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SUBSTRATE TYPE AS A SELECTIVE TOOL AGAINST COLONIZATION BY NON-NATIVE SESSILE INVERTEBRATES

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ABSTRACT

Different substrates of varying composition, color, texture and orientation may selectively influence recruitment of sessile invertebrates and thereby influence the resultant community. Thus substrates may act as a barrier to the establishment of non-indigenous species (NIS). In southern Brazil, granite is the main rock forming natural rocky walls that are available for encrusting organisms. In this study we tested whether granite selectively influences recruitment and impedes colonization by introduced and cryptogenic species that are already established on artificial substrates within the region. Plates of rough cut granite and of polyethylene were made available each month under a pier at a yacht club in Paranaguá Bay. A community is already established on concrete columns and fiber glass floats on the piers. After one, two and twelve months, the faunal composition of the plates was compared between the two treatments and other artificial substrates. Granite was recruited by all the seven introduced species found in the Bay and by 18 of 26 cryptogenic species and therefore is ineffective as a barrier to NIS colonization.

RESUMO

Substratos de diferentes materiais, cores, texturas e orientação podem influenciar seletivamente no recrutamento de invertebrados sésseis e, assim, influenciar a comunidade resultante. Deste modo, o substrato pode funcionar como barreira contra o estabelecimento de espécies não nativas (NIS, na sigla em inglês). No sul do Brasil, o granito é a principal rocha formadora de costões rochosos naturais disponíveis para organismos incrustantes. Nesta investigação, nós testamos se o granito seleciona o recrutamento de espécies e se poderia, assim, impedir a colonização de espécies introduzidas ou criptogênicas já estabelecidas em substratos artificiais na região. Placas não polidas de granito e de polietileno foram submersas a cada mês em um píer de um iate clube na Baía de Paranaguá. Há uma comunidade já estabelecida sobre colunas de concreto e sobre flutuadores de fibra de vidro presentes no iate clube. Depois de um, dois e doze meses, as espécies presentes nas placas de diferentes materiais foram comparadas entre si e também com outros substratos. O granito foi colonizado por todas as sete espécies introduzidas encontradas na região, e por 18 das 26 espécies criptogênicas, sendo então ineficaz como barreira contra a colonização de NIS.

Descriptors: Introduced species, Recruitment, Bioinvasion, Artificial substrata, Granite, Estuaries, Conservation, Paranaguá Bay.
Decritores: Espécies introduzidas, Recrutamento, Bioinvasão, Substrato artificial, Granito, Estuário, Conservação, Baía de Paranaguá.

INTRODUCTION

Non-indigenous marine species (NIS) are continuously spread throughout the world by human maritime activities and constitute one of the major global changes associated with the oceans (RUIZ et al., 1997). Ship transport is a known vector with a long history of dispersal of species throughout the oceans in a variety of ways: in ballast water, encrusted on hulls and in sea chests (COUTTS et al., 2003).

NIS, when arriving in a new environment, are subject to the availability of local resources for their survival and subsequent establishment of populations. Adverse conditions or resource limitation may be barriers that impede the establishment of NIS. For benthic sessile organisms, space for attachment and growth is one of the main limiting resources that may prevent a species from becoming established.

In coastal environments, humans continuously build new structures and add artificial hard substrates that remain immersed and, therefore, available for attachment by encrusting organisms. Such structures are usually made of concrete, wood, PVC, other plastic materials, fiberglass and so on. Recent studies find that these structures are colonized first by NIS and act as stepping stones for the colonization of natural habitats (CONNEL and GLASBY, 1999). On the other hand, even if established on artificial substrates, NIS may only become a threat to natural communities once they colonize natural substrates.

Substrates themselves can favor establishment of certain species because of their chemical composition (BAVESTRELLO et al., 2000; GLASBY and CONNEL, 2001; KNOTT et al., 2004; ANDERSSON et al., 2009), color, texture (SKINNER and COUTINHO, 2005; FLORES and FAULKNES, 2008), depth and orientation. Thus, substrate characteristics may be barriers to colonization by NIS, thereby preventing their invasion. Different substrate characteristics may be associated with different species compositions or abundances (GLASBY, 2000; CONNELL, 2001; BULLERI and CHAPMAN, 2004; BULLERI, 2005; STACHOWICZ et al., 2007). On the other hand, recruitment by some NIS is possible on a large variety of substrates and this opportunism permits their successful invasion (CREED and PAULA, 2007).

Concrete columns, floating fiberglass and floating hulls comprise distinct habitats each with their own communities of different species (NEVES et al., 2007). However, temporal variation as part of the wider picture is unknown here, but it has been shown to exert an important influence on communities and should be considered when comparing natural and artificial substrates (GLASBY, 2000). In this study, we test whether granite, the natural substrate of rocky marine habitats in southern Brazil, acts as a selective barrier to recruitment, thereby reducing colonization by introduced or cryptogenic species. The experiment was carried out over one year to include temporal variation in the recruitment process.

