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Non indigenous ascidians in port and natural environments in a tropical Brazilian bay

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ABSTRACT. Despite limited natural dispersal, some species of ascidians can be transported vast distances via oceanic petroleum platforms, ship hulls and ballast water and therefore may be good indicators of bioinvasion. Usually non indigenous species (NIS) are abundant in harbors. This is caused in part because of the higher propagule delivery rate in these areas. An alternative explanation of why invasion is enhanced in harbor and marinas is that environmental degradation commonly found in these habitats favors the establishment of NIS. Most surveys for introduced species were not comprehensive and targeted mainly ports and marinas. Angra dos Reis is an excellent system that provides an opportunity to compare the potential distribution of introduced and native species of Ascidiacea between port and natural environments. Here, we compared the colonization of experimental subtidal plates placed in harbors and marinas with the colonization of plates placed in nearby natural areas. With 27 taxa (15 identified to species), species richness was greater in port environments (25 versus 8). Six taxa were common to both environments while 19 taxa were exclusively found in ports. Among the identified species in ports, three were introduced, five were cryptogenic and only one was native. Only three species were found exclusively in the natural sites and all were cryptogenic. The presence of introduced species only in the port areas of Angra dos Reis reinforces the need for continued, periodic monitoring in the region for early detection of new, potentially invasive, species as well as for better understanding of abnormal population growth of the already known species. Management to reduce the transfer of exotics to natural habitats must be implemented.

KEY WORDS. Artificial substrate; bioinvasion; exotic species; organic pollution; Tunicata.

Research on marine bioinvasion is a relatively new topic in Brazil (Ferreira *et al.* 2009). The first list of introduced species was recently compiled (Lopes 2009) and patterns of invasion still need to be addressed. Ascidians are important bioindicators of anthropogenic transport due to their short lifespan and their lecithotrophic larvae that are usually incapable of long distance dispersal (Petersen & Svane 1995, Lambert 2005). Long distance transport of ascidians may occur in three ways: 1) ballast water, 2) fouling on the hulls of ships and on oceanic platforms, 3) transport of cultivated shellfish from one farm to another (Lambert 2001). Ballast water is probably not the most important vector due to the short larval period of ascidians. However, some species may become established in the substrate that accumulates in ballast tanks, such as the ascidian *Bostricobranchus digonas* Abbott, 1951 that was found in Paranaguá Bay in the southern Brazilian state of Paraná (Rocha 2002). Many species of ascidians are resistant to the heavy metals of the anti-fouling paints and may travel on ship hulls (Piola & Johnston 2008). Transport of adult ascidians attached to ballast tanks is also possible as it is the transport in sea-chests (Coutts *et al.* 2003, Coutts & Dodgshun 2007). Transport of non-native ascidians on cultivated shellfish has not yet been documented in Brazil.

Generally NIS are abundant in harbor areas. This is related in part to he higher propagule deliver in these areas that should lead to an increased probability of invasion success (Johnston *et al.* 2009). An alternative factor that may enhance invasion in harbor and marina areas is environmental degradation commonly found in these habitats that favors the establishment of NIS, as native species are poorly adapted to these altered conditions (Preisler *et al.* 2009). While environmental degradation has often been associated with vulnerability to invasion by exotic species since the seminal work of Elton (1958), the relationship between organic pollution and invasion has been poorly studied. This relationship is better known in plants in terrestrial (Riley & Banks 1996), fresh water (Thiébaut

2005) and marine and estuarine systems (Lapointe *et al.* 2005a, b). Recently, heavy-metal pollution has been shown to increase diversity and dominance of non-native invertebrates in rocky intertidal communities (Piola & Johnston 2008).

Introduced organisms may also be favored by a change in resource availability following eutrophication, such as space for colonization when pollution intolerant species die, increasing space for tolerant invasive species. Among the mechanisms of eutrophication that may directly favor bioinvasion is the increase in food resources for filter feeders due to the increase in phytoplankton (Herbert 1999). Reduction in native species richness in polluted systems (Piola & Johnston 2008) may also increase the invasibility of the system (Stachowicz *et al.* 1999).

