UNIVERSIDADE FEDERAL DO PARANÁ

DANIEL DE MIRANDA LINS

DISTRIBUIÇÃO E IMPACTO DE ESPÉCIES INCRUSTANTES NA MARICULTURA DE SANTA CATARINA

CURITIBA

2021

DANIEL DE MIRANDA LINS

DISTRIBUIÇÃO E IMPACTO DE ESPÉCIES INCRUSTANTES NA MARICULTURA DE SANTA CATARINA

Tese apresentada ao curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ecologia e Conservação.

Orientadora: Profª. Drª. Rosana M. da Rocha

CURITIBA

2021

Universidade Federal do Paraná
Sistema de Bibliotecas
(Giana Mara Seniski Silva – CRB/9 1406)

Lins, Daniel Miranda de
Distribuição e impacto de espécies incrustantes na maricultura de Santa Catarina. / Daniel de Miranda Lins. – Curitiba, 2021.
152 p.: il.

Orientadora: Rosana M. da Rocha.

Tese (doutorado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ecologia e Conservação.

1. Bioinvasão. 2. Aquicultura. 3. Maricultura. 4. Incrustações marinhas. I. Título. II. Rocha, Rosana Moreira da, 1962-. III. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ecologia e Conservação.

CDD (22. ed.) 639.8

MINISTÉRIO DA EDUCAÇÃO SETOR DE CIÊNCIAS BIOLÓGICAS UNIVERSIDADE FEDERAL DO PARANÁ PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO PROGRAMA DE PÓS-GRADUAÇÃO ECOLOGIA E CONSERVAÇÃO - 40001016048P6

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **DANIEL DE MIRANDA LINS** intitulada: **Distribuição e impacto de espécies incrustantes na maricultura de Santa Catarina**, sob orientação da Profa. Dra. ROSANA MOREIRA DA ROCHA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 30 de Setembro de 2021.

Assinatura Eletrônica
01/10/2021 14:01:52.0
ROSANA MOREIRA DA ROCHA
Presidente da Banca Examinadora

Assinatura Eletrônica
12/10/2021 10:23:28.0
ANDREA LOCKE
Avaliador Externo (INSTITUTE OF OCEAN SCIENCES)

Assinatura Eletrônica
06/10/2021 15:54:36.0
ANDREA DE OLIVEIRA RIBEIRO JUNQUEIRA
Avaliador Externo (UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO)

Assinatura Eletrônica
04/10/2021 14:43:21.0
CLÁUDIO MANOEL RODRIGUES DE MELO
Avaliador Externo (UNIVERSIDADE FEDERAL DE SANTA CATARINA)

Assinatura Eletrônica
06/10/2021 12:10:03.0
JOEL CHRISTOPHER CREED
Avaliador Externo (UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO)

Ahó Mitakuye Oyasin

AGRADECIMENTOS

Não existem palavras para expressarmos tamanha gratidão, por muitas pessoas que ao longo desses anos apoiaram este trabalho. Aos amigos, familiares e colaboradores, muitíssimo obrigado.

Sou eternamente grato à minha orientadora, professora Drª Rosana Rocha. –à dupla dinâmica: Ro e Jim, que insistiram tanto, cutucando minha mente e contribuíram imensamente com a produção desta tese; Valeu!

Um abraço especial aos meus companheiros do LABIMAR e do Setor de Ciências Biológicas - UFPR, toda sua compreensão na convivência diária e por conversas paralelas altamente produtivas!

Também sou grato à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao programa de pós-graduação em Ecologia e Conservação por conceder-me a bolsa de estudos e especialmente a oportunidade fantástica, através do programa de internacionalização UFPR – PRINT de fazer o doutorado sanduíche. Gratidão à professora Dra Tamara Robinson e aos colegas da universidade de Stellenbosch na África do Sul, que me receberam de portas abertas com toda confiança e suporte técnico e financeiro. Um carinho especial para Tainã Loureiro e família.

Agradeço aos coautores dos capítulos, revisores, membros titulares e suplentes da banca avaliadora.

Aos maricultores de Santa Catarina -Avante!