MATERIAL AND METHODS

Study Area

Substrate plates in two different treatments were placed at the Paranaguá Yacht Club, in the Itiberê river near its mouth in Paranaguá Bay (25º31’S, 48º30’W, Fig. 1). Paranaguá Bay is part of a large estuarine complex in the southern Brazilian coastal states of Paraná and São Paulo. This estuarine system is connected to the sea by three channels, the main one located close to Mel Island (LANA et al., 2001). Water circulation and stratification patterns inside Paranaguá Bay change during the year, with variation in salinity (12 - 29 in summer, 20 - 34 in winter), and temperature (23 - 30ºC in summer, 18 - 25ºC in winter) (LANA et al., 2001).

The Itiberê River skirts the city of Paranaguá, separating Valadares Island from the continent. Its margins were originally mangrove swamps that were destroyed as a result of the expansion of the city (CANEPARO, 2000). Now only small mangrove forest fragments remain, the roots of which, along with the columns of many piers, are covered by encrusting communities.

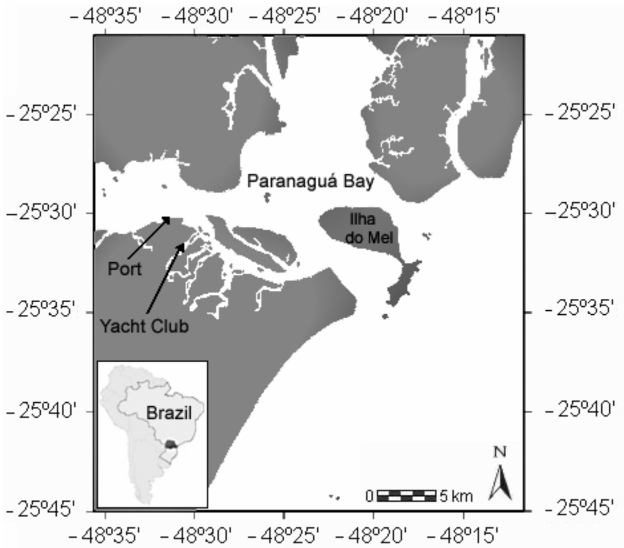

Fig.1. Map of Paranaguá Bay showing location of study area and of the port of Paranaguá. In the detail the map of Brazil showing the state of Paraná.

The Yacht Club has two main piers with a central walkway supported by concrete columns and lateral floating fiberglass docks, covered with wood. The international port of Paranaguá, the most important port in southern Brazil, is less than 1 km from the Yacht Club. As larger ships became more frequent, the port was forced to move from the city to its current location in 1935 (GODOY, 2000).

Field Experiment

Each month, 10 plates (23.0 x 11.5 cm) of rough-cut granite and six plates (14 x 14 cm) of black polyethylene plastic were hung from the floating docks at the Yacht Club from March/2007 to February/2008. Bricks were attached to each rope at 1.5 m depth to maintain the plates in a vertical position and reduce the influence of tidal drag. One month after plates had been submerged they were removed from the water, photographed (granite) or examined under a stereomicroscope (plastic), and then replaced in their previous positions, where they remained for another month (i.e., for a total of 2 months). Also, at the beginning of the experiment, another 10 granite and six plastic plates were put in place where they remained for an entire year. Upon collection, plates were fixed in formalin 4% after treatment in sea water with menthol crystals for 2 h. Plastic and granite plates were on the same ropes, and so had a slight difference in depth, but both were always submerged.

Plate Analysis

Species were counted on the exposed surfaces of the granite plates in 15 squares of a 3.6 cm^2 grid, equivalent to 30% of the total plate area. Species were counted on plastic plates in 21 squares of a 1.4 cm^2 grid (21% of the total plate area). Specialists for each *taxon* were provided with voucher samples for their identification. Species were classified as native, introduced or cryptogenic, following the literature, based on their origin and current geographical distribution (CARLTON, 1996) and on other criteria, such as presence on artificial substrates, ports and marinas, disjointed distribution and rapid dispersion in a region (CHAPMAN and CARLTON 1991, 1994). Historical data regarding encrusting species in the region are relatively recent, with the oldest study of fouling communities dating from 1987-88 (CORREIA and SILVA, 1990). We compared our list of species with those of the latter study and with those of NEVES et al. (2007) and NEVES and ROCHA (2008) to better understand the recent invasion history of the Bay and evaluate the ability of each species to colonize different types of substrates.

