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Research Article

Biodiversity of ascidians in a heterogeneous bay from southeastern Brazil

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Abstract

Ascidians are fast-growing sessile animals, frequently observed on artificial and natural substrata. Several introduced ascidians are known for monopolizing space in benthic communities, mainly in urbanized areas, where harbours act as gateways for exotic species. In the southeastern Brazil, the Arraial do Cabo Bay is the main point of coastal upwelling and one of the most visited localities during the summer due to beaches and dive sites. While the region of the botton of the bay is close to the city, being exposed to anthropogenic disturbances but warm waters, the area outside the bay is a pristine site, exposed to cold waters caused by the upwelling system. To understand how ascidian species are distributed through the temperature and anthropogenic gradients, we sampled ascidian specimens at 11 sites from three distinct regions: (1) inner bay, a harbor area exposed to intense ship and boat/platform traffic; (2) external bay, a pristine and warm area and (3) outside of the bay, a region exposed to coastal upwelling and cold water. We recorded 31 species of ascidians of which the Didemnidae and Styelidae families were the most speciose. *Symplegma rubra* and *Phallusia nigra* were the most common species. We found 22 species in the inner bay, of which nine were exclusive to this region (*Styela canopus, Clavelina oblonga, Polyclinum constellatum, Polyclinum molle, Didemnum speciosum, Botrylloides giganteum, Ascidia curvata, Ascidia sydneiensis* and *Rhodosoma turcicum*). In the external bay, we found 19 species, five exclusive to this region (*Didemnum vanderhorsti, Didemnum galacteum, Eusynstyela tincta, Eusynstyela* sp. and *Botryllus* sp.). Outside of the bay, three of the four species found (*Cystodytes dellechiajei, Didemnum granulatum* and *Didemnum rodriguesi*) were exclusive. Sixteen species were categorized as cryptogenic, seven as native and five as invasive to the Brazilian Coast. Four of the five invasive species were found only in the inner bay (*R. turcicum, S. canopus, A. sydneiensis* and *A. curvata*) close to man-made structures, while *Styela plicata*, an invasive ascidian with worldwide distribution, was also observed in the external bay. Seventeen of the 31 species were exclusive to one of the three regions, suggesting that anthropogenic impacts in the inner bay and the upwelling outside of the bay may be influencing the occurrence of different species, and increasing the local diversity of ascidians.

Keywords: Ascidiacea, coastal upwelling, anthropogenic impacts, invasive species

Introduction

Coastal waters contain an array of natural and man-made hard substrata that can serve as habitat for sessile fouling communities (Connell and Glasby 1999; Bulleri and Chapman 2010). While man-made structures can increase the amount of space available for fouling organisms, they are frequently associated with decreased water quality due to human activities, such as boat traffic and aqua-culture. As result, sessile communities from urbanized regions may have relatively low diversity, and can be composed mostly of exotic species, that have been introduced through ship hulls or ballast water (Piola and Johnston 2008).

Ascidians (Tunicata: Ascidiacea) are often dominant members of marine fouling communities from the subtidal zone in both natural and artificial substrata (Lambert and Lambert 2003; Lambert 2007; Shenkar and Loya 2009). The distribution