Café para
mudar
aquilo que posso
Vinho para
aceitar
aquilo que
não posso.
(Autor desconhecido)

RESUMO

A maricultura é responsável tanto pela introdução intencional de espécies não nativas para fins comerciais, como por fornecer substrato para espécies epibiontes/incrustantes introduzidas, que podem reduzir a qualidade dos bivalves e causar a depreciação de equipamentos (boias, cordas, embarcações, etc.). Neste sentido, o Capítulo I e II apresentam uma lista de espécies-alvo com resultados de levantamentos de distribuição, frequência e do impacto das incrustações, realizados em oito fazendas de mexilhões nos municípios de maior produtividade em Santa Catarina. *Styela plicata* foi a espécie mais difundida e ocorreu em mais de 70% de todos os substratos avaliados em cada local. De norte a sul, outras espécies muito frequentes (> 50% dos substratos) foram *Megabalanus coccopoma*, *Aplidium accarense* e *Didemnum perlucidum* na Penha, *Schizoporella errata* em Governador Celso Ramos, *Branchiomma luctuosum* e *M. coccopoma* em Palhoça e Florianópolis. As espécies-alvo eram geralmente mais frequentes em boias do que em *long-lines*, e as principais espécies incrustantes eram *M. coccopoma*, *S. plicata* e *S. errata*. Já nas pencas de mexilhões, *S. plicata* (98% das pencas), *B. luctuosum*, *M. coccopoma*, *S. errata* (>50%) e *D. perlucidum* (40%). Também descobrimos que os mexilhões eram 19-36% menores em tamanho e até 60% mais leves quando recobertos pelas espécies *D. perlucidum*, *M. coccopoma* e/ou *S. errata,* mas, considerando a classificação de impacto, *Mytilus galloprovincialis* classificou-se com maiores impactos negativos para os produtores. Mytilus foi introduzido recentemente em Santa Catarina e tem causado prejuízos sem precedentes à produção na região de Bombinhas. O monitoramento do mexilhão invasor e de *Perna perna* em Bombinhas, ao longo de um ano, revela que o recrutamento é sazonal e semelhante ao longo do ano, tornando impossível evitar a espécie invasora nos coletores de Perna. Análises moleculares apontam para a possibilidade de um único evento de introdução e o Mar Mediterrâneo como a região de origem dos indivíduos introduzidos no estado. No Capítulo final desta tese, apresentamos um estudo do potencial da maricultura de Santa Catarina em tornar-se fonte de propágulos de espécies incrustantes para outras regiões do país. Considerando dois cenários representativos de emissões de gases do efeito estufa, descobriu-se que as áreas de habitat com alta adequabilidade ambiental permanecem no futuro (2040-50). Os estados de Pernambuco e Ceará, estão em maior risco devido à alta adequabilidade às espécies *D. perlucidum* e *M. coccopoma* e conectividade relativamente maior apontada pelo volume de cargas recebido em navios de contêineres saindo de Santa Catarina.

Palavras-chave: 1. Bioinvasão 2. Espécies exóticas 3. Espécies não-indígenas 4. Aquicultura 5. Espécies não-nativas

ABSTRACT

Mariculture is responsible both for the intentional introduction of non-native species for commercial purposes, as well as for providing substrate for introduced epibiont/fouling species, which can reduce the quality of bivalves and cause depreciation of equipment (buoys, ropes, vessels, etc.). In this sense, Chapter I and II present a list of target species with results of the distribution and frequency survey, carried out in eight mussel farms in the municipalities with higher productivity and estimates of species impact in southern Brazil. The ascidian *Styela plicata* was the most widespread species and occurred in more than 70% of all substrates evaluated at each site. From north to south, other very frequent species (> 50% of substrates) were the barnacle *Megabalanus coccopoma* and the ascidians *Aplidium accarense* and *Didemnum perlucidum* in Penha, the bryozoan *Schizoporella errata* in Governador Celso Ramos, the polychaete *Branchiomma luctuosum* and *M. coccopoma* in Palhoça and Florianópolis. The target species were usually more frequent on buoys than on long-lines, and the main fouling species were *M. coccopoma*, *S. plicata* and *S. errata*. Fouling mussel socks were *S. plicata* (98% of the socks), *B. luctuosum*, *M. coccopoma*, *S. errata* (>50%) and *D. perlucidum* (40%). We found that mussels were 19-36% smaller in size and weighed 60% less when covered by species *D. perlucidum*, *M. coccopoma* and/or *S.errata* but, considering the impact classification, *Mytilus galloprovincialis* ranked higher in the negative impacts to the producers. *Mytilus* was recently introduced in Santa Catarina and has caused unprecedented damage to production in Bombinhas. The monitoring of the invasive mussel and *Perna perna* in Bombinhas, over the course of one year, reveals that similar trends in seasonal recruitment makes it impossible to avoid the invasive species in *Perna* collectors. Molecular analyses point to the possibility of a single introduction event and the Mediterranean Sea as the region of origin of the individuals introduced into the state. In the final Chapter of this thesis, we present a study on the potential of Santa Catarina mariculture becoming a source of propagules of fouling species to other regions of the country. Considering two representative scenarios of greenhouse gas emissions, we found that habitat areas with high suitability remain in the future (2040-50). The states of Pernambuco and Ceará are at greater risk due to the high environmental suitability to the species *D. perlucidum* and *M. coccopoma* and connectivity indicated by the volume of goods transported in container ships with Santa Catarina as port of call.