RESULTS

A total of sixty-two *taxa* were found. Of these, 44 species were identified, eight of which were native, seven were introduced, three historically introduced and 26 were cryptogenic (Table 1). The native species to Paranaguá Bay were: *Fistulobalanus citerosum* (Cirripedia), *Nicolea uspiana, Pseudobranchiomma paulista* and *Branchiomma patriota* (Polychaeta), *Mytella charruana*, *Crassostrea rhizophorae* and *Ostrea puelchana* (Bivalvia), and *Molgula phytophila* (Ascidiacea). The Brazilian endemics include *Nicolea uspiana*, *P. paulista*, *B. patriota* and *M. phytophila*, the first three of which are here reported for the first time in Paraná. *Fistulobalanus citerosum* occurs, in Brazil, from Pernambuco to Rio Grande do Sul. The bivalve *O. puelchana* occurs along the southeastern and southern coasts, while *M. charruana* and C. *rhizophorae* are more widespread (Table 1).

Of the introduced species, the octocoral *Carijoa riisei*, the bivalve *P. perna* and the barnacle *M. tintinnabulum* were probably introduced into Brazil long ago and are now considered naturalized. The polyp of the hydroid *H. carnea* and the serpulid *H. sanctaecrucis* are here reported for the first time in southern Brazil. The other introduced species included the hydrozoan *Garveia franciscana*, the barnacles *Amphibalanus reticulatus, Megabalanus coccopoma* and *Striatobalanus amaryllis*, and the ascidian *Styela plicata*, of which only *M. coccopoma* was previously unknown in Paranaguá Bay (Table 1).

Most of the species were classified as cryptogenic due to the lack of historical distributional information. Many have wide geographical distribution with unknown native ranges or disjunct populations. This category comprises nine hydrozoans, one anthozoan, seven bryozoans, one bivalve, one polychaete, four barnacles and three ascidians (Table 1).

Species did not occur synchronously on the granite plates, but rather some were restricted to a single month while others appeared throughout the year (Table 2). At least one introduced species appeared on granite plates each month: in summer *Garveia franciscana*, *Amphibalanus reticulatus* and *Striatobalanus amaryllis*; in the fall *A. reticulatus* and *Megabalanus coccopoma*; in winter *A. reticulatus*, *M. coccopoma* and *Styela plicata*; in the spring *H. sanctaecrucis, A. reticulatus* and *S. plicata*. Rare species were found on $\leq$ 10% of the plates, while common species were found on all plates.

Table 1. Taxa found at Paranaguá Yacht Club on granite and polyethylene plates in the years 2007-2008, with introduction status and geographical distribution.

Taxa	Status[1]	Geographical distribution	References
Porifera			
Porifera sp. 1	–	–	
Porifera sp. 2	–	–	
Hydrozoa			
Bouganvillia muscus (Allman, 1863)	C	Atlantic, Pacific, Indian Ocean, Mediterranean, Brazil (RJ- PR)	Calder, 1988; Migotto et al., 2002
Clytia gracilis (M. Sars, 1850)	C	Circumglobal, Atlantic, Indian and Pacific oceans, Brazil (PE, ES-PR, RS)	Millard, 1975; Migotto et al., 2002
Clytia linearis (Thornely, 1899)	C	Western and Eastern Atlantic, Indian Ocean, West and East Pacific, Brazil (ES, SP, PR)	Calder, 1988; Migotto et al., 2002
Ectopleura dumortieri (Van Beneden, 1844)	C	Cosmopolitan, Atlantic Ocean, Mediterranean Sea, North Sea, Brazil (RJ-RS)	Migotto, 1996; Migotto et al., 2002; Galea et al. 2007
Eudendrium carneum Clarke, 1882	C	Atlantic, Indian Ocean, East Pacific, Clarion Island, Brazil (FN, PE, BA-SC)	Calder, 1988; Migotto et al., 2002
Garveia franciscana Torrey, 1902	I	North Atlantic, Gulf of Mexico, West Africa, India, North Pacific, Mediterranean Sea, Australia, Brazil (PE, PR)	Medel and López-Gonzales, 1996; Migotto et al., 2002; Cairns et al., 2003; Neves and Rocha, 2008
Hydractinia carnea (M. Sars, 1846)	I	Cosmopolitan, Brazil - only medusa stage (SP, PR),	Migotto et al., 2002; Cairns et al., 2003; Bisby et al., 2005
Obelia bidentata Clarke, 1875	C	Circumtropical, West and East Atlantic, Indian Ocean, West and East Pacific, Brazil (PE, RJ-SP, PR)	Calder, 1988; Correia and Silva, 1990; Migotto et al., 2002
Obelia dichotoma (Linnaeus, 1758)	C	Cosmopolitan, Brazil (ES-PR, RS)	Calder, 1988; Migotto et al., 2002, Galea et al., 2007
Pinauay crocea (Agassiz, 1862)(=*P. ralphi* Bale, 1884)	C	Atlantic, Indian Ocean, South Africa, Brazil (ES-RS)	Millard, 1975; Migotto et al., 2002
Plumularia floridana Nutting, 1900	C	Cosmopolitan, Brazil (PE, ES-SP)	Calder, 1997; Migotto et al., 2002
Tubulariidae (1 species)	–	–	
Anthozoa			
Actiniaria	–	–	
Aiptasia pallida (Verrill, 1864)	C	United States, Gulf of Mexico, Brazil (RN, PE, BA-SC)	Gomes and Mayal, 1997; Schlenz et al., 1998;
Diadumene sp.	–	–	
Clavulariidae (1 species)	–	–	
Carijoa riisei (Duchassaing and Michelotti, 1860)	HI	Circumtropical, Brazil (AP-SC)	Concepcion et al 2010
Bryozoa			
Alcyonidium sp.	–	–	
Biflustra denticulata (Busk, 1856)	C	East Pacific, United States (Gulf of California and Cape Hatteras), Colombia, Brazil (ES, SP, PR, SC)	Winston, 2005; Montoya-Cadavid et al., 2007; Vieira et al., 2008
Bugula neritina (Linnaeus, 1758)	C	Cosmopolitan, Brazil (RJ, SP, PR, SC)	Orensanz et al., 2002; Ramalho et al., 2005; Wyatt et al., 2005; Vieira et al., 2008
Bugula stolonifera Ryland, 1960	C	Cosmopolitan, Brazil (RJ, SP)	Orensanz et al., 2002; Ramalho et al., 2005; Wyatt et al., 2005; Vieira et al., 2008
Conopeum reticulum (Linnaeus, 1767)	C	Cosmopolitan except polar regions, Brazil (ES-SC)	Vieira et al., 2008
Conopeum sp	–	–	

