

Research Article

Rapid Assessment Survey of introduced ascidians in a region with many marinas in the southwest Atlantic Ocean, Brazil

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Abstract

In August 2014, we performed Rapid Assessment Surveys (RAS) in seven marinas along circa 70 km of the coastline of Ilha Grande Bay (IGB), southeastern Brazil to evaluate diversity in the Ascidiacea and of introduced species. This region is very important for marine biodiversity and includes many protected areas. However, urbanization, tourism and economic development (aquaculture, fisheries, commercial ports and nuclear energy generation) are some of the impacts and drivers of species introduction. Our survey found 16 species, only one native, two introduced and eleven cryptogenic. Two species could not be identified precisely. The most species-rich marina had 11 species and the most polluted three marinas had only six species each. Comparisons with literature indicated that marinas in the IGB had fewer species and fewer introductions than marinas in the São Sebastião Channel (São Paulo), in the same biogeographic region. Future monitoring should consider complementary methods such as passive collectors deployed during summer time and RAS in at least two different times of the year.

Key words: marine introductions, fouling, RAS, tunicates, exotic species, biofouling, Ilha Grande

Introduction

The Brazilian coast is approximately 8,000 km long, between the tropical and subtropical biogeographical realms, and includes nine different ecoregions (Spalding et al. 2007). As in many other countries with a long coastline, marine commerce is a critical component of the nation's economy. Circa 97% of exports and 89% of imports are by ocean shipping. Brazil's main trading partners are countries from the Indian and Pacific Oceans, with China being the single most important partner, representing ~57% of all foreign trade. Other regions are also important, with western Europe contributing 17% of trade, Mediterranean countries 8%, and Middle Eastern countries 5% (ANTAQ 2014). Most maritime traffic to and

from Brazil passes through biogeographical realms with environmental conditions similar to those of Brazil. Thus, marine shipping is likely to be an important mechanism of species introduction to Brazilian marine habitats.

There are 33 international ports and many more private commercial terminals along the Brazilian coast. For example, at Ilha Grande Bay, Rio de Janeiro, there are one port and one oil terminal. Throughout Brazil, concern has grown about marine species introduction given the damage that invasive species can cause (Carver et al. 2003; Blum et al. 2007; Wong and Vercaermer 2012; Cordell et al. 2013). Recent invaders to Brazilian waters include the sun coral *Tubastraea* spp. (Lages et al. 2011; Silva et al. 2014), the bivalves *Myoforceps aristatus* (Breves-Ramos et al. 2010; Ignacio et al. 2012) and *Isognomon bicolor*

(López et al. 2010) and the ophiuroid *Ophiotela mirabilis* (Hendler et al. 2012; Mantelatto et al. 2015).

In addition to maritime shipping (Currie et al. 1998; Gollasch 2002; Chapman et al. 2013), recreational boating is also likely to be a vector for marine invasions. Ilha Grande Bay has the largest concentration of recreational boats and marinas in South America as well as coastal Brazil. The fleet in Rio de Janeiro and São Paulo together is estimated to have approximately 44,000 boats and Rio has approximately 28% of circa 350 marinas in Brazil (Amaral 2015). This region is included in the latitudinal range from 20 to 25°S and it is the most species-rich for ascidians in the southwestern Atlantic, with circa 61 recorded species (Moreno et al. 2014).

Given the heavy foreign ship traffic to Ilha Grande Bay, nonnative species may easily arrive. Because of the region's high biodiversity and endemic species (Creed et al. 2007), these introductions may have an important impact on native communities (e.g., Carlton 2002). Also, species that are introduced to Ilha Grande Bay may rapidly spread to nearby regions via smaller recreational and commercial watercraft (Davidson et al. 2010; Canning-Clode et al. 2013).

Several methods are currently used to detect newly arrived nonnative species, including passive collectors (such as artificial plates), visual inspections of artificial structures in marinas (by taking them out of the water or scuba), visual inspection for target species during the docking of ships and direct collection of samples (Campbell et al. 2007; Bishop and Hutchings 2011). Artificial substrates or sampling of available and accessible structures from piers is the most often employed assessment techniques. Rapid Assessment Surveys (RAS) or Rapid Assessment Protocols (RAPs) have been developed and extensively used to monitor sites and quickly detect introduced species (Arenas et al. 2006; Campbell et al. 2007).

