

Research article**Bivalve cultures provide habitat for exotic tunicates in southern Brazil**Rosana M. Rocha^{1*}, Laura P. Kremer¹, Mariah S. Baptista¹ and Rafael Metri²¹Universidade Federal do Paraná, Departamento de Zoologia, Caixa Postal 19020, 81531-980, Curitiba, Paraná, Brazil²Universidade Estadual do Centro-Oeste, Caixa-Postal 3010, Presidente Zacarias 875, 85010-990 - Guarapuava, Paraná, Brazil

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Abstract

Commercial shellfish farming is increasing in Brazil to provide for the growing local and international markets. Shellfish (mussel and oyster) production in Brazil is greatest in the state of Santa Catarina where three sea ports with national and international commerce are sources of non-indigenous species (NIS) that have the potential to find suitable habitat in the shellfish farms. Here we describe the tunicate community associated with shellfish farms during the past 10 years. Survey results identified 17 species of which only one was native. Two were clearly introduced. Of the 14 species classified as cryptogenic, 10 were probably introduced. Tunicates become very abundant in farms and as a consequence farms require periodic cleaning of the shells as well as associated equipment. *Styela plicata* and *Didemnum perlucidum* are the most abundant and may become a threat to natural communities, but they are currently not found on natural substrates. *Ciona intestinalis* and *Styela clava* were not found in shellfish farms, even though *C. intestinalis*, at least, has been introduced many times in Brazil. We recommend that a public awareness program be developed to prevent colonization by *C. intestinalis* in the bivalve farms. Additionally, we emphasize that the diversity of possibly introduced species also requires close monitoring for rapidly expanding populations, since a diversity of life cycles and biological requirements also increases the probability of the appearance of a new pest.

Key words: aquaculture, Ascidiacea, ascidian, bioinvasion, mussel, nonindigenous species, oyster, Urochordata**Introduction**

Bivalve farms (mussels and oysters) create complex novel substrates and communities due to the combination of bivalve shells and artificial substrates. Farms are usually located within calm bays and estuaries which are locations that provide high quality habitat for fouling organisms including introduced species. Additionally, farm structures are usually suspended and protected from predation by invertebrates and fish also providing refuge for exotic organisms such as tunicates. Recent studies indicate that shellfish farms offer substrate and habitat for many exotic species (reviewed by McKindsey et al. 2007).

Tunicates are commonly an important part of the community associated with bivalves and while they do not compete with bivalves for food (Lesser et al. 1992; Petersen 2007), they can compete for substrate space and may even cause bivalve mortality by growing over them (Igic 1972). Tunicates can also increase the weight of

systems causing them to sink (Getchis 2006). Thus, tunicates may often cause severe economic losses (LeBlanc et al. 2003).

Bivalve farming is increasing rapidly in Brazil (Borghetti et al. 2003), with the state of Santa Catarina being the major producer since 1990. In the year 2005, Santa Catarina was the source of 82% of the bivalves produced in Brazil. Currently, bivalve production is located within 15 bays along 1,213 ha of coastline and provides work for 786 employees, an essentially family-owned business (Manzoni 2005). In Santa Catarina, three major ports are potential sources of non-native species: São Francisco do Sul, Itajaí, and Imbituba. Ports with international ship traffic and nearby bivalve farms offer many artificial substrates for non-native species to become established. Tunicates are very common in this fouling community and some species become very abundant. In southern Brazil, in contrast with other locations, no single species becomes dominant as seen in Prince Edward Island, Canada (LeBlanc et al. 2007; Ramsay et

al. 2008). Instead, many species may increase in abundance simultaneously. In this study, we list tunicate species associated with an oyster farm and a mussel farm in the state of Santa Catarina, Brazil. We examine specifically the status (resident or introduced) of each species and their potential impact in natural communities.

Methods

Study site

The mussel farm is located on the north coast of Santa Catarina, in Armação do Itapocoroy Bay (26°46'10"S, 48°47'04"W). The mussel cultivated in this region is *Perna perna* (Linnaeus, 1758), and it was likely unintentionally introduced during the slave trade (Souza et al. 2004). Now the mussel is naturalized and occurs in the low intertidal region of rocky shores along the entire Brazilian coast. Mussels are grown on long lines characterized by a rope which is buoyed and extends horizontally near the water surface. Mussels are cultivated on 3m long vertical ropes. Armação do Itapocoroy Bay is protected from waves and receives water from the plume of the Itajai-açu River whose mouth is 20 km south. Water salinity is usually less than 34 and temperatures range from 19–28°C (Schettini et al. 1999) and water depth is 9–12 m (Marenzi et al. 2006).