Once non indigenous ascidians become established they provide large local sources of larvae for further invasions into additional harbors or nearby natural communities (Lambert & Lambert 1998, Lambert 2001). Although understanding differences in invasion rates is critical for implementation of management strategies (Preisler *et al.* 2009) the vast majority of surveys were not comprehensive and target mainly ports and marinas, which are potential inoculation sites (Campbell *et al.* 2007). For instance in Australia 41 ports have been surveyed between 1995 and 2004, in contrast to just one comprehensive survey in Port Phillip Bay conducted also in natural regions (Sliwa *et al.* 2009). The establishment of NIS outside harbors is of much greater conservation concern than invasion in harbors (Preisler *et al.* 2009). Fifty species of exotic invertebrates were found in an estuary without international shipping, illustrating the importance of intraregional transport and natural mechanisms as vectors of secondary transport (Wasson *et al.* 2001).

The use of artificial substrates to passively collect fouling communities is a common ecological tool that has been used in numerous countries. Their application to introduced species is more recent being used to act as an early warning monitoring tool (Campbell *et al.* 2007). In the present study we compared the colonization of experimental subtidal plates placed in harbors and marinas with the colonization of plates placed out of these areas. This approach facilitates comparisons since the community in each site has the same age reflecting the larval pool found in the area. The region near Angra dos Reis is reasonably well-preserved, although several foci of organic pollution are found near urban areas. Aspects of the region favor the arrival of introduced species, such as shellfish cultivation, presence of offshore oil platforms and ports (Sepetiba and Angra) with heavy marine traffic. Thus, the region provides an excellent system to compare the potential distribution of introduced and native species of Ascidiacea between ports and natural environments.

MATERIAL AND METHODS

The port of Angra dos Reis is within Ilha Grande Bay (22º55'-23º15'S, 44º00'-44º43'W) in the south of the state of Rio de Janeiro. Angra is one important and large port in southeastern Brazil, through which steel products are exported and grains are imported. In addition to the port, large marinas are also found in the bay, including Piratas and Bracuí. During the summer, many tourists visit the city of Angra do Reis and an important part of their recreational activities includes boat transport, with ~15000 boats in the area. Thus the region around the city of Angra dos Reis is strongly influenced by both, interregional transport (due to the port) and intraregional transport (due to marinas and tourism) of species. Additionally, high levels of organic particulates and of ammonia, nitrogen, phosphorus and chlorophyll a are found in these waters, due to nearby release of sewage.

In this study, we compare two sites with stressing conditions (port + eutrophication) with two, more natural sites away from the anthropic sources of degradation. The stressing sites were Piratas marina, with many recreational boats, and Capitania pier, very close to the port. The natural sites, with no anthropic sewage impact (Mayer-Pinto & Junqueira 2003) were Mombaça and Gipóia (Fig. 1).

Superficial water was collected at all sites, refrigerated and taken for analysis to the laboratory of Hydrobiology at UFRJ (Universidade Federal do Rio de Janeiro). Salinity, temperature, suspended particulates, ammonia, nitrite, total nitrogen, phosphorus and chlorophyll a were measured following standard procedures.

Ascidians were collected on experimentally placed plates. In each site, four PVC plates (12 x 12 cm) were suspended from a 1 m PVC tube, which was repeated five times. Tubes were suspended under piers because ascidian larvae tend to prefer darker places for attachment. Thus, each location had 20 plates, for a total of 80 plates which were in place for colonization from December 2005 to March 2006 (summer, at this latitude). Plates were then collected, placed in 10% formalin and taken to the laboratory for species identification and analysis. Species were classified as native, cryptogenic or introduced, following Carlton (1996) and Chapman & Carlton (1991), for the classification of new introductions.

RESULTS

Eutrophication was clear at the two port environments (Capitania and Piratas), while salinity and temperature were similar among all sites, except Piratas, where salinity was somewhat lower (Tab. I). A total of 27 ascidian taxa were found, of which 15 were identifiable to the species level. The remaining species could not be clearly identified due to the lack of mature gonads or larvae that are essential for identification. Since status classification (native etc.) requires specific identification, detailed analysis only includes those ascidians that were identified to species.