This study reports on surveys of ascidians in several Ilha Grande Bay marinas using Rapid Assessment Surveys (RAS) techniques. We also evaluate the efficiency of this method to detect introductions compared to other methods that have been used in the region and estimate the level of similarity of the biota found in these marinas as compared to other regions.

Study site

Ilha Grande Bay (IGB) is located in southeastern Brazil, in the state of Rio de Janeiro, with circa

1,000 km² area. The water in this region is mainly oligotrophic, except close to large towns such as Angra dos Reis (~170,000 inhabitants) and Paraty (~40,000 inhabitants), where sewage treatment is insufficient, leading to an increase in organic contamination and eutrophication of coastal waters (Mayer-Pinto and Junqueira 2003). The Bay has warm (from 20 to 28°C), calm waters and hosts an important tourist industry. The opening of BR101 highway in 1970 was followed by the construction of many marinas, and boating and tourism replaced the traditional fishery industry. Many shellfish farms provide suitable habitat and protection for introduced species. Other human activities in IGB waters include one shipyard, one commercial port and two nuclear power plants.

This study included seven marinas along ~70 km from Mangaratiba in the east up to Paraty in the west (Table 1, Figure 1). Total number of berths was used as a measure of marina size and this information was available from the websites of each marina (Table 1). We also obtained information about water quality, measured by fecal coliform bacteria counts by the Instituto Estadual do Ambiente during the years 2014 and 2015 (INEA 2015), based on Brazilian standards (CONAMA 1986). Water temperature and salinity data were acquired from the literature (Creed et al. 2007; Ignacio et al. 2010, 2012) or non-published data from our lab (Table 1).

Sampling

Surveys were performed in August 2014 to develop baseline data of ascidian diversity in the IGB, and to find nonnative species. Three people sampled each marina for 30 minutes. Thus, we surveyed proportionally a higher area in smaller marinas than on the large ones within this same time frame. During each assessment, representative samples from the fouling community were scraped from the walls of floating docks, immersed ropes and other structures reachable from the pier. After collecting, large clumps of ascidians, mainly *Styela plicata*, were examined and separated to find other ascidians within the clumps.

Ascidians were narcotized in a menthol solution and fixed in 4% formaldehyde with a few preserved in ethanol 95% for future molecular studies. Diving inside marinas is forbidden due to safety restrictions and bad water quality, and so this sampling method was the only method possible. Samples were deposited in the scientific collections of Rio de Janeiro State University (UERJ) and the Federal University of Paraná (UFPR).

Table 1. Names, geographical coordinates, size and environmental characteristics of each site surveyed in Ilha Grande Bay.

Site	Geographical coordinates		Number of docking places for boats	Proportion of samples over Brazilian standards of fecal coliform counting	Sea surface temperature (°C)		Sea surface salinity	
	Latitude, S	Longitude, W			Min	Max	Min	Max
Porto Real	23°02'03.53"	44°09'24.67"	60	0.7	24.3	26.2	32	34.5
Portogalo	23°02'25.26"	44°12'13.29"	57	0.0	25.1	26.9	30	34.5
Iate Clube Aquidabã	23°00'17.66"	44°18'19.72"	149	1.0	24.4	28	28.2	35.4
Cais Santa Luzia	23°00'33.94"	44°18'59.72"	250	1.0	24.4	28	28.2	35.4
Bracuhy	22°56'59.77"	44°23'43.19"	800	0.4	21.6	29.9	22.9	34.6
Tarituba Pier	23°02'44.80"	44°35'43.97"	39	0.1	24.9	27.2	32.5	35
Porto Imperial	23°13'54.21"	44°42'51.41"	150	0.9	21.5	34	35	36.5