The oyster farm (Fazenda Marinha Atlântico Sul) is located in Ribeirão da Ilha, Florianópolis (27°43'34"S, 48°33'51"W). The oyster, *Crassostrea gigas* (Thunberg, 1793), is also non-native and was introduced for cultivation purposes. Salinity here is 32–35, water temperature is 17–29°C and water depth is 4–6 m. Oysters are reared in suspended lantern nets, a 1.5 m long structure formed by six horizontal shelves of plastic discs (42 cm diameter) on which the oysters grow, covered by fish net (to avoid predation). Lantern nets hang side by side from 90 m long main ropes supported at the surface by buoys. At this farm, five sets of 18 main ropes occupy 21 ha.

Ascidian survey

Ascidians were collected during 1998 – 1999 and 2004 – 2007 at the mussel farm and during 2006–2007 at the oyster farm. Animals were scraped from ropes, buoys, and bivalve shells, anesthetized with menthol crystals and fixed in 4% buffered formalin. Vouchers were deposited in the Ascidiacea collection of the Zoology

Department, Universidade Federal do Paraná, Brazil. Abundances were estimated according to the frequency of encounters (but sampling effort was not standardized), and species were classified as rare, common or very common.

Analysis

Historical records of tunicates in southern Brazil are few, and begin in 1945 (Van Name 1945). Species status was based on Chapman and Carlton (1994), relying largely on the criteria related to geographical distribution and restriction to artificial habitats. The criterion “extension of range after first encounter” could not be used, since old tunicate reports for the region were restricted spatially and temporally. The criterion “access to human mechanism(s) of dispersal” was used for species found on boat hulls. While we did not survey boats, we did find *Didemnum perlucidum* Monniot 1983 and *Styela plicata* (Lesueur, 1823) on hulls and so kept this criterion. The criterion “insufficient active dispersion capabilities” was not used because it is true of all ascidians. We added two criteria: “found in aquaculture in other countries” and “known to be introduced in other regions” as an evidence of transport and capacity of establishment in altered environments. All criteria were based on the studied region (Santa Catarina) and a greater number of + is associated with a greater probability of a species having been introduced.

Results

With a total of 17 tunicate species found, *Polyclinum constellatum* Savigny, 1816, *Aplidium accareense* (Millar, 1953) and *Symplegma brakenhielmi* (Michaelsen, 1904) were found only on mussels and *Polycarpa spongiabilis* Traustedt, 1883 only on oysters (Table 1). The most common species were *Styela plicata* and *Didemnum perlucidum* at both farms, but neither species was dominant.

Both *Aplidium accareense* and *Botrylloides giganteum* (Aron e Sole-Cava, 1991) occasionally formed very large colonies at both farms, while *P. spongiabilis* were also very common at the oyster farm (Table 1). Tunicates were only a part of the encrusting community, sharing space with bryozoans, hydrozoans, sponges and barnacles (the latter most diverse and abundant on mussels).

Bivalve cultures as habitats for exotic tunicates

Table 1. Ascidians present on a mussel (M) and an oyster (O) farm in southern Brazil (x = rare, xx = common; xxx = very common) and their invasion status in this region.