Most of the species are known from Brazil except for *Polyclinum aurantium* Milne-Edwards, 1841 which is a first record and a new introduction. Four species are reported for the first time in Angra dos Reis – *Ascidia curvata* (Trausdedt, 1882), *Ascidia sydneiensis* Stimpson, 1855, *Polyclinum constellatum* Savigny,

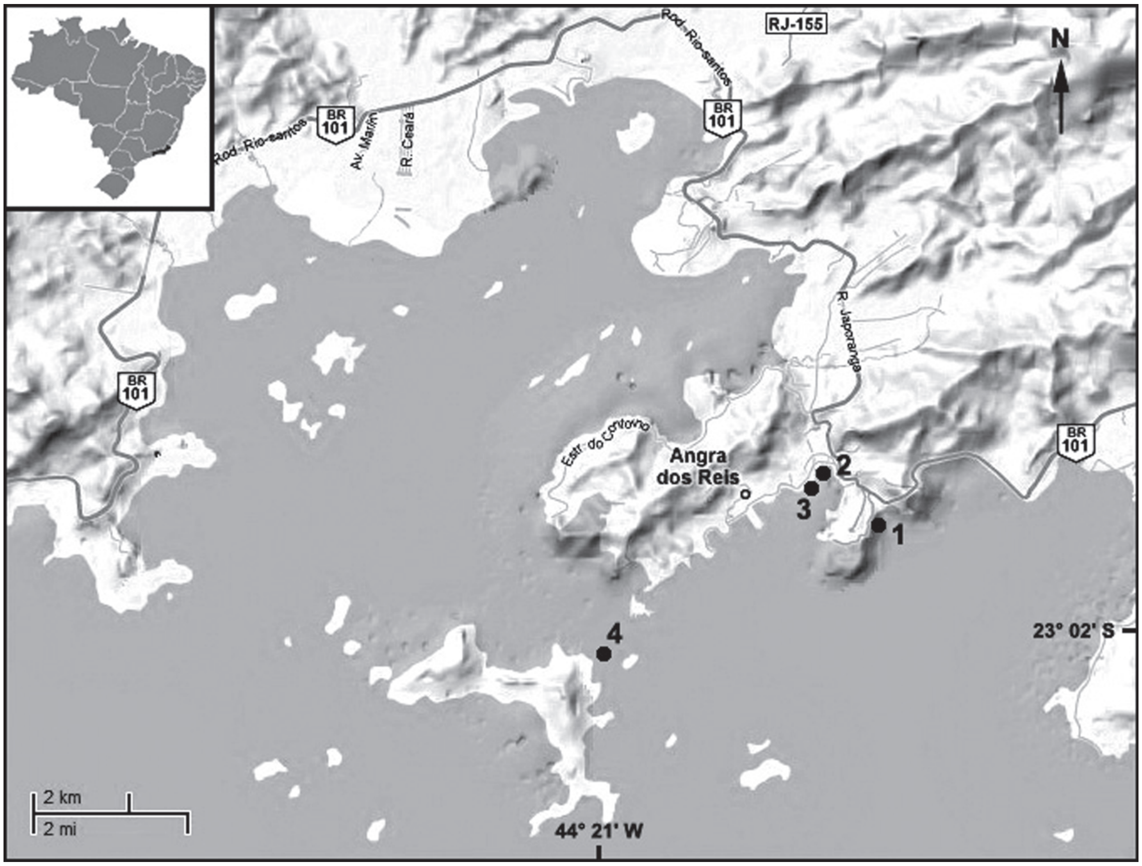

Figure 1. Study area in Ilha Grande Bay, in the region of Angra dos Reis: (1) Mombaça, non-port/non-eutrophic; (2) Piratas, port/eutrophic; (3) Capitania, port/eutrophic; (4) Gipóia Island, non-port/non-eutrophic.