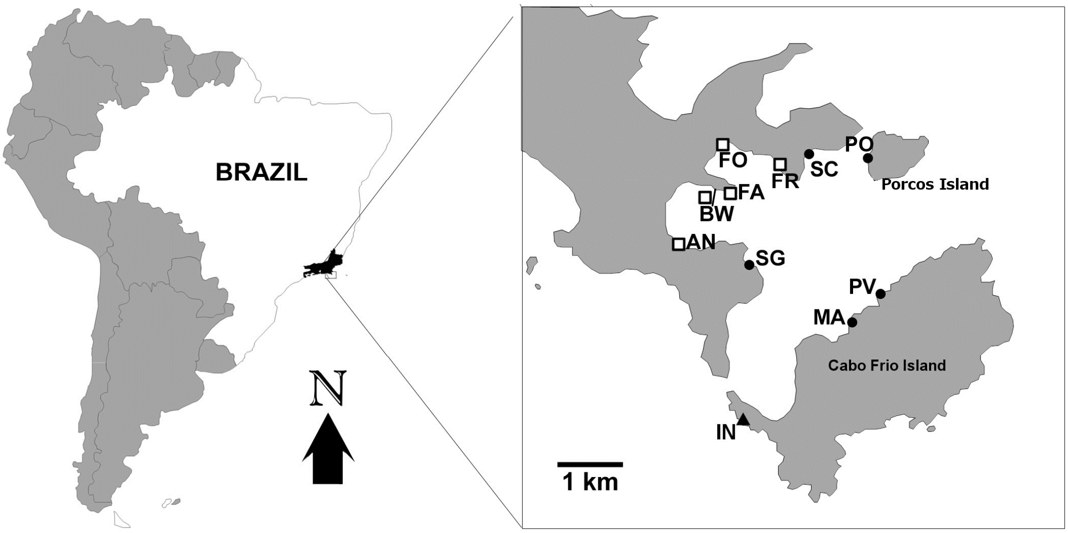

Figure 1. Map of Arraial do Cabo Bay, Brazil. White squares represent sites from inner bay: BW = Breakwater, FA = Fortaleza, FO = Forno, AN = Anjos, FR = Floating Restaurant. Black circles represent sites from the external bay: SG = Saco do Gato, SC = Saco dos Cardeiros, PO = Porcos Island, PV = Pedra Vermelha, MA = Maramutá. Black triangle represent the site outside the bay: IN = Saco dos Ingleses (for details see supplementary Table S1).

of ascidian species is affected by salinity (Lambert 2005; Gab Alla 2008), light intensity (Young and Chia 1985), hydrodynamic regimes (Shenkar et al. 2008) and temperature (Kowalke et al. 2001; Thiyagarajan and Qian 2003; Primo and Vásquez 2009). Thus, both natural and anthropogenic modifications of physical parameters on coastal waters can affect the occurrence, distribution and diversity of ascidians. While some ascidians are only found in natural pristine substrata, other species are recognized as nuisance species in several harbors of the world. Species such as *Styela plicata* and *Didemnum vexillum* (Lambert 2007) are able to survive in disturbed habitats and may dominate the sessile communities, reducing local diversity (Stachowicz et al. 1999). This is especially true for urbanized areas, where active harbours can serve as gateways for exotic species (Rocha and Kremer 2005; López-Legentil et al. 2014).

The city of Arraial do Cabo, located in tropical Southeastern Brazil, is in an area subject to coastal upwelling (Valentin 1994). This phenomenon brings cold, nutrient-rich waters to oligrotrophic surface waters (Valentin 1984). The cold waters are observed mainly outside the Arraial do Cabo Bay (ACB), creating a gradient of temperature from the exposed shores outside the bay to the more protected warm shores located inside the bay (Castro et al. 1995; Ferreira et al. 2001). During the summer, when the upwelling is more frequent, the difference of temperature between regions is usually greater than 10°C (Valentin 2001; Carriére et al. 2009). The inside and outside of ACB also experience different levels of human impact. Located at the inner end of the bay is an urbanized area with a breakwater that reduces water circulation and supports a pier and several recreational docks. At the outer end are the exposed shores of Cabo Frio Island, which are protected by the Brazilian Navy with restricted access for tourist and boat traffic.

Given that both water temperature and anthropogenic impact are important factors determining ascidian distribution and dominance, we describe here the diversity of ascidians from the ACB along these two ecological gradients. We expected that ascidian species would occupy different portions through the anthropogenic and temperature gradients, with invasive species being more frequent in the urbanized region.

Materials and methods

Study area

We conducted this study in the municipality of Arraial do Cabo (22°58'00"S, 42°01'25"W), on the Southeastern Brazilian Coast. This area is located in a transitional zone between the tropical and subtropical provinces and is bathed by a coastal

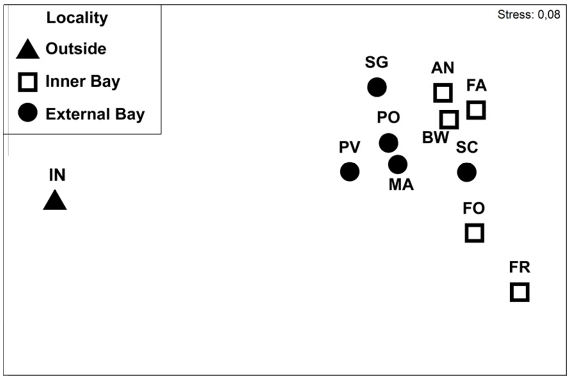

Figure 2. Two dimensional plot of the occurrence of ascidians species in Arraial do Cabo Bay and surroundings analyzed by non-metric Multidimensional Scaling (nMDS).

upwelling system. The irregular morphology of the coastline associated with the position of Cabo Frio and Porcos Islands results in a large embayment of 9.5 Km² called Arraial do Cabo Bay (ACB) (Laborel 1970) (Figure 1).

