO axis explained 19.5%. A total of 59.3% of the variation in the fish metrics for urban influence was explained by the first two PCO axes. The first PCO axis explained 38.3% of the total variation, whereas the second PCO axis explained 21%. The first axis separates satisfactorily the impacted from control sites for both impacts (Fig. 2). No significant differences in multivariate dispersions were found by PERMDISP routine within each group (impact and control) for both impacts ($p > |0.05|$).

Twenty-four and 21 metrics had high correlation with the first axis according to CAP analysis for the thermal and urban influences, respectively ($r > 0.5$). Differences between the impact and control sites (Mann–Whitney test) were found for all metrics with higher values for the control sites compared with the impacted sites (Table 4). The only exception was found for the metric "density of individuals with heavy fishing pressure" that did not differ between the control and thermal impacted sites.

The metrics selected after verifying redundancy using Spearman rank correlation ($r > 0.85$) for the thermal influence were: (1) total number of species (diversity/composition); (2) number of cryptic species; and (3) number of water column species (habitat association); (4) number of transient species (mobility/behavior); (5) density of individuals with "low" and "very low" resilience (resilience); (6) density of

Table 6
Scores obtained with each metric of the multimetric index for the urban influence.

Location	Sites	Selected metrics						Observed score	Relative score	Ecological condition
		Total density	Number of rare species/total number of species	Density of individuals with heavy fishing pressure	Number of cryptic species	Number of residentspecies	Density of herbivores			
Impact	1	1	3	1	4	2	1	12	0.40	Poor
	2	1	2	1	3	3	1	11	0.37	Poor
	3	1	4	1	3	2	2	13	0.43	Poor
	4	1	2	1	4	3	1	12	0.40	Poor
	5	1	4	1	2	3	1	12	0.40	Poor
	6	2	4	2	4	3	2	17	0.57	Moderate
	7	1	4	1	3	4	3	16	0.53	Moderate
	8	2	3	1	3	3	1	13	0.43	Poor
	9	2	4	2	1	2	1	12	0.40	Poor
	10	2	4	2	4	3	1	16	0.53	Moderate
	11	1	3	1	3	3	1	12	0.40	Poor
	12	1	4	1	4	2	1	13	0.43	Poor
Control	1	2	4	1	4	3	2	16	0.53	Moderate
	2	5	4	1	5	5	3	23	0.77	Acceptable
	3	3	3	2	5	5	2	20	0.67	Moderate
	4	3	5	1	5	5	4	23	0.77	Acceptable
	5	3	5	1	5	5	3	22	0.73	Acceptable
	6	5	4	1	5	5	3	23	0.77	Acceptable
	7	2	4	5	3	3	3	20	0.67	Moderate
	8	1	4	2	4	5	4	20	0.67	Moderate
	9	4	4	5	5	4	4	26	0.87	Good
	10	2	5	1	5	5	4	22	0.73	Acceptable
	11	2	5	2	5	5	5	24	0.80	Acceptable
	12	4	5	3	5	5	4	26	0.87	Good

omnivores, (7) density of carnivores; and (8) density of herbivores (trophic structure). The selected metrics for the urban impact were: (1) total density and (2) ratio between the number of rare or uncommon species/total number of species (diversity/composition); (3) density of individuals with heavy fishing pressure (fishing pressure); (4) number of cryptic species (habitat association); (5) number of resident species (mobility/behavior); and (6) density of herbivores (trophic structure) (Table 4). Overall, the set of metrics selected to assess the thermal influence differed from those selected for the urban influence, except the number of cryptic species and the density of herbivores metrics that were selected for both types of impacts.

3.2. Scores and ecological condition

Significant differences ($p < 0.01$) were found between the relative scores (control and impacted sites) for both impacts according to the Mann-Whitney test (Table 4). Overall, the ecological condition at the impacted sites by thermal influence were classified as "bad" (50% of sites) and "poor" (50%), whereas the control sites were classified as "acceptable" (37%) and "good" (50%) (Table 5). Differences in the ecological conditions between the control sites and the sites affected by thermal influence indicate a severe influence in the area. The ecological condition "bad" was indicated mainly by the low scores in metrics in the category trophic structure as consequence of low species abundance and imbalanced trophic structure observed for these groups. The control sites that had ecological conditions "acceptable" and "good" had high punctuation mainly for metrics total number of species, number of water column species and number of transient species (Table 5).