Table I. Comparison of physical and chemical water conditions between port/eutrophic and non-port/non-eutrophic (natural) sites. (N) Total nitrogen (NH_4), ammonia, (NO_2) nitrate, (NO_3) nitrite, (P) phosphorus, (SPM) suspended particulate material.

Conditions	Site	Physico-chemical Conditions								
		Temperature (°C)	Salinity (S)	Chlorophyll (µg/l)	SPM (mg/l)	NH_4 (µM)	NO_2 (µM)	NO_3 (µM)	N (µM)	P (µM)
Port + Eutrophication	Capitania	28	24.0	1.564	282.0	4.27	0.19	0.05	15.97	0.79
	Piratas	28	32.5	2.708	282.0	6.96	0.51	2.27	20.95	1.67
Natural	Mombaça	27	33.5	0.004	57.0	0.57	0.11	0.14	5.36	0.27
	Gipóia	27	34.5	0.171	61.8	0.60	0.08	0	4.02	0.45

1816 and *P. aurantium* –, while *A. curvata* is also a first record for the state of Rio de Janeiro.

More taxa were found in the port sites (n = 25) than in the natural sites (8, Tab. II). Six taxa were common to both conditions with *Symplegma brakenhielmi* (Michaelsen, 1904), *Botrylloides nigrum* Herdman, 1886, *Diplosoma listerianum* (Milne-Edwards, 1841), and *Herdmania pallida* (Savigny, 1816) occurring in all sites. In contrast, 19 taxa, with nine identified species – *Styela plicata* (Lesueur, 1823), *Styela canopus* (Savigny, 1816), *P. aurantium*, *P. constellatum*, *Clavelina oblonga* Herdman, 1880, *Phallusia nigra* Savigny, 1816, *A. curvata*, *A. sydneiensis* and *Didemnum vanderhorsti* Van Name, 1924 –, were found only in the port sites. Among these, three are introduced, five are cryptogenic and only one is native. In the natural sites, all species were cryptogenic (Tab. III) and only three taxa were found exclusively in these areas (Tab. II).

Table II. Ascidiacea found during this study at Angra dos Reis, Rio de Janeiro. Species richness was greatest in port/eutrophic environments (G = 6.4, df = 1, p < 0.05).

Species	Port/Eutrophic		Natural	
	Capitania	Piratas	Mombaça	Gipóia
Didemnum vanderhorsti	x	x		
Didemnum sp. 1	x			
Didemnum sp. 2				x
Didemnum sp. 3		x		
Didemnum sp. 4			x	
Didemnum sp. 5	x			
Diplosoma sp.		x		
Diplosoma listerianum	x	x	x	x
Clavelina oblonga	x			
Lissoclinum sp.		x		
Polyclinum sp.		x		
Polyclinum constellatum	x	x		
Polyclinum aurantium		x		
Ascidia curvata		x		
Ascidia sydneiensis	x			
Phallusia nigra	x			
Herdmania pallida	x	x	x	x
Microcosmus exasperatus	x	x	x	
Botrylloides nigrum	x	x	x	x
Botryllinae 1	x			x
Symplegma rubra	x	x		
Symplegma brakenhielmi	x	x	x	x
Styela canopus	x	x		
Styela plicata	x	x		
Polyandrocarpa sp.		x		
Styelidae 1	x	x		
Molgula sp.		x		

DISCUSSION

As expected due to the local marine context of ports and offshore oil rigs, introduced ascidian species were found at Angra dos Reis (20% of the total), along with several cryptogenic species (73%), with only one native species. Moreover, the three introduced species were only found in port sites.

It is well-known that artificial substrates, such as buoys, ropes and floats, near and in marinas often have large numbers of introduced ascidians (Lambert & Lambert 1998, 2003) and one experimental study demonstrated that over time and on artificial, but not natural, substrates two introduced species increased to the detriment of the natives (Tyrrel & Byers 2007). Our experiment here followed a similar protocol and thus should have favored introduced species over natives. That no introduced species were found in the natural areas suggests that none has become established there, despite the nearness to possible sources including those with eutrophic input and transport by local boats (Fig. 1). Similar results, with introduced species in ports but not in nearby better-preserved areas are known (Lambert & Lambert 2003, Turon *et al.* 2003).