Sampling design

To assess the ascidian assemblages in the region of the ACB and through the anthropogenic and temperature gradients, we collected specimens from April to July, 2011 at 11 different sites inside and outside the ACB. Each site was visited once, when a SCUBA diver searched for ascidians in rock crevices, over, underneath boulders and pillars of pontoons for one hour. For sites in natural areas, the diver swam in a zigzag pattern from the surface to the sandy bottom, up to 16 meters deep (modified from Ferreira et al. 2001). All observed specimens were collected and transported to the lab in plastic bags. At the Instituto de Estudo do Mar Almirante Paulo Moreira (IEAPM), collected specimens were anesthetized with menthol crystals for one hour and then fixed in 70% alcohol for further examination under a dissecting microscope. After identification, the ascidian species were categorized as native, introduced or cryptogenic according to current literature and previous data.

The 11 sampling sites were divided into three regions according to exposure and to anthropogenic (harbor area and domestic sewage) and upwelling influences (Figure 1). The first region, hereafter referred to as the inner bay, was located inside the ACB, close to urban areas and thus exposed to intense runoff material and international and local boat traffic. Because it was also located at the inner end of the bay it is therefore less affected by the upwelling, presenting warm waters. Sites in the inner bay included Anjos (AN), Forno (FO) and Fortaleza (FA), the man-made breakwater (BW) and the shores in the proximities of the floating restaurant (FR). The second region, the external bay, was also inside ACB, but far from urban areas; thus, it was less affected by anthropogenic impacts but still possessed warm waters. Sites in the external bay included: Pedra Vermelha (PV), Maramutá (MA) Sacos do Gato (SG), Saco dos Cardeiros (SC) and Porcos (PO). Pedra Vermelha and Maramutá are located on Cabo Frio Island, which is protected by the Brazilian Navy that restricts tourist access. The third region, outside the bay, was characterized by a single shore site: Saco dos Ingleses (IN), which is under strong influence of the coastal upwelling (Coelho-Souza et al. 2012) and far from anthropogenic influences.

Statistical analyses

We developed a presence/absence matrix of ascidian species per site and calculated the Sorensen similarity coefficient for non-metric Multidimensional Scaling (nMDS) to clarify the relationships among localities and regions. The differences among regions observed in the nMDS were tested using an ANOSIM test. To describe which species contributed to the dissimilarity among regions, we performed a SIMPER analysis. All tests were performed with Primer 6.0 software (Plymounth Marine Laboratory, UK) (Clarke and Gorley 2006).

Results

We sampled 31 species of ascidians belonging to nine families, from which Didemnidae and Styelidae were the most representative, each with 10 species (Table 1). *Symplegma rubra* Monniot, 1982 and *Phallusia nigra* Savigny, 1816 were the most widely distributed species, being observed respectively in nine and eight of the 11 sites. Forno Beach, Breakwater and Maramutá sites possessed the highest abundance of species, with 13, 12 and 12 species respectively, while Saco dos Ingleses was the least speciose site with only four species. Among regions, the inner bay showed 10.8 ± 0.9 (mean ± SE) species per site, 22 in total, while the external bay showed 9.4 ± 0.9 (mean ± SE), 19 in total. Only four species were recorded outside the bay.

As well as differing in species richness, regions also differed in species composition, with the two regions inside of the bay being different to the

Table 1. Occurrence of ascidians from Arraial do Cabo Bay and status (C = cryptogenic, I = introduced, N = native, ? = no information available), in the inner bay (BW = breakwater, FA = Fortaleza , FO = Forno, AN = Anjos, FR = Floating Restaurant), external bay (SG = Saco do Gato, SC = Saco dos Cardeiros, PO = Porcos Island, PV = Pedra Vermelha, MA = Maramutá) and outside the bay (IN = Saco dos Ingleses).