Taxa	M	O	1 ^a	2	3	4	5	6	7	8	Status ^b
Clavelinidae											
<i>Clavelina oblonga</i> Herdman, 1880	xx	xx	-	?	+	-	+	-	+	-	C
Holozoidae											
<i>Distaplia bermudensis</i> Van Name, 1902	x	x	+	?	-	+	+	-	-	-	C*
Polyclinidae											
<i>Polyclinum constellatum</i> Savigny, 1816	x		+	?	-	+	+	+	+	+	C*
<i>Aplidium accarensis</i> (Millar, 1953)	xx		+	?	-	-	+	-	-	-	C*
Didemnidae											
<i>Didemnum perlucidum</i> Monniot 1983	xxx	xxx	?	+	+	+	+	+	-	+	C*
<i>Trididemnum orbiculatum</i> (Van Name 1902)	x	xx	+	?	-	-	-	-	-	-	N
<i>Lissoclinum fragile</i> (Van Name, 1902)	x	x	?	?	-	-	+	+	-	+	C
<i>Diplosoma listerianum</i> (Milne-Edwards, 1841)	xx	xx	+	?	+	+	+	+	+	+	C*
Asciidiidae											
<i>Ascidia sydneiensis</i> Stimpson, 1855	x	x	+	?	?	+	+	+	+	+	I
Styelidae											
<i>Botrylloides giganteum</i> (Aron e Sole-Cava, 1991)	xx	x	+	?	-	+	+	+	-	-	C*
<i>Botrylloides nigrum</i> (Herdman, 1886)	x	x	-	?	+	-	-	+	-	+	C
<i>Symplegma brakenhielmi</i> (Michaelsen, 1904)	x		-	?	+	-	+	+	-	+	C
<i>Symplegma rubra</i> Monniot, 1972	x	x	+	?	-	+	+	+	-	-	C*
<i>Styela canopus</i> (Savigny, 1816)	x	x	+	?	-	+	+	+	+	?	C*
<i>Styela plicata</i> (Lesueur, 1823)	xxx	xxx	-	+	+	+	+	+	+	+	I
<i>Polycarpa spongiabilis</i> Traustedt, 1883		xx	-	?	-	+	+	-	-	-	C*
Pyuridae											
<i>Microcosmus exasperatus</i> Heller, 1878	x	x	-	?	-	+	+	+	+	+	C*

^a1-6 modified from Chapman and Carlton (1994): (1) not recorded before in the region; (2) access to human mechanism(s) of dispersal; (3) association with known introductions; (4) prevalence in or restriction to artificial or altered environments; (5) discontinuous or restricted regional distribution; (6) global distribution; new criteria: (7) presence in aquaculture in other countries; (8) known as introduced in other regions. + for yes, - for no, ? for unknown.

^bStatus: N = native, C = cryptogenic, C* = cryptogenic but probably introduced, I = introduced.

Only *Clavelina oblonga* Herdman, 1880, *Distaplia bermudensis* Van Name, 1902, *Trididemnum orbiculatum* (Van Name 1902) and *Symplegma rubra* Monniot, 1972 are apparently native species to the Atlantic Ocean. However, *C. oblonga* and *D. bermudensis* along with *S. rubra* have disjunct distributions and so were classified as cryptogenic in southern Brazil. Further, *S. rubra* was not found in natural substrates at Arvoredo Marine Reserve (Rocha et al. 2005a) suggesting a possible human-mediated

southern extension of its distribution. *Didemnum perlucidum*, *Lissoclinum fragile* (Van Name, 1902), *Diplosoma listerianum* (Milne-Edwards, 1841), *Botrylloides nigrum* (Herdman, 1886), *Styela canopus* (Savigny, 1816) and *Microcosmus exasperatus* Heller, 1878 are cryptogenic because of their disjunct distributions either in the western Atlantic Ocean or globally (Figure 1). Introduced species found included *Ascidia sydneiensis* Stimpson, 1855 and *Styela plicata*.