In addition to the greater propagule delivery to the port areas, an alternative and not exclusive hypothesis for the differences between port and natural environments is eutrophication, which may favor the establishment of exotic species. The mechanism by which introduced species are favored in eutrophic areas is unclear and merits further study. It is probable that increased particulate matter in suspension in the water column is important because when overloaded, the filtration capacity of many species declines (Petersen 2007). Thus, perhaps the small pool of introduced species in ports all over the world (including species found here, *A. sydneiensis*, *S. canopus*, *Microcosmus exasperatus* Heller, 1878 and *H. pallida*) is adapted to these conditions. Our data do not allow a rigorous test of the role of eutrophication here due to complex conditions required for such a test. That is, one needs ports with and without eutrophication as well as “natural areas” with and without eutrophication, with all areas being reasonably geographically close to one another. Such conditions are seldom available anywhere, and are not available in the region of Angra dos Reis. On the other hand, our experimental approach with settlement plates controls for time and reflects the pool of larvae that is available in the area.

Another clear trend was that solitary species were favored in port/eutrophic areas (five species of the seven solitary species were only found in these sites) and were often extremely abundant, as also observed in other locations (Monniot *et al.* 1991). Colonial species in general have smaller zooids and are thus even further hindered by large quantities of particulate matter in suspension as mentioned above.

While *S. plicata* and *A. sydneiensis* are already known from Brazil as introduced species (Rocha & Kremer 2005, Lopes 2009), the colonial *P. aurantium* must be considered a new introduction in Brazil. This species has never been seen in Brazil nor reported from any other place in the western Atlantic Ocean. The species occur in the eastern Atlantic, reported from France (Milne-Edwards 1841), Senegal (Pérès 1949) and Sierra Leon (Millar 1956) and thought to be introduced in the Azores (Cardigos *et al.* 2006). Thus, the logic for considering this species a new introduction follows Chapman & Carlton (1991), in which a species is considered introduced when: it appears in places where never before found, human mechanisms of transport are likely (this species was probably brought on fouling of ship hulls), artificial substrates are available and facilitate its colonization (this

Table III. Distribution and status of the species of Ascidiacea found during this study at Angra dos Reis, Rio de Janeiro.

Species	Distribution		Status
	Brazil	Global	
Didemnum vanderhorsti	CE, PE, AL, BA, ES, RJ, SP, SC	West Atlantic	Native
Diplosoma listerianum	RN, PE, AL, BA, ES, RJ, SP, PR, SC	Atlantic, Pacific, Indian	Cryptogenic
Clavelina oblonga	ES, RJ, SP, PR, SC	West and East Atlantic	Cryptogenic
Polyclinum constellatum	CE, PE, ES, RJ, SP, SC	West Atlantic, Pacific, Indian	Cryptogenic
Polyclinum aurantium	RJ	East and North Atlantic	Introduced
Ascidia curvata	PE, BA, ES, RJ, PR	West Atlantic	Cryptogenic
Ascidia sydneiensis	RJ, SP, PR, SC	Atlantic, Pacific, Indian	Introduced
Phallusia nigra	CE, AL, BA, RJ, SP	Atlantic, Pacific, Indian, Red Sea, Mediterranean	Cryptogenic
Herdmania pallida	CE, AL, BA, RJ, SP	Atlantic, Pacific, Indian	Cryptogenic
Microcosmus exasperatus	CE, PE, AL, BA, RJ, SP, PR, SC	Atlantic, Pacific, Indian, Mediterranean	Cryptogenic
Botrylloides nigrum	PE, AL, BA, ES, RJ, SP, PR, SC	Atlantic, Pacific, Indian	Cryptogenic
Symplegma rubra	ES, RJ, SP, PR	West Atlantic, Pacific, Indian	Cryptogenic
Symplegma brakenhielmi	CE, PB, AL, PE, BA, ES, RJ, SP, SC	West Atlantic, Pacific, Indian	Cryptogenic
Styela canopus	RN, PE, BA, RJ, SP, PR, SC	Atlantic, Pacific, Indian	Cryptogenic
Styela plicata	BA, RJ, SP, PR, SC	Atlantic, Pacific, Indian, Mediterranean	Introduced

species was found on artificial substrates), and the natural dispersal ability is insufficient to explain species distributions (larvae are lecithotrophic and short lived, thus unable to traverse large distances (in this case, crossing the Atlantic Ocean).