Table 1. Continuation.

Taxa	Status1	Geographical distribution	References
Electra tenella (Hincks, 1880)	C	United States (Florida), Puerto Rico, Colombia, Mediterranean, India, Japan, New Zeland, Brazil (SP, PR)	Winston, 1982; Badve and Sonar, 1995; Streftaris et al., 2005; Montoya-Cadavid et al., 2007; Vieira et al., 2008
Hippoporina verrilli Maturo and Schopf, 1968	C	West Atlantic, East Pacific, Galapagos, Brazil (SP, PR)	Winston, 1982; Vieira et al., 2008
*Sinoflustra annae (*Osburn, 1953)	C	United States (Florida, Texas, California), Canada (Vancouver, Queen Charlotte Islands), Panama, West Africa, Japan, Brazil (SP, PR)	Hastings, 1930; McCann et al., 2007; Vieira et al., 2008
Ctenostomata (1 species)	–	–	
Bivalvia			
Brachidontes sp.	–	–	
Crassostrea rhizophorae (Guilding, 1828)	N	Venezuela, Suriname, Uruguay, Brazil (AP-RS)	Rios, 1994
Hiatella sp.	–	–	
Musculus lateralis (Say, 1822)	C	United States (North Carolina-Texas), West Indies, Brazil (PE-SC).	Rios, 1994
Mytella charruana (Orbigny, 1842)	N	Mexico, Ecuador, Galapagos, Venezuela, Suriname, Brazil (PB-PR), Uruguay, Argentina,	Rios, 1994; Junqueira et al., 2004
Ostrea puelchana Orbigny, 1841	N	Brazil (RJ-RS), Argentina	Rios, 1994
Perna perna (Linnaeus, 1758)	HI	Mediterranean, Senegal, South Africa, Brazil (ES-RS).	Rios, 1994
Polychaeta			
Branchiomma patriota Nogueira *et al.*, 2006	N	Brazil (SP)	Nogueira et al., 2006
Hydroides sanctaecrucis Krøyer *in* Mörch, 1863	I	Dutch Antilles, French Guiana, Haiti, Gulf of Mexico, Panama; Mexico (Pacific coast), Hawaii, Australia, Brazil (PR)	Hayes et al., 2005; Lewis et al., 2006
Neanthes cf. *succinea* (Frey and Leuckart, 1847)	C	Cosmopolitan, Brazil (RJ-PR, RS)	Fauchald, 1977
Nicolea uspiana Nogueira, 2003	N	Brazil (PE, SP)	Nogueira, 2003; Nascimento et al., 2007
Nicolea sp			
Pseudobranchiomma paulista Nogueira et al., 2006	N	Brazil (SP)	Nogueira et al., 2006
Pseudobranchiomma sp.	–	–	
Serpula sp.	–	–	
Cirripedia			
Amphibalanus amphitrite (Darwin, 1854)	C	Cosmopolitan, Brazil (AP-RS)	Rocha, 1999; Farrapeira, 2009
Amphibalanus eburneus (Gold, 1841)	C	Cosmopolitan, Brazil (PE, RJ, SP, PR)	Farrapeira, 2009
Amphibalanus improvisus (Darwin, 1854)	C	Cosmopolitan, Brazil (CE-RS)	Farrapeira, 2009
Amphibalanus reticulatus (Utinoni, 1967)	I	Circumtropical, Brazil (PE, BA, RJ -SC)	Farrapeira, 2009
Balanus trigonus (Darwin, 1854)	C	Cosmopolitan, Brazil (AP-RS)	Apolinário, 2002; Orensanz et al., 2002; Zullo, 1992
Fistulobalanus citerosum (Henry, 1974)	N	Brazil (PB-RS)	Neves and Rocha, 2008
Megabalanus coccopoma (Darwin, 1854)	I	United States (East coast), Gulf of Mexico, Belgium, Northeast Pacific, Brazil (PE-RS)	Apolinário, 2002; Farrapeira et al., 2007
Megabalanus tintinnabulum (Linnaeus, 1758)	HI	Cosmopolitan, Brazil (PI-RS)	Farrapeira, 2009