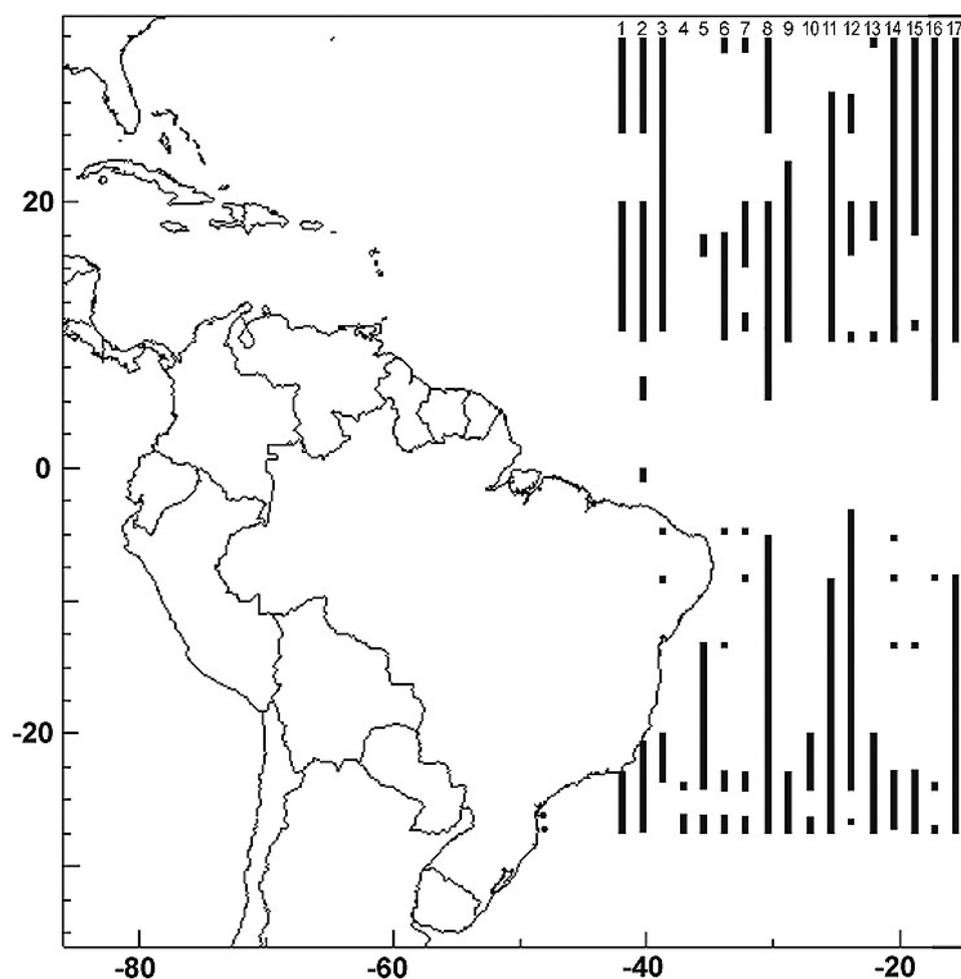


Figure 1. Southwestern Atlantic Ocean showing the latitudinal distribution of the species found in the shellfish cultures of Santa Catarina (dots on the south coast of Brazil). 1. *Clavelina oblonga*, 2. *Distaplia bermudensis*, 3. *Polyclinum constellatum*, 4. *Aplidium accarensense*, 5. *Didemnum perlucidum*, 6. *Trididemnum orbiculatum*, 7. *Lissoclinum fragile*, 8. *Diplosoma listerianum*, 9. *Ascidia sydneiensis*, 10. *Botrylloides giganteum*, 11. *Botrylloides nigrum*, 12. *Symplegma brakenhielmi*, 13. *Symplegma rubra*, 14. *Styela canopus*, 15. *Styela plicata*, 16. *Polycarpa spongiabilis*, 17. *Microcosmus exasperates*.

Discussion

The dynamics of colonization and use of shellfish farm substrates by tunicates suggest that shellfish farms are potential havens for introduced and possibly invasive species. While tunicate species richness ($n = 17$) was low when compared to that of a nearby marine reserve (26 species, Rocha et al. 2005a) and to São Sebastião Channel, in the state of São Paulo (54 species, Rodrigues et al. 1998), this shellfish farm community comprises mostly cryptogenic species, many of which are probably introduced. The genus *Styela*, here represented by *S. plicata*, comprises very invasive species, such as *S. clava* which can reach high densities in shellfish cultures (Getchis 2006; Ramsay et al. 2008). Although records of *S. plicata* in Brazil begin in 1883 (Van Name 1945), evidence suggests that it was introduced in the south (Rocha and Kremer 2005; Barros et al. 2009) (Table 1). The oldest record for *S. plicata* in Santa Catarina is from Florianópolis in 1962 (Rodrigues 1962). The species is known to foul bivalves in Spain (Perera et al. 1990), Japan (Arakawa 1990) and Hong Kong (Huang 2003) (Table 2).

The first Brazilian record of *Clavelina oblonga* was in 1910 in São Paulo by Hartmeyer (Van Name 1945), prior to the construction of the port of São Sebastião. *Clavelina oblonga* is common along the entire southern coast of Brazil (Rodrigues et al. 1998; Rocha and Nasser 1998, Lotufo 2002, Rocha and Faria 2005, Rocha and Kremer 2005) and frequently found on continental natural rocky substrates. Since *C. oblonga* is abundant and easily identified, its absence along the northern Brazilian coast indicates a disjunct distribution, as it is also found in the Caribbean. *C. oblonga* has not yet been found in the Alcatrazes archipelago which is 36 km from the coast in São Paulo (Rocha and Bonnet, in press), while it is common on continental natural rocky substrates nearby. In Santa Catarina *C. oblonga* is often found on the natural substrates of the Arvoredo Marine Reserve, but not usually in high abundance (Rocha et al. 2005a). In São Paulo, populations decline during the winter, possibly due to mortality caused by thermal minimum of this species (R. Rocha, unpub. data).