The impacted sites by urban influence were classified mainly as "poor" whereas the control sites were mainly classified as "acceptable" (Table 6). The main metric associated to this condition were "total density", "density of individuals with high fishing pressure" and "density of herbivores". For the control sites, the metric "density of individuals with high fishing pressure" had the lowest punctuation. For the impacted sites, 75% of the ecological condition was classified as "poor" and 25% as "moderate" indication the high level of disturbance in the area. The control sites had 75% proportion for the ecological condition as "acceptable" and 25% as "good".

4. Discussion

The fish-based metrics incorporated into the multimetric index succeed in evaluating the ecological condition and discriminating sites impacted by thermal and urban influences in the Ilha Grande Bay. The final index scores attributed a better ecological condition for control than impacted sites. To our knowledge, this is the first study to propose developing and applying a multimetric index based on tropical rocky reef fish assemblages for assessing ecological condition for a coastal area affected by these disturbances. This is the first step to supply information to establish a bioassessment programs in rocky reef coastal areas of South America. We lack reliable information about physical and chemical thresholds that precisely indicate disturbance. Instead, a single index summarizing thermal and urban influences is a quick and practical way to describe the pressure on individual sites and to make objective comparisons with others sites.

4.1. Effects of thermal influence

We detected that the thermal discharges from the Brazilian cooling water nuclear power plants alter fish composition and habitat structure with significant differences observed between impacted and control sites as indicated by the eight selected metrics. In two previous studies in this area (Teixeira et al., 2009, 2012), we found that thermal discharge decreases benthic cover, and consequently, fish richness and diversity. However, we also found that the effects of thermal discharge are minimized on fish communities in sites with complex physical structure and abundant benthic cover. Some selected metrics responded objectively in separating control and impacted sites. For example, the total number of species showed higher values for the control sites compared with the impacted sites. Impacted sites had poor benthic cover and consequently, low fish richness, where opportunist and tolerant species dominated (Teixeira et al., 2012). Changes in water temperature alter fish assemblages by decreasing species richness (Rong-Quen et al., 2001). Furthermore, rocky shores have a variety of microhabitats, which increase fish diversity (Gratwicke and Speight, 2005; Kovalenko et al., 2012). Thermally polluted rocky substrate are unable to support sessile invertebrates or microalgae (Ras et al., 2013) and will have a negative impact on fish using the habitat for shelter, food, nesting and juvenile settlement.

The metric number of water column species had high scores at the control sites, an indication of the resources availability effect on habitat choice by mobile species. The thermal impact strongly influenced the benthic cover, limiting food and shelter resources as well as increasing water column temperature (Teixeira et al., 2012). Floeter et al. (2004) stated that high mobility fishes, usually swimming in large schools, are less dependent on the reef substratum for protection, and seem to respond more readily to environmental fluctuations, moving among reefs and vertically in the water column in response to the availability of resources or to satisfy their environmental preferences, such as optimal temperature. The omnivorous schooling sparids (*Diplodus argenteus*) and the planktivorous (*Chromis multilineata*) are typical water column species exclusively found in the control sites of this study, an indication of the predictive potential of the metric number of water column species.

Most metrics of the trophic structure category were selected for assessing thermal influence (density of omnivores, density of carnivore and density of herbivores) which are associated to the response of the high temperatures that trigger fish metabolism processes among them and changes the trophic structure via increasing/decreasing feeding rates (Munday et al., 2009). Aerobic capacity of reef fishes was found to decline at elevated water temperatures (31, 32 or 33 °C) compared with controls (29 °C) (Nilsson et al., 2009). The increasing in the oxygen demand at higher temperatures causes a reduction in the capacity for aerobic functions, allowing less energy to be devoted to feeding, growth and reproduction (Pörtner and Knust, 2007). Major stressors driving the degradation of reefs have included altered trophic structures (Jackson et al., 2001; Pandolfi et al., 2003). Omnivores feed on a variety of organisms, including animal and plant material. These fishes are extremely plastic in their diet and can shift between food sources as a result of seasonal environmental disturbance (Ferreira et al., 2004).

We found an effect of the thermal influence on the number of transient species that had higher values at the control sites. Transient species are primarily pelagic feeders (e.g. jacks, Carangidae) typical of coastal areas and loosely associated with reefs (Yeager et al., 2011). They regularly move into reefs and other coastal systems (e.g. estuaries, sand beaches), performing incursions searching for food. It thus indicates that a better habitat quality (in terms of availability of food) are found in the control sites that in some extent are also important for non-typical reef species.