The first record of *S. plicata* in Brazil is from the 1800s (Traustedt 1883). Along the southern coast, *S. plicata* is usually found in anthropic environments on artificial substrates (Mayer-Pinto & Junqueira 2003, Rocha & Kremer 2005, Barros *et al.* 2009). In Algeciras Bay, southern Spain, *S. plicata* was found as much on artificial as on natural substrates, but only in areas that had suffered some human impact (Naranjo *et al.* 1996, Carballo & Naranjo 2002). Still, this species is rarely found on natural substrates in Brazil, and only in Rio de Janeiro (Junqueira, non published data); but it is very common and abundant in eutrophic areas on experimental plates where it forms dense clumps.

Indeed, dense clumps may be found on any surface, including sea shells and carapaces of other animals. In the southern Brazilian state of Santa Catarina, *S. plicata* is commonly very abundant in cultivated mussels and oysters (Rocha & Kremer 2005, Rocha *et al.* 2009). In Mexico, in San Quintin Bay, several introduced ascidians, including *S. plicata*, were found on the shells of the commercially important oyster, *Crassostrea gigas* (Rodriguez & Ibarra-Obando 2008). When ascidians are abundant on bivalve cultures they may result in decreased productivity due to competition for space and food, which may reduce and deform the shells (Enright 1993, Taylor *et al.* 1997). That is not to mention the great efforts and money that then must be spent in cleaning the shells of the encrusting organisms. Since cultivation of bivalves takes place at and near Angra dos Reis, the ever increasing urbanization brings with it domestic sewage and eutrophication, which then may lead to a problem with introduced species that then colonize the cultivated shellfish.

Ascidia sydneiensis was first recorded in Brazil in the state of São Paulo (Bjornberg 1956) and its distribution is restricted to the southern and southeastern coast (Millar 1958, Rodrigues 1962, Rocha & Nasser 1998, Rocha & Kremer 2005, Rocha & Costa 2005). This introduced species is also usually found on artificial substrates (Rocha & Kremer 2005), but also may be found on natural substrates, as in Paranaguá Bay, in the state of Paraná (Rocha & Kremer 2005) and at Arraial do Cabo, Rio de Janeiro (Rocha & Costa 2005). Here we report its first record for Angra dos Reis.

The colonial *D. vanderhorsti* was the only species among all these found that is considered native to Brazil,. This species is amply distributed in the western Atlantic Ocean (Van Name 1945, Millar 1962) and in Brazil was first found in São Paulo by Bjornberg (1956) and then registered by Millar (1958), Rocha & Monniot (1995) and Rodrigues *et al.* (1998). Subsequently, this species was found in Santa Catarina (Rocha *et al.* 2005) and Tito M.C. Lotufo (pers. comm. 2002) also collected it on natural substrates in northeastern and southeastern Brazil (Pernambuco, Alagoas, Bahia, Espírito Santo and Rio de Janeiro). In Rio de Janeiro, Marlene Simões (pers. comm. 1981) found *D. vanderhorsti* on natural substrates in Niterói (Boa Viagem Beach) and Lotufo (pers. comm. 2002) on natural substrates in Angra dos Reis (Redonda and Porcos Islands). While published records only report this species from the states of São Paulo and Santa Catarina, unpublished records suggest that the true distribution in Brazil is probably along the entire coast. While this species was only found in port areas in this study, colonies were attached to solitary ascidians, which suggests that they are unlikely to attach to artificial substrates. If true, the lack of adequate substrate may explain why they were not found in natural areas, where very few solitary species were found.