Keywords: 1. Biological invasion 2. Exotic species 3. Non-indigenous species 4. Aquaculture 5. Non-native species

LISTA DE FIGURAS

Figure 2 – The impact due to fouling was mainly due to reduced growth (filled circles are fouled samples) as shown in scatter plots of tissue dry weight by shell dry weight, by fouling species (filled circles). A) *Didemnum perlucidum*, B) *Megabalanus coccopoma*, C) *Schizoporella errata*.

Figure 3 – Comparison of effect size (mean control value minus the individual fouled sample values) among fouling species in three size and quality variables in the brown mussel (*Perna perna*). A) Tissue dry weight (g), B) Shell weight (g) and C) Shell length (cm). Statistical results in Table 2.

Capítulo 3

Figure 1 – Detail of the coast of Santa Catarina including Florianópolis Island and study sites: 1. Zimbros and 2. Canto Grande (CGB), both in Bombinhas; 3. Sambaqui (SAF) and 4. Ribeirão da Ilha (RIF), Florianópolis north and south bays; 5. Palhoça (PAL).

Figure 2 – *Perna perna* (brown) and *Mytilus galloprovincialis* (Mediterranean) mussels from ~30-day-old collectors from Santa Catarina, Brazil with scale.

Figure 3 – Neighbour joining tree (NJ tree) of the four mussel populations from Brazil and reference populations of different species from the northern and southern hemispheres, based on the F_{ST} distance matrix from allele frequencies of the SNP loci. NJ tree obtained with POPTREEW and visualized with MEGA version 6. Population/sample codes as shown in Table 1 and Fig. 1. Bootstrap values are shown at nodes as percentages of 100000 replicates. Numbers at each node are well supported. Scale Bar represents 0.05 changes per nucleotide position.

Figure 4 – Structure plot (K = 3, 5) for four Brazil mussel populations and reference populations of different species from the northern and southern hemispheres.

Figure 5 – Neighbour joining tree of the four mussel populations from Brazil and reference samples of *Mytilus galloprovincialis* from different locations, based on the F_{ST} distance matrix from allele frequencies of the SNP loci. NJ tree obtained with POPTREEW and visualized with MEGA version 6. Population/sample codes as shown in Table 1 and Fig.

1. Bootstrap values are shown at nodes as percentages of 100000 replicates. Scale Bar indicates 0.005 changes per nucleotide position.

Figure 6 – Correspondence analysis of a) populations, and, b) individuals from four mussel samples from Brazil and reference samples of *Mytilus galloprovincialis* from different locations. Population/sample codes as shown in Table 1.

Figure 7 – Abundance of invasive (*Mytilus galloprovincialis*) and local (*Perna perna*) mussels recruited on experimental plates deployed at mussel farms from November 2018 until November 2019 in Zimbros and Canto Grande at Bombinhas municipality, south Brazil. Each point is the total count of recruits in 12 monthly replicates for each of the sites. Note the logarithmic scale of axis Y.

Figure 8 – Correlation of the abundance of recruits with maximum and minimum sea surface temperatures. Dots represent the sum of recruits on experimental plates deployed monthly at mussel farms from November 2018 until November 2019 in each of two sites (Zimbros and Canto Grande at Bombinhas municipality, south Brazil). Seawater temperature (monthly averages for each plate deployment period) obtained from https://SeaTemperature.org at Tijucas, Santa Catarina.

Capítulo 4

Figure 1 – *Aplidium accarense*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk, light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

Figure 2 – *Botrylloides giganteus*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

Figure 3 – *Didemnum perlucidum*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP2,6 in year 2050. D. future climate scenario, RCP6,0 in year 2050.

Figure 4 – *Styela plicata*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

Figure 5 – *Schizoporella errata*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP2,6 in year 2050. D. future climate scenario, RCP6,0 in year 2050.

Figure 6 – *Watersipora subtorquata.* A. Domestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP2,6 in year 2050. D. future climate scenario, RCP6,0 in year 2050.

Figure 7 – *Megabalanus coccopoma*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector

Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP2,6 in year 2050. D. future climate scenario, RCP6,0 in year 2050.

Figure 8 – *Mytilus galloprovincialis*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP2,6 in year 2050. D. future climate scenario, RCP6,0 in year 2050.