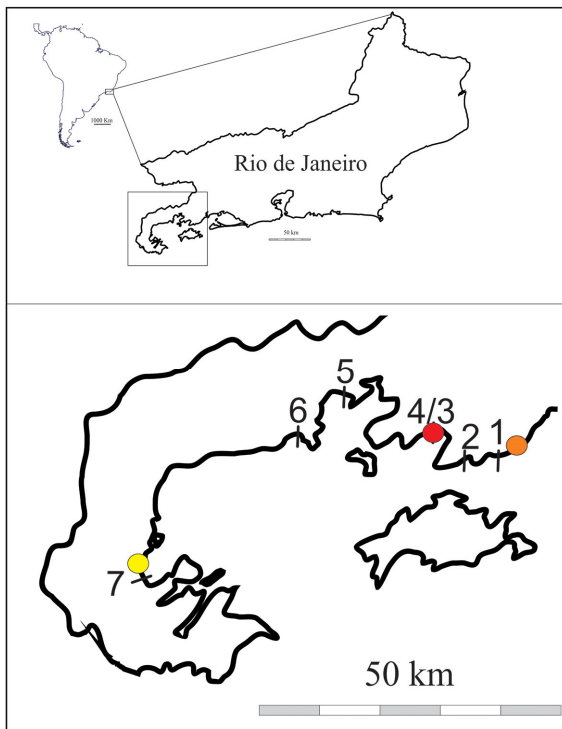


Figure 1. Map of studied area indicating its location related to South America and Rio de Janeiro coast. The marinas surveyed were, from east to west: Porto Real (1) and Portogalo (2), at Mangaratiba municipality (orange dot); Aquidabã Yacht Club (3), Santa Luzia public marina (4) and Marina Bracuhy (the largest marina in South America - 5) in Angra dos Reis (red dot); Tarituba public pier (6) and Porto Imperial Marina (7) in Paraty (yellow dot).

Data analysis

Species presences were analyzed by multivariate methods including the calculation of similarity between marinas using the Jaccard index for the

presence/absence matrix using Primer 6.0 (Clarke and Gorley 2006). Results are illustrated by non-metric multidimensional scaling with our data and with our data combined with those obtained by Marques et al. (2013) in the São Sebastião region. These two data sources are the only existing ascidian biodiversity data for southeastern Brazil using similar RAS methodology. Environmental data and biota matrices were examined using Principal Components Analysis (PCA).

Results

We found 14 ascidians that could be identified to species and two others that could be identified to genus (one *Styela* sp. and one *Ascidia* sp.) (Table 2). Three species (*Styela plicata* (Lesueur, 1823), *Styela canopus* (Savigny, 1816) and *Polyclinum constellatum* (Savigny, 1816)) were found in all marinas sampled. Bracuhy (with 12 species) and Portogalo (11 species) were the two marinas with the greatest ascidian diversity. Aquidabã, Santa Luzia and Porto Imperial (six species each) had the lowest species diversity.

Of the 14 species, one (*Trididemnum orbiculatum* (Van Name, 1902)) is considered native to the region and was found in two marinas: Portogalo and Porto Real. Two species were classified as introduced; *Clavelina oblonga* was found in only one marina, while *Styela plicata* was found in all marinas. The remaining 11 species were classified as cryptogenic (Table 2), six of which (*Polyclinum constellatum*, *Diplosoma listerianum*, *Styela canopus*, *Symplegma brakenhielmi*, *Herdmania pallida* and *Microcosmus exasperatus*) are globally widespread and have a long history of introductions throughout the world.

Table 2. Ascidiacea species recorded on seven marinas in Ilha Grande Bay during Rapid Assessment Surveys (RAS) in August 2014, including species status as N = native, I = introduced or C = cryptogenic.