The remaining species found in port areas are cryptogenic: *C. oblonga*, *A. curvata*, *P. constellatum*, *P. nigra*, and *S. canopus*. *Clavelina oblonga* is found along the southern and southeastern coast of Brazil, in Bermuda, Caribbean, northern Atlantic (Monniot 1974) and eastern Atlantic (Monniot 1969). Here, we only found a small colony on one plate in the port area. Nevertheless, this species is common in Rio de Janeiro, reported from several places: Niterói (Bjornberg 1956, Millar 1958, Simões pers. comm. 1981), Ponta do Arpoador (Rodrigues 1962) and Arraial do Cabo (Rocha & Costa 2005). Lotufo (pers. comm. 1998) also found this species at Cabo Frio (Papagaio Island), Angra dos Reis (Redonda Island) and Niterói (Itapuã Beach).

Ascidia curvata is widely distributed in the tropical western Atlantic Ocean. First recorded in Brazil in the state of Paraná (Rocha & Nasser 1998) and again found later (Rocha & Kremer 2005), it was also found in the northeast, in the states of Bahia and Pernambuco and in the southeast, in Vitória (Lotufo pers. comm. 2002), always on natural substrates. Here, *A. curvata* is reported for the first time in the state of Rio de Janeiro, but only found in port sites. This appears to be a recent occurrence in Angra dos Reis because it was not seen during the preceding 10 years, when several studies of ascidians were carried out. It is not known whether it underwent a natural range expansion from nearby areas or whether its arrival at Angra occurred by human transport.

Polyclinum constellatum is wide spread in the tropical American Atlantic Ocean, it has been reported in the Indian Ocean (Millar 1954) and is considered to be introduced in Guam, in the western Pacific (Lambert 2003). In Brazil, it has been reported in the states of Rio de Janeiro and São Paulo (Van Name 1945, Bjornberg 1956, Rodrigues 1962, Rocha & Costa 2005) and has been found in Pernambuco and Ceará on natural substrates (Lotufo pers. comm. 2002). Evidence suggests that it is a tropical species quite possibly introduced in the south, since it is only found on artificial substrates there (Rocha *et al.* 2009). Here, we report *P. constellatum* for the first time in Angra dos Reis, surprisingly considering the more than 10 years of benthic studies in the area. Thus we suggest that it might also be a recent occurrence, although it is not clear whether its occurrence is result of natural dispersion or human transport. Thus we decided classify the species as cryptogenic.

The solitary *P. nigra* is also widely distributed in the tropical western Atlantic Ocean (Van Name 1921, 1945, Berrill 1932, Millar 1962, Goodbody 2000, 2003), but has only been reported from Ceará (Lotufo & Silva 2005), Rio de Janeiro (Rodrigues 1962, Rocha & Costa 2005) and São Paulo (Van Name 1945, Bjornberg 1956, Millar 1958, Rodrigues 1962) in Brazil. However, it was also found in Alagoas (Lotufo pers. comm. 2002) and Bahia (R.M. Rocha pers. obs.). It is considered to be introduced in Hawaii (Hawaii Biological Survey, www2.bishopmuseum.org/HBS/invertguide/ascidians.htm) and Guam (Lambert 2003). The geographical origin of this species remains unknown and so it must be considered cryptogenic. Their great abundance from Florida to Brazil and its genetic flow throughout this distribution (Nóbrega *et al.* 2004) suggests that its original range is widespread in the western Atlantic. Here, again this species was only found in port sites. But, with its seasonal reproduction with a peak in the fall (Rocha *et al.* 1999) we think it is likely that it will be found in all locations after continued study.

Another solitary species, *S. canopus*, is widespread globally and cryptogenic (Rocha & Kremer 2005). However, it was quite likely to be introduced in São Paulo and Paraná due to its rarity there as well as only being found on artificial substrates. Published records only exist for Rio de Janeiro (Mayer-Pinto & Junqueira 2003, Monniot 1969), São Paulo (Rodrigues *et al.* 1998), Paraná (Rocha & Kremer 2005) and Santa Catarina (Rocha *et al.* 2005), yet it has been found on natural substrates in the Brazilian northeast (Bahia, Pernambuco, Rio Grande do Norte) and southeast (Espírito Santo and Rio de Janeiro, Lotufo pers. comm. 2002). At Angra dos Reis it was found in both port and natural environments (Sergio H.Gonçalves da Silva pers. comm. 1998, Maria P. Curbelo Fernandez pers. comm. 2002, Mayer-Pinto & Junqueira 2003).