Distaplia bermudensis is another species found along the southeastern and southern coast of Brazil (Millar 1958; Rodrigues et al. 1998,

Rocha et al. 2005a; Rocha and Kremer 2005, Rocha and Costa 2005). *Distaplia bermudensis* was first recorded in 1958 by Millar and displays a continuous distribution on natural substrates from its northern limit in the state of Espírito Santo south to Paraná (Rocha and Kremer 2005). However *D. bermudensis* demonstrates a disjunct distribution with the Caribbean populations, as it is not found in Brazilian northeast coast (Figure 1). In Santa Catarina, *D. bermudensis* does not occur on natural rocky substrates and was first found in Penha, February 2004, indicating a recent extension of its distribution. *D. bermudensis* is not abundant in any region along the Brazilian coast.

Polyclinum constellatum was first found in Brazil in 1945 (in the state of Rio de Janeiro, Van Name 1945), it has one record in the state of Ceará and is common from Espírito Santo to Santa Catarina (Lotufo 2002), with the exception of Paraná (Rocha and Nasser 1998; Rocha and Faria 2005; Rocha and Kremer 2005). *Polyclinum constellatum* occurs usually in tropical waters. It was found in shellfish cultures in Hong Kong (Huang 2003, Table 2), ports in French Polynesia (Monniot et al. 1985), altered environments (e.g. shrimp farms, Monniot and Monniot 1987) and was introduced in Texas, USA (Lambert et al. 2005). The wide geographical distribution of *P. constellatum*, the absence in the oyster farm in Florianópolis and on natural substrates in Santa Catarina (Rocha et al. 2005a) suggest that this species was probably introduced in southern Brazil (Table 1), but since colonies are small and infrequent it seems that this species is not undergoing rapid establishment.

The geographical distribution of *Aplidium accareense* includes the western coast of Africa (Senegal and Monniot 1969; Lafargue and Wahl 1986; Ghana and Millar 1953) and the Brazilian coast (São Paulo, Rodrigues et al. 1998 – as *Aplidium* sp.; Santa Catarina, Rocha et al. 2005a). While little information is available describing *A. accareense* habitat in Africa, presumably this species was found in natural settings. In São Paulo and in Santa Catarina, this species occurs on natural substrates and in shellfish farms. Since localities in Africa are very tropical, *A. accareense* absence in Rio de Janeiro is surprising, since the tunicate fauna is well-known there and there are no barriers for dispersal between São Paulo and Rio de Janeiro.

Table 2. Records of locations in which tunicates form incrustations on aquaculture: M = mussels, O = oyster, P = *Pinctata*, S = scallops.

Taxa	M	O	P	S	Location	Latitude	Source
Cionidae							
<i>Ciona intestinalis</i> (Linnaeus, 1767)	X	X			HK, NZ, Japan, Canada, Spain, South Africa, Chile	Temp, subtr	Huang 2003, Forrest 2007, Arakawa 1990, Howes et al. 2007, Carver et al. 2003, Perera et al. 1990, Castilla et al. 2005, Ramsay et al 2008
Corellidae							
<i>Corella eumyota</i> Traustedt, 1882	X				NZ	Temp	Forrest 2007
<i>Corella japonica</i> Herdman, 1880		X			Japan	Subtr	Arakawa 1990
Asciidiidae							
<i>Ascidia longistriata</i> Hartmeyer, 1906	?			?	HK	Subtr	Huang 2003
<i>Asciidiella scabra</i> (Muller, 1776)				X	Isle of Mann	Temp	Ross et al. 2004
<i>Asciidiella aspersa</i> (Muller, 1776)		X			Spain	Trop	Perera et al. 1990
Perophoridae							
<i>Perophora japonica</i> Oka, 1927		X			Japan	Subtr	Arakawa 1990
<i>Perophora viridis</i> Verrill, 1871		X			Spain	Trop	Perera et al. 1990
Clavelinidae							
<i>Clavelina lepadiformis</i> Muller, 1776	X	X			Spain	Trop	Perera et al. 1990
Polyclinidae							
<i>Polyclinum constellatum</i> Savigny, 1816	?			?	HK	Subtr	Huang 2003
<i>Aplidium densum</i> (Giard, 1872)	X	X			Spain	Trop	Perera et al. 1990
Didemnidae							
<i>Diplosoma listerianum</i> (Milne-Edwards, 1841)	X	X		X	Isle of Man, Adriatic Sea, Japan, HK	Temp, Subtr	Ross et al. 2004, Igic 1972, Arakawa 1990, Huang 2003
<i>Didemnum aspersum</i> Tokioka 1953	?			?	HK	Subtr	Huang 2003
<i>Didemnum</i> sp.					Australia		Guenther et al. 2006
<i>Didemnum vexillum</i> Kott, 2002	X		X		USA, NZ	Temp	Bullard et al. 2007, Coutts and Forrest 2007
<i>Trididemnum cereum</i> (Giard, 1872)		X			Spain	Trop	Perera et al. 1990
<i>Lissoclinum perforatum</i> (Giard 1872)	X	X			Spain	Trop	Perera et al. 1990
Styelidae							
<i>Botrylloides leachi</i> (Savigny, 1816)	X	X			NZ, Spain	Temp, Trop	Forrest 2007, Perera et al. 1990
<i>Botrylloides violaceus</i> Oka, 1927	X	X		?	Japan, HK, USA, Canada	Subtr	Arakawa 1990, Huang 2003, Bullard et al. 2006, MacNair et al. 2007
<i>Botryllus schlosseri</i> (Pallas, 1766)	X				NZ, Canada	Temp	Forrest 2007, Ramsay et al. 2008
<i>Polyandrocarpa zorritensis</i> (Van Name, 1931)		X			Spain	Trop	Perera et al. 1990
<i>Polycarpa pomaria</i> (Savigny, 1816)		X			Spain	Trop	Perera et al. 1990