Table 1. Continuation.

Taxa	**Status1**	**Geographical distribution**	**References**
Striatobalanus amaryllis Darwin, 1854	I	Cosmopolitan, Brazil: PI, PE, BA, PR	Farrapeira, 2009
Amphipoda			
Jassa sp.	–	–	
Laticorophium sp.	–	–	
Monocorophium sp.	–	–	
Ascidiacea			
Diplosoma listerianum (Milne-Edwards, 1841)	C	Cosmopolitan, Brazil (BA-SC)	Rocha and Kremer, 2005
Microcosmus exasperatus Heller, 1878	C	Circumtropical, Brazil (PE, BA, RJ-SC)	Rocha and Kremer, 2005
Molgula phytophila Monniot 1970	N	Brazil (RJ-SC)	Rocha and Kremer, 2005; Rocha and Moreno, 2000
Polycarpa sp.	–	–	
Styela plicata (Lesueur, 1823)	I	Cosmopolitan, Brazil (PE, BA, RJ-SC)	Rocha and Kremer, 2005; Farrapeira et al., 2007; Barros et al., 2009
Symplegma rubra Monniot, 1972	C	United States, Caribbean, Indian ocean, Pacific, Brazil (RJ-SC)	Rocha and Kremer, 2005

[1] Status: I = introduced, HI = historic introduction, N = native, C = cryptogenic

Table 2. *Taxa* present on granite plates with frequency and period of occurrence. NIS species in bold type.

Taxa	Frequency (%)	Months of occurrence	Taxa	Frequency (%)	Months of occurrence
Clytia gracilis + *Obelia* spp	89.7	Apr – Jan	*Branchiomma patriota*	10	Feb
Ectopleura dumortieri	30	Aug - Sep	***Hydroides sanctaecrucis***	**30**	**Nov**
Garveia franciscana	**25**	**Dec - Mar**	*Nicolea uspiana*	55.1	Feb - Dec
Hydractinia carnea	**62**	**Feb -May, Sep**	*Pseudobranchioma* sp.	49.5	Feb - Dec
Pinauay crocea	10	Aug	*Pseudobranchiomma paulista*	10	Feb
Clavulariidae	71.6	Feb - May, Oct - Dec	*Amphibalanus amphitrite*	57.5	Jun - Sep
Actiniaria	53	all	*Amphibalanus improvisus*	82.5	Apr - Jan
Aiptasia pallida	14.8	Feb, Aug, Nov	***Amphibalanus reticulatus***	**75.9**	**May - Jan**
Biflustra denticulata	12.5	Feb, Apr, May, Oct	*Balanus trigonus*	13.3	Jul - Sep
Bugula neritina	55.0	May - Aug	*Fistolobalanus citerosum*	74.4	all
Bugula stolonifera	25.0	Jul - Aug	***Megabalanus coccopoma***	**60**	**May - Aug**
Electra tenella	88.9	all	***Striatobalanus amaryllis***	**20**	**Feb**
Hippoporina verrili	77.6	Feb - Jul, Sep - Jan	*Monocorophium* sp, *Laticorophium* sp, *Jassa* sp	99.1	all
Synoflustra annae	21.3	Feb - May, Nov	*Diplosoma listerianum*	16.7	Mar - Apr, Sep
Hiatella sp.	48.7	all	*Microcosmus exasperatus*	24.7	Mar - Jun, Sep - Dec
Musculus lateralis	52.9	Feb - Aug	*Molgula phytophila*	53.2	Jul - Nov
Mytella charruana	71.8	all	***Styela plicata***	**31.6**	**Jun - Nov**
Ostrea puelchana	59.8	Jan - Jul, Sep - Nov	*Symplegma rubra*	30	Mar – May, Sep - Oct
Perna perna	45	May - Jul, Sep	Styelidae	100	Apr

Frequent *taxa* (> 70% occurrence on granite plates) were *Hippoporina verrili, Electra tenella, Amphibalanus reticulatus, A. improvisus, Molgula phytophila* and Styelidae (Table 2). The amphipods *Monocorophium* sp., *Laticorophium* sp. and *Jassa* sp., and the hydrozoans *Clytia gracilis, Obelia dichotoma* and *Obelia bidentata* were pooled because they could not be distinguished in the photographs; both groups were very common (99% and 90% respectively). Of the introduced species, *A. reticulatus* was very frequent (75.9%) on granite plates, while *G. franciscana*, *H. sanctaecrucis*, *S. plicata* and *M. coccopoma* were less frequent and *S. amaryllis* was rare (Table 2).