Degraded systems have also a few species with low or very low resilience than in less affected areas (Henriques et al., 2008a). This metric indicates that control areas despite not being pristine, had better environmental quality because of shelters species of low resilience. On the other hand, species with high resilience, such as *Sphoeroides greeleyi* and *Eucinostomus argenteus* were more abundant in the impacted sites.

Besides the indirect effect of increasing water temperature on fish communities through the degradation of reef habitats, increasing temperature also have a direct effect on fish communities. Populations of thermally tolerant species are likely to persist at higher temperatures, whereas thermally sensitive species could decline, indicating that the community structure of reef fish assemblages might change significantly as temperatures increase (Nilsson et al., 2009). Four of the eight metrics selected for the thermal influence, four were related to the number of species (total number of species, number of cryptic species, number of water column species and number of transient species), which suggest that these metrics reflect the influence of the increasing temperature on species-specific composition.

4.2. Effects of urban influence

Urban influence from tourism, fishing and urban development in coastal areas alter fish composition and habitat structure with significant differences observed between impact and control sites as detected by the six selected metrics. The total density has been considered a good indicator of community integrity, because the increasing stress in the environment generally produces a decline in abundance (Keough and Quinn, 1991; Harrison and Whitfield, 2004; Coates et al., 2007). High abundance at the control sites can be attributed to species represented mainly by typical fish families of rocky reefs, such as the members of the families Pomacentridae, Haemulidae and Sparidae (e.g., *Abudefduf saxatilis*, *Haemulon steindachneri* and *Diplodus argenteus*). The importance of fish abundance discriminating sites according to sewage influence was highlighted by Smith et al. (1999), who observed significant effects of sewage outfalls on the decline of the abundance of several common resident species of reef fish. However, some studies have demonstrated a higher density of planktivorous fishes and particulate organic matter (POM) feeders at the locations affected by the sewage than at the control sites (Grigg, 1994; Guidetti et al., 2002). They indicated that the nutrient enrichment caused by the outfall enhances the food availability for POM feeders and also the zooplankton abundance, which is the main food source for planktivores fish.

Metrics associated to species occurrence such as the Number of rare species/number of total species are difficult to be analyzed because they depend on the sampling effort. However, metrics associated to occurrence of rare species are important because a great number of species in rocky coastal reefs are rare. The presence of rare or threatened species and their occurrence imparts additional conservation value to the ecosystem (Harrison and Whitfield, 2004).

Density of individuals with heavy fishing pressure is a metric closely associated to impact caused by commercial and sport fisheries on the community structure. Artisanal fishery in Brazil is an important economic activity that corresponds in average to 50% of the total fisheries production of the whole country and in some regions corresponds to 70% (Begossi et al., 2011). In the study area, different types of fisheries occur irrespective of some legal restrictions, such as artisanal, recreational, spearfishing, trawlers and industrial fisheries (Begossi et al., 2011). In rocky coastal reefs, recreational fishing using line and hook and submarine hunting are the most common fishing types because of the structural complexity of the habitat. Spearfishing is selective and therefore impacts only some species and families. Meyer (2007), studying the impacts of spearfishing and line and hook in Hawaii detected the fish biomass and mean size caught by harpoon had significant higher size compared with those caught by line and hook. The main targets of spear fishing are members of the Serranidae and Scaridae families because of their large size, good quality as food and their great commercial importance. Serranidae is a large and important fish group, considering their diversity, biomass and role as top predators (Randall, 1998; Gibran, 2007). The species *Epinephelus marginatus* and *Mycteroperca acutirostris* are under heavy fishing pressure in our study area. They have considerable importance for spear and commercial fisheries and are highly vulnerable to overexploitation. Such species are very appreciated as food, and are well represented in southeastern Brazil mainly by small-sized individuals (up to 400 mm TL), which is, probably as a consequence of overexploitation (Gibran, 2007). Fishing typically targets large-bodied individuals, resulting in declines in target species size, density, and biomass (Russ, 2002). Targeting of predatory species may further result in trophic cascades, whereby abundance of prey species increases, reducing the nature and quality of primary production (Mumby, 2006).