Table 2 (continued)

Taxa	M	O	P	S	Location	Latitude	Source
<i>Cnemidocarpa bicornuata</i> (Sluiter, 1900)	X				NZ	Temp	Forrest 2007
<i>Asterocarpa humilis</i> (Heller, 1878)		X			Chile	Temp	Castilla et al. 2005
<i>Styela canopus</i> (Savigny, 1816)	?			?	HK	Subtr	Huang 2003
<i>Styela clava</i> Herdman, 1881	X	X			Canada, Japan	Temp, Subtr	LeBlanc et al. 2007, Locke et al 2007, Bourke et al 2007, Ramsay et al 2008, Arakawa 1990
<i>Styela plicata</i> (Lesueur, 1823)	X	X		?	Spain, Japan, HK	Trop, Subtr, Temp	Perera et al. 1990, Arakawa 1990, Huang 2003
<i>Styela rustica</i> (Linnaeus, 1767)	X				Russia	Temp	Khalaman 2001
Pyuridae							
<i>Microcosmus australis</i> Herdman, 1899	?			?	HK	Subtr	Huang 2003
<i>Microcosmus exasperatus</i> Heller, 1878		X			Spain, HK	Trop, Subtr	Perera et al. 1990, Huang 2003
<i>Microcosmus squamiger</i> Hartmeyer and Michaelsen, 1928		X			Mexico	Trop	Rodriguez 2005
<i>Herdmania momus</i> (Savigny, 1816)	?			?	HK	Subtr	Huang 2003
<i>Pyura dura</i> (Heller, 1877)		X			Spain	Trop	Perera et al. 1990
<i>Boltenia echinata</i> (Linnaeus, 1767)	X				Russia	Temp	Khalaman 2001
Molgulidae							
<i>Molgula ficus</i> (Macdonald, 1859)		X			Chile	Temp	Castilla et al. 2005
<i>Molgula</i> sp.	X				Russia	Temp	Khalaman 2001
<i>Molgula</i> sp.	X				Canada	Temp	LeBlanc et al. 2003

NZ – New Zealand, HK – Hong Kong, ? – not clear from the reference on which culture the species was found; Temp – temperate, Subtr – subtropical, Trop – tropical

In Africa *A. accarensis* absence farther south is explained by accumulation of sand and mud between the mouths of the Niger and Congo Rivers (Longhurst 2007) resulting in the reduction of adequate substrate. This evidence suggests the possibility of an introduction, however until evidence is available, we classify the species as cryptogenic.

In Brazil *Didemnum perlucidum* was first recorded in São Paulo (Rocha and Monniot 1995), is found in Bahia (Lotufo 2002), but has not been observed in Paraná or on natural substrates in Santa Catarina (Rocha et al. 2005a). *Didemnum perlucidum* is common in disturbed habitats and artificial substrates (Monniot and Monniot 1997) and is an opportunistic colonizer (L. Kremer, unpub. data). The species often forms very large colonies that grow over other organisms such as bivalves, barnacles and *S. plicata* and so it is possibly damaging the

bivalve industry. Its high abundance on artificial substrates in São Paulo (Lotufo 1997) and Santa Catarina suggests invasive potential, but only small colonies in cryptic habitats occur in natural substrates. Local transportation on boat hulls was observed.