Discussion

Granite is usually ineffective as a natural barrier to recruitment and was colonized by most non-indigenous species. Yet, in some circumstances, granite seemed to be selective because plates were colonized by fewer species than available in Paranaguá Bay. Nonetheless, NIS colonized many plates and so we should consider colonization rate to be low, rather than accidental. Nis colonized at least one plate every month.

Studies of the fouling community in Paranaguá Bay are rather recent, with the earliest in 1987 (CORREIA and SILVA, 1990). In that study acrylic plates were suspended from buoys at three different locations, and were either replaced monthly or left for a year. One of those study sites was at the entrance to the bay which is a euhaline region having some ascidians and hydrozoans. The second was close to the yacht club and a third within the bay. In that study, 35 encrusting species were identified, of which four were native, 28 cryptogenic and three introduced (the ascidians *Styela plicata* and *Diplosoma singulare,* and the naturalized bivalve *Perna perna*). *Diplosoma singulare* has not been seen again in the bay, while *S. plicata* was found on about 30% of the granite plates from June to November (though not abundant, Table 2). It is worth noting that this NIS was found only on granite (not plastic) plates, while it was also seen on ropes and within the holes in the bricks. *Styela plicata* is known as a NIS in Brazil where it was first seen on Mel Island at the mouth of Paranaguá Bay in 1954 (MOURE et al., 1954). In Brazil, it is now found from Santa Catarina in the south to Pernambuco in the northeast, though less commonly north of Rio de Janeiro (FARRAPEIRA, 2007). It was not found in 2004 in samples taken from floats, concrete columns or underneath boats (NEVES et al., 2007; NEVES and ROCHA, 2008), which may be due to the timing of those collections (April, when it does not reproduce). A contemporaneous study in the bay found *S. plicata* only on Mel Island, growing on the shells of a small mussel culture and on an old pier (ROCHA and KREMER, 2005).

In 2004, different substrates were sampled, but only once, at the Paranaguá Yacht Club, where half the number (19) of encrusting invertebrate species was found, as compared to other studies. This included four introduced species: the hydrozoan *G. franciscana*, the polychate *Polydora cornuta* and the barnacles *A. reticulatus* and *S. amaryllis* (NEVES et al., 2007; NEVES and ROCHA, 2008). With the exception of the polychaete, all occurred on granite plates with frequencies of 25%, 76% and 20%, respectively (Table 2). Thus, again granite is ineffective as a barrier to colonization.

It is interesting to note that the earlier study found no introduced species of barnacles (CORREIA and SILVA, 1990). *Amphibalanus reticulatus* was first reported in Brazil in the northeast (Pernambuco) and then in more southern states (Bahia - YOUNG, 1998; Rio de Janeiro - MAYER-PINTO and JUNQUEIRA, 2003). Similarly, *S. amaryllis* was first reported in Brazil in 1987 from northern (Piauí –YOUNG, 1989) and northeastern coasts (YOUNG, 1998). Thus, their occurrence in southern Brazil may be the result of continuous range extension to the south. The state of Paraná is the southern limit of the reported geographical range of *S. amaryllis.* Nonetheless, *A. reticulatus* has been found on polyethylene plates in a mussel farm in the more southern state of Santa Catarina (L. P. KREMER, personal communication 2008).

This study detected an additional three introduced species in Paranaguá Bay: the hydrozoan *Hydractinia carnea*, the serpulid *Hydroides sanctaecrucis* and the barnacle *Megabalanus coccopoma.* All occupied granite plates (62%, 30% and 60% of the plates, respectively) and the least common serpulid was found only on granite. *Hydractinia carnea* is known from tropical waters of the western Atlantic Ocean (CAIRNS et al., 2003) and Mediterranean Sea (BOUILLON et al., 2004). Medusae were recently encountered in the state of São Paulo in the first record of the species in Brazil (MIGOTTO et al., 2002). Hydrozoans have been studied on rocky shores in the state of Paraná since 1983 by one of our team (MAH) and *H. carnea* has never been found, not even on artificial substrates in more oceanic waters.

Hydroides sanctaecrucis is a tropical sedentary fouling serpulid from the Caribbean known from the Dutch Antilles, French Guiana, Mexico, Haiti, the Gulf of Mexico and Panama, where it also occurs in the Pacific. It has also been reported from Hawaii, and recently from Singapore and Australia (LEWIS et al., 2006). The species forms large agglomerations and can become a nuisance (LEWIS et al., 2006).