	PR ¹	PG	AQ	SL	BR	TA	PI	Status
Number of species	10	11	6	6	12	7	6	
<i>Ascidia</i> sp.				X	X		X	--
<i>Ascidia curvata</i> (Traustedt, 1882)			X	X	X		X	C
<i>Botrylloides nigrum</i> Herdman, 1886	X	X	X	X	X			C
<i>Clavelina oblonga</i> (Herdman, 1880)	X							I
<i>Didemnum granulatum</i> (Tokioka, 1954)	X							C
<i>Didemnum perlucidum</i> Monniot, 1983					X	X		C
<i>Diplosoma listerianum</i> (Milne-Edwards, 1841)		X	X		X	X	X	C
<i>Herdmania pallida</i> (Heller, 1878)		X			X			C
<i>Microcosmus exasperatus</i> Heller, 1878	X	X			X			C
<i>Phallusia nigra</i> Savigny, 1816	X	X			X	X		C
<i>Polyclinum constellatum</i> (Savigny, 1816)	X	X	X	X	X	X	X	C
<i>Styela canopus</i> (Savigny, 1816)	X	X	X	X	X	X	X	C
<i>Styela plicata</i> (Lesueur, 1823)	X	X	X	X	X	X	X	I
<i>Styela</i> sp.		X				X		--
<i>Symplegma brakenhielmi</i> (Michaelsen, 1904)	X	X			X			C
<i>Trididemnum orbiculatum</i> (Van Name, 1902)	X	X						

¹ Sites: PR: Porto Real, PG: Portogalo, AQ: Aquidabã Yacht Club, SL: Santa Luzia, BR: Bracuhy, TA: Tarituba, PI: Porto Imperial.

Figure 2. nMDS plot based on Jaccard presence-absence matrix of ascidian species in marinas surveyed at Ilha Grande Bay (current work) and São Sebastião Channel (Marques et al. 2013), on southeastern Brazil. The most similar marinas (>70% resemblance) are circled.

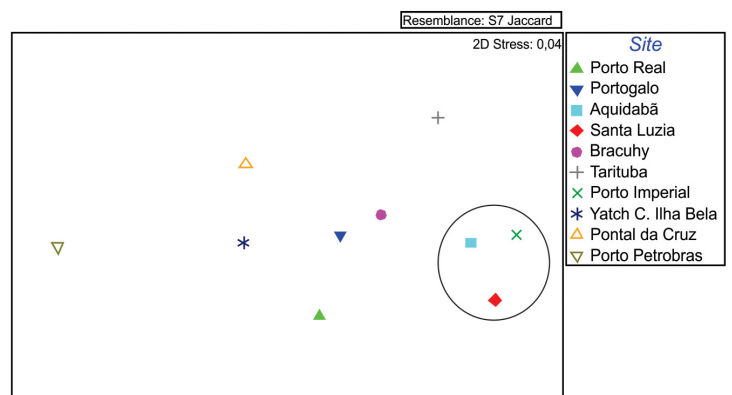
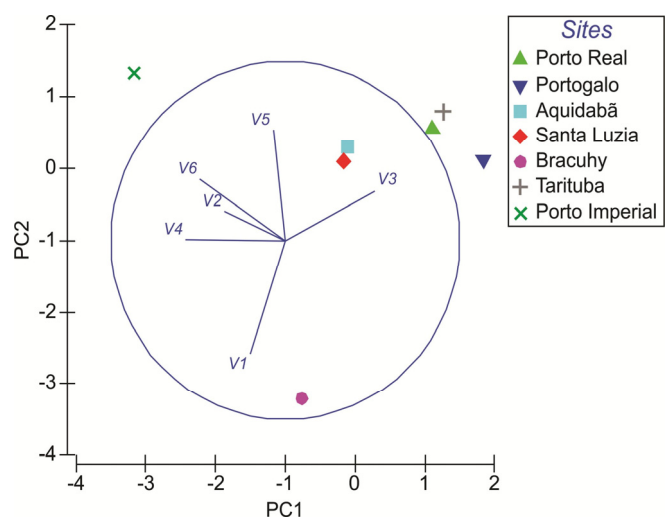


Figure 3. Principal components analysis (PCA) for the environmental variables: V1. number of mooring positions at marina; V2. water quality based on fecal coliforms analysis (number of analyses for which the coliform countings was higher than good quality standards divided by the total number of analyses), V3. minimum temperature, V4. maximum temperature, V5. minimum salinity, V6. maximum salinity.



The ordination plot obtained from Jaccard similarity among marinas revealed the formation of one group including the marinas of Santa Luzia, Aquidabã and Porto Imperial, closely associated with urban areas. These marinas have similar environmental conditions, and all are polluted (Figure 2, Table 2). Tarituba and Porto Real marinas shared four species: *Phalusia nigra*, *Polyclinum constellatum*, *Styela plicata* and *S. canopus*. The marinas Pontal da Cruz and Yatch Club Ilha Bela, at São Paulo (Marques et al. 2013) were more similar to the largest and non-polluted marinas from IGB.