Lissoclinum fragile is found on vertical rocky walls in the Arvoredo Marine Reserve (Rocha et al. 2005a). The first record of *L. fragile* in Brazil is recent (Rodrigues et al. 1998) and its distribution along the Brazilian coast is scattered. *Lissoclinum fragile* is an abundant species in port regions, suggesting ship transport as a potential vector (Monniot et al. 1985; Rocha and Kremer 2005). Because *L. fragile* is found worldwide, it is not possible to determine its original distribution. Genetic study of this species could resolve its dispersion pattern. This species is not abundant on aquaculture equipment or bivalves.

Diplosoma listerianum is now found globally (Lambert 2001) including Brazil. A complete description of *D. listerianum* status in Brazil can be found in Rocha and Kremer (2005). The species is also found encrusting bivalve shells in other shellfish cultures (Table 2). The colonies usually stops growth at the margin of the bivalve shelves, and since it is very thin it is probably harmless to bivalve growth, but a study testing this hypothesis is still wanting.

The introduced *Ascidia sydneiensis* is only observed between Rio de Janeiro and Santa Catarina on the Brazilian coast (Figure 1) and was found in Florianópolis in 1962 (Rodrigues 1962). While rare, it is found mostly on artificial substrate (Rocha and Kremer 2005). Records worldwide include only tropical and warm temperate regions (Van Name 1945). In Penha, *A. sydneiensis* was found in 1998, only on shells of an experimental culture of *Nodipecten nodosus* (Linnaeus, 1758). In recent surveys, *A. sydneiensis* was always found inside lantern nets of both oysters and this pectinid. In the oyster culture gear, the species was abundant in early summer in lantern nets that were left submerged over four months without cleaning, but was rare on cultures during winter. *A. sydneiensis* apparently requires unoccupied space to settle, since plates with pre-existing encrusting communities deployed for another experiment were not invaded by this species during summer. While introduced, this species does not appear to be rapidly establishing in Brazilian waters.

Botrylloides giganteum on the Brazilian coast is restricted to the states of Espírito Santo, Rio de Janeiro (Aron and Sole-Cava 1991; Lotufo 2002), São Paulo (Rodrigues and Rocha 1993), and now Santa Catarina, but not Paraná (Rocha and Nasser 1998; Rocha and Faria 2005; Rocha and Kremer 2005). This species is also found in Senegal (Pérès 1949; Monniot 1969) and South Africa (Millar 1962). In both São Paulo and Santa Catarina, specimens were only found on artificial substrates. The species was not found in Penha during surveys in 1998 – 1999, or in older surveys in São Paulo and Santa Catarina (Rodrigues 1962). It is quite likely that *B. giganteum* was introduced to Brazil and that its distribution is now spreading along the coast. Colonies of *B. giganteum* may be large and thick and, if uncontrolled, this species could cause important losses to shellfish cultures, by increasing weight and smothering mussel shells.

Although *Botrylloides nigrum* occurs in the Indian and Pacific Oceans, most records suggest

its original distribution is the western Atlantic (see Figure 17 in Rocha and Kremer 2005). *Botrylloides nigrum* usually grows on other organisms (Rodrigues and Rocha 1993), both animals and plants, and we observed that it can be transported by hull fouling. This species is also common on artificial substrates such as ship wrecks and PVC structures (R. Rocha, pers. obs.), although not yet reported on other shellfish culture gear as observed for other species of the Botryllinae (Table 2). Colonies are very thin and delicate suggesting that they do not harm bivalves, unless in very high densities, but this hypothesis needs to be tested.

Symplegma brakenhielmi is observed in natural and artificial substrates along the Brazilian coast, from Paraíba to Santa Catarina (Rodrigues et al 1998; Lotufo 2002; Rocha and Faria 2005; Rocha et al 2005). This species has a wide geographical distribution, with most of the records from the Indian and the Pacific Ocean under the name *S. oceanica* (Kott 1985; Monniot and Monniot 1997). In the Atlantic it has also been confounded with *S. viride*, which does not occur in Brazil (Couto 2003).