Reef systems are among the most sensitive marine habitats to anthropogenic disturbances, since their communities are comprised by many site-attached species intimately linked with benthic habitat structure, with relatively small home ranges (Claudet et al., 2006). In this way, local impacts can have harmful effects on reef fishes concerns are raised on this subject. The metric number of resident species included in the urban influence assessment can be considered a good measure for assessing community health. This metric is considered particularly affected by local perturbations (Henriques et al., 2013) and can be included as an efficient measure of community health in rocky shores. Resident species are those that live on rocky reef for feeding, shelter, and mainly for spawning and parental care, mostly in nests (Belmaker, 2009). Habitat losses caused by pollutants or the dominance of invaders species can jeopardize relationship between species and the rocky reef during the life cycle of these species.

Metrics such as the number of cryptic species was important in distinguishing control and impacted sites of both thermal and urban influences. Cryptic fishes comprise small-sized species, which are visually and behaviorally cryptic and live in close association with the substratum (e.g. Blenniidae, Labrisomidae and Gobiidae) (La Mesa et al., 2006; Dalben and Floeter, 2012). The low scores of this metric at the impacted sites are probably associated with poor benthic cover, characterized by low macroalgae cover and few mobile and sessile invertebrates. Furthermore, due to their small home ranges (0.25–2 m^2) and benthic behavior, small cryptic fish assemblages are expected to show even stronger relationships with habitat characteristics (Depczynski and Bellwood, 2004). The low scores of this metric at the impacted sites may indicate small-scale changes in the benthic cover, probably related to the availability of shelter, which is considered especially important for small reef fishes to mitigate normally high rates of predation (Almany and Webster, 2006). Dalben and Floeter (2012) reported that cryptic fishes were positively correlated with microhabitat complexity (e.g., number of holes), which is an indication of the suitability of this metric to assess changes physical structure and benthic cover.

The importance of the density of herbivores fishes to assess thermal and urban influence must be related to different causes. Algal-availability seems may be the main reason for decreasing herbivore density in the thermal-influenced sites, where the benthic cover is dominated by vermetid molluscs of the *Petaloconchus* genus and calcareous algae of the *Jania* genus (Teixeira et al., 2012), whereas algal turfs (epilithic algal matrix) are the main feeding substratum for herbivorous fish species (Bonaldo and Bellwood, 2008; Francini-Filho et al., 2010). However, in the sites impacted by anthropogenic influences, low density of herbivorous fishes is likely to be influenced by multiple factors. Fishing pressure has lead strong population declines of herbivorous parrotfishes, mainly because of spearfishing (Ferreira and Gonçalves, 1999; Ferreira et al., 2004). In our study, herbivores species (e.g., Scaridae) are target by spearfisher mainly in the urban-impacted sites, indicated by the low abundance and small-sized individuals. Besides overfishing, eutrophication caused by urban and tourism developments has been demonstrated to affect herbivorous density by the increase in macro-algae density (Chabanet et al., 1995; Williams and Polunin, 2001). High abundances of fleshy macroalgae are being negatively correlated with herbivorous density (Williams and Polunin, 2001). However, land-based pollution may not affect in the same way all herbivorous fish. Some more tolerant species were found to increase, whereas others, usually considered more sensitive, declined (Chabanet et al., 1995). Guidetti et al. (2002) reported that some fish categories responded to sewage by avoiding polluted waters or reducing their abundance (e.g. sparids of the genus *Diplodus* and labrids).

The multimetric index developed in this study succeeds in discriminating sites under thermal and urban influences, but this is only a first step to develop a robust biotic indicator to assess disturbance in tropical rocky reefs. There are many sources of pollution in rock reef areas that increase with activities associated to expansion of population and urbanization. Deforestation, sewage discharge, shipyard and oil terminal are some activities that contribute to such impacts. Moreover, industrial fisheries, including destructive bottom trawling and recreational fisheries targeting rocky reef fishes (spearfishing and hook-and-line) also contribute to changes in rocky-reef communities. In this sense, establishing an index to assess the effects of such anthropogenic influences is a main goal to formulate policies of conservation in the area. Further studies are necessary to assess different types of disturbance in tropical coastal rocky reefs based on fish assemblages to get a more holist feature on the effects of anthropogenic activities on these areas. Such studies are necessary to the understanding of the degree of alteration of rock reef coastal areas. In this context, this index is a useful tool to assess different types of impacts that alter structural/functional characteristics of the fish community and have potential as a preliminary multimetric index to be used in tropical rocky reefcoastal areas.

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