Principal components analysis (PCA) revealed that three axes were responsible for 97% of the variation. Axis I (marina size) explained 47% of the variation, axis II (water quality as measured by fecal coliform bacteria) explained 36%, and axis III (minimum temperature) explained 14%. The biota of most marinas was influenced by minimum temperature and minimum salinity, while only that in Bracuhy was influenced by marina size (Figure 3).

Discussion

Many of the species found in this study are very invasive and found in harbors and marinas worldwide, suggesting that boat and vessel transportation may have led to the introduction of these species to these Brazilian sites. Our sampling surveys are the most extensive assessment of the ascidians found in Ilha Grande Bay. All species found in this study were reported from other marinas and harbor environments all along the Brazilian coast, but mainly in the southeast (Marins et al. 2010; Ignácio et al. 2010; Marques et al. 2013).

RAS protocols used here are frequently used in initial surveys or in monitoring programs, especially those that cover long distances and when results and decisions must be obtained quickly (Cohen et al. 2005; Pederson et al. 2005; Arenas et al. 2006). However, they generally restrict sampling to the first meter of the water column, or somewhat deeper (long ropes). Compared with other surveys, we found that all marinas had a similar total number of species: Cohen et al. (2005) found 5–17 species per site in California, Pederson et al. (2005) found 4–14 in New England and López-Legentil et al. (2014) found 5–16 species on the coast of Catalonia.

Comparison of our results with older studies in the same region (Marins et al. 2010; Ignacio et al. 2010) suggests that the survey method used

may influence species detections. Marins et al. (2010) used passive collectors (plates) deployed for three months during the summer at Aquidabã (named Piratas) and Santa Luzia (called Capitania). They found 16 and 15 total species, with two and three introductions, respectively. We found six species and one introduction in the same sites, while we conducted a single RAS assessment in the winter. The total distance covered by Marins et al. (2010) study (maximum 6 km) was smaller than the area surveyed in this study, but included a temporal component. Ignacio et al. (2010) surveyed Santa Luzia (called Anil) in 2004 with SCUBA divers and detected six species, two introduced and similar to our results of six species and one introduction. However, Ignacio et al. (2010) also surveyed Bracuhy marina, where they only found five species, none introduced, whereas we found 12, one introduced. In Ignacio et al. (2010) they scraped many 0.1 m² areas at different depths, but did not target specific clumps of ascidians. As an overall comparison of surveys, of the 26 known ascidians species for the IGB we found 16, Marins et al. (2010) found 21, and Ignacio et al. (2010) found 10.

While all species recorded by Ignacio et al. (2010) were found in our survey, there were some differences with the species list by Marins et al. (2010). We did not find *Didemnum vanderhorsti*, *Polyclinum aurantium*, *Ascidia sydneyensis* and *Symplegma rubra*, yet two species, *D. granulatum* and *T. orbiculatum*, were only recorded in our survey (Table 2, Supplementary Table S1). More work is needed to determine the ideal method for rapid species detection, but our results indicate complementarity of the detecting methods and that long term monitoring programs should probably include both passive collectors and RAS surveys. We also call attention to the importance of collecting clumps of dominant species during RAS surveys to look for other species hidden within. In our study, *S. plicata* formed large clumps, in which we also found *S. canopus* and *Ascidia curvata*.

Three introduced species known to occur in Rio de Janeiro have not yet been found in IGB. *Botrylloides giganteum* and *Ciona intestinalis* were recorded in Guanabara Bay (Aron and Sole-Cava 1991; Marins et al. 2009) and *Rhosodoma turcicum* was recently found in Arraial do Cabo (Skinner et al. 2013). While not found in our surveys, they may easily arrive in IGB in the near future.