Symplegma rubra has a scattered distribution (Rocha and Kremer 2005) even in the Caribbean where it is well known (Rocha et al. 2005b). In Brazil, the northernmost record was in Espírito Santo until 2002 (Lotufo 2002). Recently *S. rubra* was reported on rocks in the intertidal region in Paraíba (Gama et al. 2006) and in Baía de Todos os Santos, Bahia on riprap during surveys in 1999 and 2004 (R. Rocha, unpub. data). If these are new arrivals or rather fortuitous findings of a rare species is difficult to say. *Symplegma rubra* is not abundant on shellfish farms in Santa Catarina and did not appear in the survey of 1998-1999. It was also not found on natural substrates of Arvoredo Marine Reserve (Rocha et al. 2005a), all suggesting a possible recent introduction.

Styela canopus is found in Brazil from Rio Grande do Norte to Santa Catarina (Rocha and Kremer 2005), but is never highly abundant and is typically associated with altered environments including artificial substrates (Rocha and Kremer 2005). This species has been reported in shellfish farms in Hong Kong (Huang 2003) (Table 2).

The first report of *Polycarpa spongiabilis* in Brazil, Rio de Janeiro, was by Traustedt (1883), who described a specimen collected in 1836 (Van der Sloot 1969). The next record of *P. spongiabilis* (as *P. obtecta*) was in 1945 from São Sebastião, São Paulo and São Francisco,

Santa Catarina (Van Name 1945). This species today is also found in tropical Pernambuco (Lotufo 2002) and Bahia (R. Rocha, unpub. data) and is very common in the Caribbean (Van der Sloom 1969; Rocha et al. 2005b), which suggests a possible tropical origin. Our observations support this hypothesis as this species appeared on experimental plates in Florianópolis only during the summer, when surface water temperature peaked at 29°C. *Polycarpa spongiabilis* was unexpected in Florianópolis and it was probably introduced. This species has not been reported on bivalve farming in other countries, but two other species of *Polycarpa* have been known to encrust shellfish cultures in Spain (Table 2).

Microcosmus exasperatus has been documented from Pernambuco (Millar 1977) to Santa Catarina (Lotufo 2002) in Brazil, but is rare south of São Paulo, even in ports and shellfish farms. Its geographical distribution suggests that it originates from tropical and subtropical waters (Rocha and Kremer 2005) and that Santa Catarina is the southern limit of its distribution where the species appears on both shellfish farms and natural substrates (Rocha et al. 2005a). This species has been observed in oyster farms in the Mediterranean (Perera et al. 1990) and in Hong Kong (Huang 2003) (Table 2). In the Mediterranean *M. exasperatus* was introduced in or before 1963 (Monniot 1981).

Some surprising absences of species are notable including *Ciona intestinalis* and *Styela clava*. These species are often significant fouling species in shellfish farms and have been found to occupy nearly 100% of the available substrate at times (Howes et al. 2007; LeBlanc et al. 2007; Ramsay et al. 2008). Even though bivalve farms in Brazil are subtropical and fouled by a diverse community of exotic species, neither of these species were found in this study. However, *C. intestinalis* was found in São Paulo (Rodrigues et al. 1998) and Paraná (sample from 1968 in the Ascidiacea collection of Zoology Department, Universidade Federal do Paraná – DZUP CION01), and has become established in Rio de Janeiro between 1960-80 (A. Junqueira, personal communication). *Ciona intestinalis* was also recently found in the Alcatrazes Archipelago, São Paulo (Rocha and Bonnet, in press). Thus, *C. intestinalis* has been transported to the Brazilian coast and has established many times suggesting that future establishment in Santa Catarina is likely.

Although many of the species reported here are now apparently harmless, they pose a

potential threat to the shellfish culture industry and to native communities, since any change of environmental conditions could trigger exponential population growth. For example, exotic tunicate reproduction and growth has been shown to be favored due to warming of coastal waters in a temperate region (Stachowicz et al. 2002). Many of the species found in this study are apparently tropical and would be favored by an increase in water temperatures expected due to global warming.

Risk analysis (RA) is an important tool for designing and justifying management actions (McKindsey et al. 2007). We are not aware of any risk analysis for bioinvasions in the state of Santa Catarina. Therefore, a risk analysis to examine the risks of species introductions at each phase of bivalve culture and protocols to minimize introductions in this region are needed. While the invasive *Ciona intestinalis* and *Styela clava* are yet to be found in these waters, *C. intestinalis* has arrived many times in Brazil and a campaign toward public awareness is imperative to prevent its colonization of bivalve farms. Also, the diversity of introduced species in Brazil also requires constant monitoring for rapid population expansion as the diversity of life history and biological settings increases the probability of the appearance of a new pest.

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