Of the six species found in both environments, four were considered to be cryptogenic: *Diplosoma listerianum*, *M. exasperatus*, *B. nigrum*, *Symplegma rubra* Monniot, 1972 (Rocha & Kremer 2005). The two remaining species that were not previously classified are *H. pallida* and *S. brakenhielmi*. *Herdmania pallida* was very abundant in almost all study sites with the exception of one of the natural sites, where we found only colonial species. This species is very tolerant of organic pollution and has been seen in eutrophic and oligotrophic waters of the region on artificial and natural substrates (Silva pers. comm. 1998, Fernandez pers. comm. 2002). *Herdmania* Lahille, 1888 has recently been revised, yet there is still doubt as to the identity of the Atlantic species. Kott (2002) states that the Atlantic populations are different from those in the Indian and Pacific Oceans, but she does not provide the characters that show that differentiation, nor does she suggest a new name. On the other hand, Monniot (2002) affirmed that those animals from Guadalupe, Panamá and Brasil are *H. pallida* with an ample distribution in all oceans. The taxonomic uncertainty and lack of knowledge of geographic origins require that it be classified as cryptogenic. In Brazil, it is found from the northeast (Millar 1977) to the southeast, in Rio de Janeiro (Rodrigues 1962, Rocha & Costa 2005) and São Paulo (Van Name 1945, Bjornberg 1956, Millar 1958, Rodrigues 1962). It has been found on natural substrates in Rio de Janeiro, Bahia and Alagoas.

The colonial *S. brakenhielmi* is widely distributed globally, with records from the Atlantic (Van Name 1921, Monniot 1983, Goodbody 2000, 2003, Rocha *et al.* 2005), Indian (Monniot & Monniot 1997) and Pacific Oceans (Monniot & Monniot 2001, Lambert 2003). This species also has uncertain taxonomy and many records from the Pacific are under the name *S. oceanica* (Monniot & Monniot 1997). In Brazil, it is widely distributed in the northeast (Lotufo pers. comm. 2002), southeast (Rodrigues *et al.* 1998, Rocha & Costa 2005) and south (Rocha *et al.* 2005, Rocha & Faria 2005). At Angra dos Reis, *S. brakenhielmi* is very common and often found during the last 10 years (Silva pers. comm. 1998, Fernandez pers. comm. 2002). Lambert (2003) states that the species is introduced in Guam. Here, *S. brakenhielmi* was found in all study areas with apparently wide tolerance for pollution. This species must also be classified as cryptogenic pending further study.

Human impact in coastal regions, especially near large urban centers, may increase the susceptibility to introduction of exotic species. In the specific case of Angra dos Reis we found that three introduced species were only found in port/eutrophic areas. Additionally, two more species that were found for the first time in the region, suggesting introduction, were only found in these areas. Thus, species richness was greater in port/eutrophic areas and additional data from the plates show that biomass is greater in eutrophic sites (unpublished data) indicating faster growth and increased survival to reproductive age. All of these details indicate that port/eutrophic areas can favor introduced species. Future study will be needed to address the potential impact that these introduced species may have on other benthic organisms, but it has already been shown that at least one species, *S. plicata*, can cause a large detrimental impact in cultivated shellfish (Rocha *et al.* 2009).

The large number of species classified as cryptogenic impedes understanding of the ascidian community in the region and may camouflage the presence of other NIS in anthropogenic areas. This reinforces the need for continued, periodic monitoring in the region for early detection of new, potentially invasive species as well as for better understanding of abnormal population growth of the already known species. Reduction in eutrophication is essential to reduce the likelihood of invasion as well as to permit the continued cultivation of shellfish. Enhanced educational effort aimed at cleaning boat hulls, fishing and sporting gear for the reduction of possible local transport and to eliminate the discarding of bait or other living organisms are also urgent management procedures for the region.

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