LISTA DE TABELAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

SUMÁRIO

INTRODUÇÃO GERAL

A maricultura muitas vezes enfrenta desafios financeiros devido à incrustação de espécies epibiontes porque esta reduz a produtividade e aumenta os custos associados à limpeza e manutenção de equipamentos. Fazendas marinhas são reservatórios de espécies incrustantes e desempenham um papel fundamental no estabelecimento e disseminação de espécies não nativas, introduzidas intencionalmente ou não em águas costeiras. A disseminação de espécies é ainda facilitada por um processo de trampolim, pelo movimento de cordas de recrutamento, lanternas de ostras e pencas de mexilhão entre as fazendas, e por embarcações de pesca e recreação que possam transitar nas proximidades de instalações de aquicultura (Wasson et al., 2001; Minchin 2007; Darbyson et al., 2009; Davidson et al., 2010, Adams et al., 2014; Mesel et al., 2015; Castro et al., 2017).

Um exemplo de uma espécie introduzida que se tornou invasora e causa impactos devastadores à maricultura é a ascídia *Ciona intestinalis* no Canadá, onde pode se tornar extremamente abundante e aumentar a mortalidade de mexilhões nativos *Mytilus edulis* (Daigle & Herbinger 2009), reduzindo a produtividade e aumentando os custos de produção. Os custos das incrustações variam geograficamente porque são consequência da composição da comunidade epibionte, dos regimes de abundância e recrutamento das espécies envolvidas e de sua dinâmica temporal de crescimento, que variam regionalmente (Lacoste & Gaertner-Mazouni 2015). Até o momento, estimativas sobre os impactos das espécies incrustantes, muitas vezes incluindo ascídias, sugerem que até 30% dos custos de produção são destinados para o combate de organismos incrustantes (Watson et al. 2009; Dürr & Watson 2010; Adams et al. 2011; Fletcher et al. 2013).

Um grande número de espécies incrustantes é introduzido fora de sua distribuição nativa e muitas se tornam invasoras (Rocha et al. 2009; Fitridge et al. 2012). As espécies se tornam invasoras se as barreiras que anteriormente limitavam o crescimento populacional forem removidas (Blackburn et al. 2011). Em muitas regiões, várias espécies introduzidas podem estar presentes. Como o oceano é um ambiente aberto, muito raramente uma invasão está associada a um único evento de introdução. As espécies são frequentemente introduzidas por navios mercantes que viajam entre oceanos (Hewitt & Campbell 2010) e são consideradas modelos para o estudo de bioinvasão. A taxa em que uma espécie é transportada como incrustação em áreas de nicho protegidas em cascos de navios e tanques de lastro (Coutts & Dodgshun 2007; Hulme 2009; Davidson et al., 2010) pode determinar a pressão de propágulos fundamental em uma determinada região e, eventualmente, a probabilidade de invasão, reduzindo os efeitos estocásticos relacionados à demografia e condições ambientais nas regiões receptoras (Simberloff 2009). Mesmo com muitos eventos de transporte e introdução, as espécies devem superar uma série de barreiras ecológicas e ambientais que, em geral, limitam o crescimento populacional e apenas uma fração das espécies transportadas e introduzidas se torna invasora (Blackburn et al., 2011). A ampla plasticidade fisiológica é uma característica comum às espécies invasoras (Broennimann et al., 2007), que permite a persistência de espécies introduzidas em alguns ambientes receptores de propágulos como portos, marinas e baías de fundeio (Piola & Johnston 2008). A abundância de indivíduos disponíveis na região doadora e a pressão propágulos introduzidos determinam, em grande parte, a probabilidade de invasões devido aos efeitos genéticos envolvidos na fase de estabelecimento (Blackburn et al., 2015). Uma vez que uma nova região é alcançada, a disponibilidade de substrato rígido para o estabelecimento de espécies incrustantes é imprescindível. Assim, a ampla disponibilidade de estruturas artificiais em áreas costeiras cultivadas aumenta a complexidade do habitat e fornece abrigo e recursos para muitas espécies introduzidas (Mckindsey et al., 2007; Ruiz et al., 2009; Mineur et al., 2012).