Megabalanus coccopoma is from the coasts of the tropical eastern Pacific Ocean in Central and South America and was introduced in coastal waters of southeastern and southern Brazil (JUNQUEIRA et al., 2004). Now it is apparently moving northwards and has recently been found on ship hulls in Pernambuco (FARRAPEIRA et al., 2007).

The polychate *Polydora cornuta*, first found in 2004, was not seen in this study, suggesting either local extinction or that competition prevented its recruitment. Alternatively, because it is cryptic and lives in bivalve shells, it may have been overlooked in this study.

Of the total of 43 cryptogenic species identified for Paranaguá Bay to date, 16 were not found in this study. Among them, *O. geniculata, R. horsti, S. evelinae, B. nigrum, C. oblonga* and *P. constellatum* do not tolerate wide variation in salinity and were mostly found on plates at the bay entrance (CORREIA and SILVA, 1990). They probably did not occur at the yacht club due to variable salinity. *Corophium acherusicum* is a case of taxonomic confusion, because it was found to comprise a complex of species of different genera. The remaining species either disappeared from the area, indicating that they were probably introduced, or were not found due to different sampling techniques. Finally, three of the cryptogenic species occurred only on granite plates: the bivalve *M. lateralis* and the ascidians *D. listerianum* and *S. rubra*, also suggesting that granite, as a substrate, does not limit settlement.

While we find that natural substrates probably do not limit introductions, additional lessons arise from this study. First, detectability of introduced species depends on the temporal scale of the study. A recent review of the methods used to survey ports and marinas around the world showed that all used short time intervals, usually days (CAMPBELL et al., 2007). Even when a study was repeated at a given location, the repeat was not seasonal, but rather was after a very long time interval, often years. Thus, two surveys per year (dry and rainy seasons) are recommended to avoid this problem (CAMPBELL et al., 2007). We would add the additional recommendation that subtropical sites should also be surveyed seasonally since the reproductive season is not as highly synchronized as in temperate regions. Moreover, the use of the “passive sampling method” (*sensu* CAMPBELL et al., 2007) also permits the collection of specimens on different temporal scales (days, months or years) – our study found some species only on the short term plates and others only on the long term plates (Table 3). Thus a combination of temporal scales should be used.

Table 3. Comparison of registers of encrusting species known from Paranaguá Bay.

Taxa	Status[1]	Substrate[2]			
		Acrilic[3] 1987-88	Various[3, 4] 2004	Polyethylene 2007-08	Granite 2007-08
Hydrozoa					
Bougainvillia muscus	C	X			
Clytia gracilis (= C. sp in Correia and Silva, 1990; =*C. hemisphaerica* in Neves et al., 2007)	C	X	X	X	X
Clytia linearis	C			X	
Ectopleura dumortieri	C	X		X	X
Eudendrium carneum	C	X*		X	
Garveia franciscana	I		X	X	X
Hydractinia carnea	I			X	X
Lafoeina almirantensis Millard and Bouillon, 1973	C	X			
Obelia bidentata	C	X	X	X	X
Obelia dichotoma	C	X	X	X	X
Obelia geniculata Linnaeus, 1758	C	X			
Pennaria disticha Goldfuss, 1820 (=*Halocordyle disticha*)	C	X*			
Pinauay crocea (=*Ectopleura warreni* in Correia and Silva, 1990)	C	X		X	X
Plumularia floridana	C			X	
Anthozoa					
Aiptasia pallida	C			X	X
Carijoa riisei	HI	X*		X*	
Tricnidactis errans Pires, 1987	C	X*			
Bryozoa					
Alcyonidium polyoum (Hassall, 1841)	C		X		

Table 3. Continuation.