species/sites	AN	FO	FA	BW	FR	SG	SC	PV	PO	MA	IN	status
Clavelinidae												
Clavelina oblonga Herdman, 1880			x	x								C
Polycitoridae												
Cystodytes dellechiajei (Della Valle, 1877)											x	C
Holozoidae												
Distaplia bermudensis Van Name, 1902		x	x		x		x					N
Polyclinidae												
Polyclinum constelatum Savigny, 1816		x			x							C
Polyclinum molle Rocha and Costa, 2005	x		x	x								N
Didemnidae												
Didemnum galacteum Lotufo and Dias, 2007										x		N
Didemnum granulatum Tokioka, 1954											x	C
Didemnum ligulum Monniot, 1983								x		x	x	C
Didemnum perlucidum Monniot, 1983	x	x	x	x		x	x			x		C
Didemnum rodriguesi Rocha and Monniot, 1993											x	C
Didemnum speciosum Herdman, 1886			x									N
Didemnum vanderhorsti Van Name, 1924						x	x	x				C
Didemnum sp.			x	x		x			x	x		?
Diplosoma listerianum Milne-Edwards, 1841		x		x	x			x				C
Diplosoma sp.	x			x				x	x			?
Ascidiidae												
Ascidia curvata (Traustedt, 1882)	x				x							I
Ascidia sydneiensis Stimpson, 1855		x										I
Phallusia nigra Savigny, 1816	x	x	x	x	x	x	x	x	x	x		C
Styelidae												
Botrylloides giganteum (Pérès, 1949)		x										C
Botrylloides nigrum Herdman, 1886		x	x	x				x		x		C
Botryllus sp.										x		?
Eusynstyela tincta (Van Name, 1902)							x					C
Eusynstyela sp.							x					N
Polyandrocarpa anguinea (Sluiter, 1898)	x		x	x		x	x		x	x		N
Styela canopus Savigny, 1816	x											I
Styela plicata (Leuseur, 1823)		x			x		x		x	x		I
Symplegma brakenhielmi (Michaelsen, 1904)	x	x		x		x		x	x	x		C
Symplegma rubra Monniot, 1972	x	x	x	x	x		x	x	x	x		N
Pyuridae												
Herdmania pallida (Heller, 1878)	x	x		x			x					C
Microcosmus exasperatus Heller, 1878			x			x	x	x	x	x		C
Corellidae												
Rhodosoma turcicum (Savigny, 1816)		x			x							I
Number of species	10	13	11	12	8	7	11	9	8	12	4	

region outside (R global = 0.52, $p<0.001$) (Figure 2). Three out of the four species (*Cystodytes dellechiajei* (Della Valle, 1877), *Didemnum granulatum* Tokioka, 1954 and *Didemnum rodriguesi* Rocha and Monniot 1993) were exclusively found in this region. Therefore, the region outside the bay presented a high dissimilarity to the inner and external bay (100 and 92.3% respectively), with just one of the 28 species found inside the ACB occurring outside of it (*Didemnum ligulum* Monniot, 1983). Among the sampled species, 16 were categorized as cryptogenic, seven as native and five as introduced to the Brazilian Coast (Table 1). An additional three species could not be identified to species level. All species from outside the bay were categorized as cryptogenic.

Three (16%) of the 19 morphospecies reported in the external bay were not determined to specific level, 26% were native, 53% cryptogenic and only one species (5%) classified as introduced. Similarly, in the inner bay 2 (9%) of the 22 morphospecies were not determined to species level, 23% were native, 45% were cryptogenic, but 23% (five species) were introduced.

Discussion

Differences in anthropogenic and upwelling/ temperature regimes among regions in the ACB appeared to contribute to differences in regional diversity of ascidians. Fifty five percent of all ascidian species were exclusive to one of the threeregions, which is a similar pattern to those described for echinoderms, cnidarians (Castro et al. 1995) and macroalgae (Yoneshigue-Valentin and Valentin 1999; Brasileiro et al. 2009) in the ACB. Similar to previous studies, we observed more species inside the bay than outside, although our sampling effort was focused mainly inside the bay, and thus we may have undersampled the species outside the bay. On the other hand, we observed three species outside the ACB (IN) that were not present in any of the 10 sampled sites within the bay, suggesting that the differences in species composition are not only a result of sampling effort. In addition, the site within the ACB with the fewest number of species, still held twice the number of species observed outside the ACB.

Shenkar and Swalla (2011) recently reported 56 ascidian species on the Brazilian coast, a number that was later upgraded to 104 (Rocha et al. 2011). We found a high number of ascidian species in the ACB (31) compared to findings from other well-studied areas on the Brazilian Coast such as São Paulo (62 species) (Rodrigues et al 1998; Dias et al. 2012), Santa Catarina (26 species) (Rocha et al. 2005), Paraná (19 species) (Rocha and Kremer 2005) and Ceará States (19 species) (Lotufo and Bezerra-Silva 2005). Furthermore, we updated the previously documented ascidian diversity in the ACB from 19 (Rocha and Costa 2005) to 31. Considering that only 36 ascidian species are recorded for the whole of the Rio de Janeiro State (Björnberg 1956; Millar 1958; Monniot 1969-70; Rocha and Costa 2005; Marins et al. 2010; Skinner et al. 2013), ACB is an area of high ascidian diversity. Our survey also documents the first occurrence of six species from the Didemnidae family (*Didemnum perlucidum* Monniot, 1983, *Didemnum speciosum* Herdman, 1886, *Didemnum galacteum* Dias and Lotufo 2007, *D. granulatum, D. ligulum*, and *D. rodriguesi*), one styelid species – *Styela canopus* Savigny, 1816 and one pyurid species *Ascidia curvata* (Traustedt, 1882) which were not observed for the ACB in previous studies (Rocha and Costa 2005). Some of the species reported for the first time in ACB in this study are conspicuous and abundant ascidians that would have been easily observed by Rocha and Costa (2005), so their occurrence today is probably the result of recent introductions.