As ameaças à produção do estado de Santa Catarina incluem o impacto de espécies introduzidas, possivelmente através de portos internacionais localizados ao norte (Porto de Itajaí) e ao sul (Porto de Imbituba) do estado, que acarreta nos elevados custos de manejo das incrustações nas estruturas e leva à redução do crescimento dos mexilhões cultivados (Lins & Rocha 2020; Lins et al., 2021), assim como florações de algas (Alves & Mafra 2018). O número crescente de exemplos de espécies introduzidas associadas a impactos à aquicultura tem impulsionado avanços nas pesquisas que buscam identificar espécies prioritárias através das lentes da Ciência da Invasão. Recentemente, uma avaliação global de risco de introdução e disseminação de ascídias previu que as espécies *Didemnum vexillum* e *Styela clava* têm alto risco em invadir o sul do Oceano Atlântico (Lins et al., 2018). Ambas as espécies podem crescer rapidamente e em densidades extremas e são consideradas uma séria ameaça ao cultivo de bivalves. *Styela clava* pode atingir densidades de 500-1500 indivíduos por metro quadrado ao incrustar estruturas de fazendas marinhas na Ilha Prince Edward (Locke et al., 2007). Coutts e Forrest (2007) documentaram ações de manejo que não conseguiam gerenciar a invasão de *D. vexillum* na Nova Zelândia. Lições dessas experiências permitiram que esses autores listassem requisitos específicos para o sucesso de erradicação, que deveriam ser adotados para outras regiões. Sugerem as seguintes ações: aquisição de conhecimento de base e um regime de monitoramento eficaz de espécies introduzidas; o estabelecimento de linhas claras de autoridade e tomada de decisões rápidas; e quarentena eficaz para evitar a propagação. À medida que a gestão se torna cada vez mais difícil inerentemente ao desenvolvimento da cultura de mexilhão e conectividade entre regiões, medidas preventivas como essas devem ser efetivamente implementadas para reduzir impactos adicionais.

Embora a incrustação por espécies nativas em si exija o manejo periódico das estruturas como boias, cordas e embarcações, negligenciar a origem das espécies presentes e o fato de que espécies não nativas devem ser monitoradas de forma diferente poderia causar mais problemas com impactos à biodiversidade (Fitridge et al., 2012). Por exemplo, a propagação inadvertida de propágulos (fragmentos, larvas ou gametas) durante a limpeza de incrustações poderia facilitar a dispersão e colonização de ambientes naturais adjacentes às fazendas de marinhas (Coutts et al., 2010; Paetzold & Davidson 2010). A falta de uma resposta imediata à chegada da espécie invasora atrasa as ações de controle e pode resultar em perdas significativas com risco de as espécies se espalharem para áreas adjacentes (Coutts & Forrest 2007). Em muitos lugares, introduzidas pela aquicultura intencionalmente ou não, algumas espécies como o mexilhão do mediterrâneo *Mytilus galloprovincialis* e a ostra do Pacífico *Magallana gigas* (Thunberg, 1793) invadiram habitats naturais e deslocaram espécies nativas (Robinson et al., 2007; Herbert et al., 2016).

No sul do Brasil, a aquicultura de mexilhões é muito importante para a economia local (Suplicy et al., 2015), mas a instalação de estruturas aquícolas criou um novo impacto para a paisagem costeira, que tem favorecido a prevalência de espécies não nativas (Rocha et al., 2009). Embora a introdução intencional de espécies marinhas não nativas para o cultivo tenha um efeito importante para o desenvolvimento econômico e o crescimento da aquicultura marinha em nível mundial, a introdução não intencional de epibiontes/incrustantes, tem um impacto negativo para a rentabilidade da indústria (Fitridge et al., 2012). O impacto econômico negativo direto da incrustação está em grande parte relacionado ao custo de remoção de espécies indesejadas e à redução da produtividade (Bannister et al., 2019). Além disso, impactos negativos indiretos como a diminuição da biodiversidade nativa são mal compreendidos e não contabilizados, resultando em uma percepção inflada do benefício ecológico dos cultivos no estado de Santa Catarina (Suplicy et al., 2015).

A introdução de *Mytilus galloprovincialis* na África do Sul é um bom exemplo. Uma única fazenda introduziu o mexilhão na região sul do país de onde a espécie se espalhou para ambientes naturais adjacentes (McQuaid & Phillips 2000) com efeitos negativos à biodiversidade (Sadchatheeswaran et al., 2015). A importância da população cultivada nesta fazenda como fonte de propágulos ficou evidente após o abandono da atividade no local, o que levou à subsequente redução drástica da densidade das espécies invasoras nos ambientes naturais adjacentes (Rius et al., 2011). Assim, a maricultura pode criar problemas para a biodiversidade mediando a disseminação de propágulos de espécies epibiontes e também de espécies comerciais.

Nesta tese, focamos na distribuição e impacto associados à oito espécies incrustantes nos municípios de maior produtividade no estado de Santa Catarina com o objetivo de identificar espécies alvo e locais prioritários para ações de manejo. Nos quatro capítulos que seguem abordamos questões relacionadas aos impactos ambientais e econômicos de espécies invasoras em cultivos de mexilhões *Perna perna*.