Taxa	Status[1]	Substrate[2]			
		Acrilic[3] 1987-88	Various[3, 4] 2004	Polyethylene 2007-08	Granite 2007-08
Bryozoa	C	X*			X
Biflustra denticulata (Busk, 1856) (*=Acanthodesia tenuis* in Correia and Silva, 1990)					
Biflustra savartii (Audouin, 1826) (= *Acanthodesia savartii* in Correia and Silva, 1990)	C	X			
Bugula neritina	C	X		X	X
Bugula stolonifera	C			X	X
Bugula turrita (Desor, 1848)	C	X			
Conopeum reticulum (Linnaeus, 1767)	C		X		
Electra tenella	C	X		X	X
Hippoporina verrilli	C		X	X	X
Rimulostoma horsti (Osburn, 1927) (= *Schizoporella horsti* in Correia and Silva, 1990)	C	X			
Sinoflustra annae	C			X	X
Schizoporella unicornis (Johnston, 1847)	C	X			
Smittoidea evelinae (Marcus, 1937) (= *Smittina evelinae* in Correia and Silva, 1990)	C	X*			
Bivalvia					
Brachidontes cf. *rodriguezi* (d'Orbigny, 1846)	C		X		
Crassostrea rhizophorae	N	X			X
Musculus lateralis	C				X
Musculus viator (d'Orbigny, 1846)	N	X			
Mytella charruana	N		X		X
Ostrea puelchana	N				X
Perna perna	HI	X			X
Sphenia antillensis Dall and Simpson, 1901	N	X			
Polychaeta					
Branchiomma patriota	N				X
Hydroides sanctaecrucis	I				X
Neanthes cf. *succinea*	C		X	X*	
Nicolea uspiana	N				X
Polydora colonia Moore, 1907	C		X		
Polydora cf. *cornuta* Bosc, 1902	I		X		
Pseudobranchiomma paulista	N				X
Cirripedia					
Amphibalanus amphitrite	C		X	X*	X
Amphibalanus eburneus	C	X		X*	
Amphibalanus improvisus	C	X	X	X*	X
Amphibalanus reticulatus	I		X	X*	X
Balanus trigonus	C	X			X
Fistulobalanus citerosum	N		X	X*	X
Megabalanus coccopoma	I			X*	X
Megabalanus tintinnabulum	HI			X*	
Striatobalanus amaryllis	I		X		X
Amphipoda					
Corophium acherusicum (Costa, 1851)	C	X	X		
Ascidiacea					
Botrylloides nigrum (Herdman, 1886)	C	X			
Clavelina oblonga Herdman, 1880	C	X			
Didemnum speciosum (Herdman, 1886)	N	X			
Diplosoma listerianum	C				X
Diplosoma singulare Lafargue, 1968	I	X*			
Microcosmus exasperatus	C				X
Molgula phytophila	N		X	X	X

Table 3. Continuation.

Taxa	Status[1]	Substrate[2]			
		Acrilic[3] 1987-88	Various[3, 4] 2004	Polyethylene 2007-08	Granite 2007-08
Ascidiacea	C	X*			
Polyclinum constellatum Savigny, 1816					
Styela plicata	I	X*			X
Symplegma rubra	C				X
Symplegma viride Herdman, 1886	C	X			

[1] Status: I = introduced, HI = historic introduction, N = native, C = cryptogenic
[2] Source of the list of species: 1987-88 (Correia and Silva, 1990); 2004 (Neves et al., 2007; Neves and Rocha, 2008); polyethylene (present study); granite (present study).
[3] Only encrusting, tube dwellers or boring species considered; no associated fauna considered for comparison.
[4] Concrete columns, fiberglass floats, fiberglass or wood boat hulls.
* species observed only on succession plates

The second lesson is that when the "passive sampling method" is used to detect NIS, collections should include a variety of substrate types. Although most of the NIS detected in Paranaguá Bay colonized all available substrates, *H. sanctaecrucis* and *S. plicata* did not occupy polyethylene plates and would have remained undetected if only this type of substrate had been used. The species assembly was slightly different on each substrate analyzed and many cryptogenic species were seen on only one. The physical and chemical properties of the substrate can indeed influence recruitment (CONNELL and GLASBY, 1999; MAUGHAN, 2001; PERKOL-FINKEL and BENAYAHU, 2005) and our study suggests that the differences of species recruited on the different substrates were probably due to the substrate composition.

The baseline survey of the fouling community in Paranaguá Bay is 20 years old (CORREIA and SILVA, 1990) and we note that two of the species in that list were, in fact, introduced. In this study we re-surveyed only one of the sites of the previous study, yet we found seven new introductions in Paranaguá Bay. This rate, one new introduction every three years, seems low in comparison with those of other estuaries (COHEN and CARLTON, 1998; HEWITT et al., 2004). Still, we recognize that our study has limits to understand bioinvasion in Paranaguá Bay as it was restricted to the sessile fauna already established and reproducing. The true number of introduced species is probably greater and will be found when additional habitat types are evaluated and further taxonomic resolution is developed.

We are also concerned that natural hard substrates are uncommon in the bay, yet with the construction of piers and the port, hard substrates are rapidly becoming common. If what we know of the fouling community is very little, then we know even less of natural communities on hard substrates in the bay. We are aware of only two studies that examined natural substrates: one of intertidal hydrozoans (HADDAD, unpublished data) and the more recent study of tunicates on rocky walls (ROCHA and KREMER, 2005). The first found no species that are today considered to be introduced, and the other found one tunicate on artificial substrate. Thus we do not yet know whether the other NIS found here are already established on natural substrates.

In conclusion, Paranaguá Bay (as in other estuaries with ports) is an entrance for introduced species - the number of sessile non-native invertebrates in the area has increased in the last 20 years. Granite proved to be ineffective as a barrier to the colonization of NIS and so clearings that become available in natural communities on rocky shores are subject to their recruitment. Subsequent biological interactions are more important to determine the fate of the NIS in natural communities and more research is needed to better understand these interactions and to propose better management programs.

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