In comparing the diversity of ascidians in marinas in IGB and in São Paulo (Marques et al. 2013), Ilhabela Yacht Club and Pontal da Cruz were found to be similar to IGB (nMDS, Figure

2B). Twelve species (3 introduced, 9 cryptogenic) were recorded in both São Paulo and IGB: *A. sydneyensis*, *S. plicata*, *C. oblonga*, *S. canopus*, *B. nigrum*, *D. perlucidum*, *D. listerianum*, *S. brackenhielmi*, *P. constellatum*, *P. nigra*, *H. pallida* and *M. exasperatus* (Table S1). These have a wide geographic distribution and tend to be common in marinas and harbors in tropical and sub-tropical regions (Monniot et al. 1985; Rocha and Kremer 2005; Pineda et al. 2011; Pérez-Portela et al. 2013).

Some marinas are located close to urban centers with strong organic sewage influence. Data from the Instituto Estadual do Ambiente (INEA) that monitor water quality in the region confirmed the effect of domestic discharges on coastal waters close to Porto Real, Aquidabã, Santa Luzia and Porto Imperial, while water quality is better in Tarituba, Bracuhy and Portogalo (INEA 2015).

The several cryptogenic species in surveys in Brazil reflect the lack of knowledge of local faunas and previously unrecorded historical introductions. This is in stark contrast to surveys in the Mediterranean (López-Legentil et al. 2014), North Europe (Arenas et al. 2006; Minchin and Nunn 2013; Nall et al. 2015) and the United States of America (Cohen et al. 2005; Pederson et al. 2005, Tracy and Reynolds 2014) in which most species can be clearly classified as introduced or native. Many of the cryptogenic Brazilian species probably represent old introductions because they are commonly invasives and occur in many biogeographical ecoregions (sensu Spalding et al. 2007), especially in altered and artificial environments (Lambert and Lambert 2003).

One of the important impacts of bioinvasion is the homogenization of the global biota (Olden and Poff 2003, 2004). Port and marina faunas have long been observed to be very similar across different geographic regions (Monniot et al. 1985; Ignacio et al. 2010). However, in this study, the marinas remain quite different from each other. Only one of the two introduced species was found in all locations, and the other was only found in one marina. Additionally, only five comparisons of 21 had greater than 60% similarity (Jaccard) and those were relatively close and in the same coastal embayment. The most similar marinas were those with less diversity and poorer environmental conditions. This suggests that an environmental filter might have a role in selecting the same pool of pollution-tolerant species. In our survey, *S. plicata*, *S. canopus* and *P. constellatum* occurred on both polluted and clean environments. In the IGB region, Marins et

al. (2010) reported *D. listerianum*, *H. pallida*, *B. nigrum* and *S. brackenhielmi* occurred in both situations. McKinney and Lockwood (1999) also emphasized the role of cosmopolitan, pollution-tolerant species replacing local endemics as a driver of homogenization.

In the studied marinas we found tolerant species in five or more marinas, suggesting that homogenization is likely to be ongoing. Since the IGB and its coastline are relatively recently developed (Souza 2003), it might be that many exotic and cryptogenic species have not yet had the opportunity to spread across the region. On the other hand, the native fauna in the region is poorly known and local extinction has not been reported. Thus, homogenization in the region has followed scenario II of Olden and Poff (2004) - introductions of the same species without local species extinctions.

Recreational boats and marinas are important in the intra-regional spread of invasive marine species (Ashton et al. 2006; Acosta et al. 2010; Bishop and Hutchings 2011). Since 1970, after the construction of the highway between Santos and Rio de Janeiro, coastal development of the IGB has increased along with the number of marinas (Quadros et al. 2009). Currently, there are ~25 marinas between Mangaratiba and Paraty, the greatest concentration of this kind of development along the Brazilian coast (Amaral 2015). The availability of floating structures and vessel activities has also been recently correlated with the number of fouling non-native species in Scotland (Nall et al. 2015). This indicates the high potential for species dispersal inside the IGB. Our results can serve as a baseline to allow future investigators to assess changes in the ascidian diversity of the IGB. Additional work should be undertaken to assess additional marinas and to conduct continuing assessments of the marinas sampled in this survey.

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The following supplementary material is available for this article:

Table S1. Comparison of species of Ascidiacea detected at Ilha Grande Bay (present study, Ignacio et al. 2010, Marins et al. 2010) and at São Sebastião Chanel (Marques et al. 2013).

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