REFERÊNCIAS

Adams, C.M., Shumway, S.E., Whitlatch, R.B., Getchis, T., 2011. Biofouling in Marine Molluscan Shellfish Aquaculture: A Survey Assessing the Business and Economic Implications of Mitigation. J. World Aquac. Soc. 42, 242–252. https://doi.org/10.1111/j.1749-7345.2011.00460.x

Adams, T.P., Miller, R.G., Aleynik, D., Burrows, M.T., 2014. Offshore marine renewable energy devices as stepping stones across biogeographical boundaries. J. Appl. Ecol. 51, 330–338. https://doi.org/10.1111/1365-2664.12207

Bannister, J., Sievers, M., Bush, F., Bloecher, N., 2019. Biofouling in marine aquaculture: a review of recent research and developments. Biofouling 35, 631–648. https://doi.org/10.1080/08927014.2019.1640214

Blackburn, T.M., Pys, P., Bacher, S., Carlton, J.T., Duncan, R.P., Wilson, J.R.U., Richardson, D.M., 2011. A proposed unified framework for biological invasions 26. https://doi.org/10.1016/j.tree.2011.03.023

Castro, M.C.T. De, Fileman, T.W., Hall-Spencer, J.M., 2017. Invasive species in the Northeastern and Southwestern Atlantic Ocean: A review. Mar. Pollut. Bull. 116, 41–47. https://doi.org/10.1016/j.marpolbul.2016.12.048

Coutts, A.D.M., Dodgshun, T.J., 2007. The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. Mar. Pollut. Bull. 54, 875–886. https://doi.org/10.1016/j.marpolbul.2007.03.011

Coutts, A.D.M., Forrest, B.M., 2007. Development and application of tools for incursion response: Lessons learned from the management of the fouling pest Didemnum vexillum. J. Exp. Mar. Bio. Ecol. 342, 154–162. https://doi.org/10.1016/j.jembe.2006.10.042

Daigle, R.M., Herbinger, C.M., 2009. Ecological interactions between the vase tunicate (Ciona intestinalis) and the farmed blue mussel (Mytilus edulis) in Nova Scotia, Canada. Aquat. Invasions 4, 177–187. https://doi.org/10.3391/ai.2009.4.1.18

Darbyson, E., Locke, A., Hanson, J.M., Martin Willison, J.H., 2009. Marine boating habits and the potential for spread of invasive species in the Gulf of St. Lawrence. Aquat. Invasions 4, 87–94. https://doi.org/10.3391/ai.2009.4.1.9

Davidson, I.C., Zabin, C.J., Chang, A.L., Brown, C.W., Sytsma, M.D., Ruiz, G.M., 2010. Recreational boats as potential vectors of marine organisms at an invasion hotspot. Aquat. Biol. 11, 179–191. https://doi.org/10.3354/ab00302

De Mesel, I., Kerckhof, F., Norro, A., Rumes, B., Degraer, S., 2015. Succession and seasonal dynamics of the epifauna community on offshore wind farm foundations and their role as stepping stones for non-indigenous species. Hydrobiologia 756, 37–50. https://doi.org/10.1007/s10750-014-2157-1

Fitridge, I., Dempster, T., Guenther, J., de Nys, R., 2012. The impact and control of biofouling in marine aquaculture: A review. Biofouling 28, 649–669. https://doi.org/10.1080/08927014.2012.700478

Fletcher, L.M., Forrest, B.M., Bell, J.J., 2013. Impacts of the invasive ascidian Didemnum vexillum on green-lipped mussel perna canaliculus aquaculture in New Zealand. Aquac. Environ. Interact. 4, 17–30. https://doi.org/10.3354/aei00069

Herbert JH, et al. (2016) Ecological impacts of non-native Pacific oysters (Crassostrea gigas) and management measures for protected areas in Europe. Biodiversity Conservation, vol. 25, pp. 2835–2865.

Hewitt, C.L. & Campbell, M.L., 2010. The relative contribution of vectors to the introduction and translocation of invasive marine species Keeping marine pests out of Australian waters.

Hulme, P.E., 2009. Trade, transport and trouble: Managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46, 10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x

Lacoste, E., Gaertner-Mazouni, N., 2015. Biofouling impact on production and ecosystem functioning: A review for bivalve aquaculture. Rev. Aquac. 7, 187–196. https://doi.org/10.1111/raq.12063

Letters, E., 2007. Evidence of climatic niche shift during biological invasion 701–709. https://doi.org/10.1111/j.1461-0248.2007.01060.x

Lins, D., Rocha, R., 2020. Cultivated brown mussel (Perna perna) size is reduced through the impact of three invasive fouling species in southern Brazil. Aquat. Invasions 15, 114–126. https://doi.org/10.3391/ai.2020.15.1.08

Lins, D.M., de Marco, P., Andrade, A.F.A., Rocha, R.M., 2018. Predicting global ascidian invasions. Divers. Distrib. 24, 692–704. https://doi.org/10.1111/ddi.12711