The high ascidian diversity in the ACB may be related to the spatial heterogeneity of physical conditions in the region. The inner region, which was the most speciose area, is exposed to significant anthropogenic impacts (domestic sewage discharge and ship/boat traffic). The area experiences seasonal euthrophication due to organic sewage, with high levels of bacterial production mainly during summer (Coelho-Souza et al. 2013). The intensive marine traffic and associated pollution in the harbor area are also likely to cause negative impacts on the benthic community (Rogers et al. 2014). Harbor areas are also recognized as major introduction sites of non-native species (Ruiz et al. 1997). As a result of invasive species having the ability to outcompete native species under stressfull conditions (Gittenberger and Moon 2011), and because invasives do not have a shared past with native predators (Epelbaum et al. 2009), non-native species can dominate heavily disturbed areas such as the inner ACB. This in turn can lead to a reduction in the diversity of native species in these areas (Piola and Johnston 2008).

The non-indigenous ascidians *A. curvata*, *S. canopus, Ascidia sydneiensis* Stimpson, 1855 and *Rhodosoma turcicum* (Savigny, 1816) were found exclusively in the inner bay. The cryptogenic species, *Clavelina oblonga* Herdman, 1880 was also found to be abundant on man-made structures at this site. The increase in cargo ship transportation and oil and gas companies activities in the last few years have likely favored the establishment of these non-native species in the ACB region (Ferreira et al. 2004). *Styela plicata* (Lesueur, 1823), which is categorized as a harmful invasive species in many regions around the world (Pineda et al. 2011), was also present in the ACB. Previously, *S. plicata* was restricted to harbor areas on breakwater and pillars of pontoons (Rocha and Costa 2005), but the species is now present along rocky shores near the ACB, reinforcing the idea that harbors and marinas can act as gateways for invasive species that can further spread to pristine areas (López-Legentil et al. 2014).

The region outside the ACB is characterized by waters with low temperatures <18°C, high nutrient concentrations, and phytoplankton blooms (Valentin 2001; Coelho-Souza et al. 2012). We found the ascidians *C. dellechiajei*, *D. granulatum* and *D. rodriguesi* exclusively outside of the ACB. These three species have previously been reported in both warm and cold waters around the world (Shenkar and Swalla 2011). On the Brazilian coast, both *C. dellechiajei* and *D. granulatum* occur from Ceará (03° S) to Santa Catarina (27° S). *D. rodriguesi* was reported from New Caledonia (Rocha and Monniot 1993) and in Brazilian waters occurs from Rio de Janeiro to Santa Catarina, showing subtropical affinities (Rocha et al. 2011). *D. ligulum* was observed occurring both outside and inside the bay. This species lives in tropical and subtropical conditions in Brazil (Rocha et al. 2011), displaying a high dispersal capacity, and wide tolerance to temperature.

Within the ACB, the external bay had a few exclusive species, the majority of which were cryptogenic. Cryptogenic species are defined as those which have "unknown origin", and the introduced status is based on criteria such as geographical distribution, occurrence on natural or artificial substrates, and dispersal capacities of the species (Chapman and Carlton 1991). In general, species categorized as cryptogenic in this study were more abundant than natives and introduced species together, following the worldwide pattern (Shenkar and Swalla 2011). Besides several records for ascidians species (Moure et al. 1954; Björnberg 1956; Millar 1958, 1961, 1977; Monniot 1969-70; Rocha and Nasser 1998; Rodrigues et al. 1998; Rocha and Costa 2005; Rocha et al. 2005; Dias et al. 2012), most of the ascidians along the Brazilian Coast are still poorly known and their original distribution remains unclear.

Our study provides a baseline for future surveys in the ACB and highlights the dispersal of invasive species, mainly *S. plicata,* through most of the ACB. In addition, our results suggest that the different physical conditions between regions may select different species within each region, which could account for the high diversity of ascidians in the ACB. The differences in species among our three regions seems to be caused mainly by species occurring exclusively in the upwelling region (outside the bay) and to invasive and cryptogenic species in the anthropogenically affected inner bay.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Survey locations and observations of non-native species.

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