Lins, D.M., Zbawicka, M., Wenne, R., Poćwierz-Kotus, A., Molina, J.R.A., Alves, L.P., Rocha, R.M., 2021. Ecology and genetics of Mytilus galloprovincialis: A threat to bivalve aquaculture in southern Brazil. Aquaculture 540. https://doi.org/10.1016/j.aquaculture.2021.736753

Locke, A., Carman, M.R., Stefaniak, L., Lambert, G., Gittenberger, A., Zhang, H., Lin, S., Whitlatch, R.B., 2009. Genetic conspecificity of the worldwide populations of Didemnum vexillum Kott, 2002. Aquat. Invasions 4, 29–44. https://doi.org/10.3391/ai

Mckindsey, C.W., Landry, T., Beirn, F.X.O., Davies, I. a N.M., Davies, I. a N.M., 2007. Bivalve Aquaculture and Exotic Species : a Review of Ecological Considerations and Management Issues Bivalve Aquaculture and Exotic Species : a Review of Ecological Considerations and Management Issues 26, 281–294.

McQuaid, Christopher D., Arenas, F., 2009. Biological invasions: Insights from marine benthic communities. Mar. Hard Bottom Communities Patterns, Dyn. Divers. Chang. 321–332. https://doi.org/10.1007/978-3-540-92704-4

McQuaid, C.D., Phillips, T.E., 2000. Limited wind-driven dispersal of intertidal mussel larvae: In situ evidence from the plankton and the spread of the invasive species Mytilus galloprovincialis in South Africa. Mar. Ecol. Prog. Ser. 201, 211–220. https://doi.org/10.3354/meps201211

Minchin, D., 2007. Aquaculture and transport in a changing environment: Overlap and links in the spread of alien biota. Mar. Pollut. Bull. 55, 302–313. https://doi.org/10.1016/j.marpolbul.2006.11.017

Mineur, F., Cook, E., Minchin, D., Bohn, K., MacLeod, A., Maggs, C., 2012. Changing coasts 189–234. https://doi.org/10.1201/b12157-5

Paetzold, S.C., Davidson, J., 2010. Viability of golden star tunicate fragments after high-pressure water treatment. Aquaculture 303, 105–107. https://doi.org/10.1016/j.aquaculture.2010.03.004

Piola, R.F., Johnston, E.L., 2008. Pollution reduces native diversity and increases invader dominance in marine hard-substrate communities. Divers. Distrib. 14, 329–342. https://doi.org/10.1111/j.1472-4642.2007.00430.x

Rius, M., Turon, X., Bernardi, G., Volckaert, F. a M., Viard, F., 2015. Marine invasion genetics: from spatio-temporal patterns to evolutionary outcomes. Biol. Invasions 17, 869–885. https://doi.org/10.1007/s10530-014-0792-0

Robinson, T.B., Branch, G.M., Griffiths, C.L., Govender, A., Hockey, P.A.R., 2007. Changes in South African rocky intertidal invertebrate community structure associated with the invasion of the mussel Mytilus galloprovincialis. Mar. Ecol. Prog. Ser. 340, 163–171. https://doi.org/10.3354/meps340163

Rocha, R.M., Kremer, L.P., Baptista, M.S., Metri, R., 2009. Bivalve cultures provide habitat for exotic tunicates in Southern Brazil. Aquat. Invasions 4, 195–205. https://doi.org/10.3391/ai.2009.4.1.20

Sadchatheeswaran, S., Branch, G.M., Robinson, T.B., 2015. Changes in habitat complexity resulting from sequential invasions of a rocky shore: implications for community structure. Biol. Invasions 17, 1799–1816. https://doi.org/10.1007/s10530-014-0837-4

Santos, A.A. dos, Mondardo, M., Marchiori, N. da C., 2019. Presença de Mytilus cf. edulis platensis em fazendas marinhas de Bombinhas, Santa Catarina, Brasil. Agropecuária Catarinense 32, 53–55. https://doi.org/10.22491/rac.2019.v32n3.6

Sethi, S.A., Branch, T.A., Watson, R., 2010. Global fishery development patterns are driven by profit but not trophic level. Proc. Natl. Acad. Sci. U. S. A. 107, 12163–12167. https://doi.org/10.1073/pnas.1003236107

Simberloff, D., 2009. The Role of Propagule Pressure in Biological Invasions. Annu. Rev. Ecol. Evol. Syst. 40, 81–102. https://doi.org/10.1146/annurev.ecolsys.110308.120304

Suplicy, F.M., Vianna, L.F.D.N., Rupp, G.S., Novaes, A.L.T., Garbossa, L.H.P., de Souza, R. V., Guzenski, J., da Costa, S.W., Silva, F.M., dos Santos, A. a., 2015. Planning and management for sustainable coastal aquaculture development in Santa Catarina State, south Brazil. Rev. Aquac. 1–18. https://doi.org/10.1111/raq.12107

Wasson K, et al., 2001. Biological invasions of estuaries without international shipping: the importance of intraregional transport. Biological Conservation 102(2):143-153. [Okenia plana, Tenellia adspersa p. 146]. [. Biol. Conserv. 102, 143–153.

CAPÍTULO 1

INVASIVE SPECIES FOULING MUSSEL FARMS IN SOUTHERN BRAZIL

Formatado segundo normas da revista *Marine Pollution Bulletin*

Abstract

The southern Brazilian state of Santa Catarina is the leading producer of bivalve mollusks. The cities Penha, Governador Celso Ramos (GCR), Florianópolis and Palhoça are responsible for over 90% of the national Brown mussel (*Perna perna*) production which is also of extreme importance to the local economy. Despite this economic importance, challenges for the mussel production include the impact of invasive fouling species that increase management costs and can reduce mussel growth jeopardizing aquaculture profits. We evaluated the distribution of fouling non-native marine species in eight mussel farms between October and December 2017, through active search and visual census of buoys, long-line ropes and socks of mussels to determine the stage of the species invasion in the region and used a framework (SEICAT) to evaluate the ranges of impacts to mussel producers. The eight targeted introduced species occurred in all farms, with the exception of *Mytilus galloprovincialis*, which did not occur at the northern-most farms in Penha. The ascidian *Styela plicata* was the most widespread species and occurred in more than 70% of all substrates evaluated at each site. From north to south, other very frequent species (> 50% of substrates) were the barnacle *Megabalanus coccopoma* and the ascidians *Aplidium accarense* and *Didemnum perlucidum* in Penha, the bryozoan *Schizoporella errata* in GCR, the polychaete *Branchiomma luctuosum* in Palhoça, and *M. coccopoma* in Florianópolis. The target species were usually more frequent on buoys than on long-lines, and the main fouling species were *M. coccopoma, S. plicata* and *S. errata*. Fouling mussel socks were *S. plicata* (98% of the socks), *B. luctuosum*, *M. coccopoma*, *S. errata* (>50%) and *D. perlucidum* (40%). Considering the impact classification, *M. galloprovincialis* ranked higher in the negative impacts to the producers.

Introduction

Marine farms are reservoirs of fouling species and play a key role to the establishment and spread of species introduced in coastal waters. Farmed areas may extend over large ocean regions where their suspended artificial structures increase habitat complexity and provide shelter and resource to many taxa in the water (Mckindsey et al., 2007). The spread of non-indigenous marine species (NIMS) invasions are later facilitated by a stepping stone process used by natural dispersal, by stock movement between farms, and by both fishing and recreational vessels that may transit in the vicinity of aquaculture facilities (Wasson et al., 2001; Darbyson et al., 2009; Davidson et al., 2010).

The southern Brazilian state Santa Catarina (SC) is the leading producer of bivalve mollusks, an essentially family-owned business responsible for over 90% of the national Brown mussel (*Perna perna*) production which is also of extreme importance to the local economy (FAO 2014). Threats to the production include the impact of invasive NIMS introduced in international ports located to the north (Port of Itajaí) and to the south (Port of Imbituba) of the state, on management and mussel yield (Lins and Rocha 2020; Lins et al., 2021) and harmful algae outbreaks (Alves and Mafra 2018). The increasing number of examples of introduced species associated with impacts to shellfish farming has driven advances in research that seeks to identify priority species through the lenses of Invasion Science. Even though fouling by native species *per se* requires management, neglecting the presence of NIMS and the fact that they should be managed differently could cause further problems with impacts varying among habitats and locations and with the density of fouling species (Fitridge et al., 2012). For example, the inadvertently spread of propagules (fragments, larvae or gamets) during cleaning of NIMS from artificial substrates could enhance the colonization of natural environments adjacent of aquaculture farms (Coutts et al., 2010; Paetzold and Davidson 2010).

Over ten years passed since Rocha et al. (2009) published a call for public awareness and the development of preventive measures such as the monitoring the impacts of tunicate NIMS in Santa Catarina bivalve aquaculture but not much has been done. Stakeholders still perceive NIMS as pristine native species and not as introduced menaces from overseas. For instance, a major set-back from a precaution perspective was the erroneous identification of the NIMS *Mytilus galloprovincialis* mistaken in the region for the Southern Atlantic mussel *Mytilus platensis* (Santos et al., 2019). The interpretation that this species could be expanding naturally within the Warm Temperate Southwestern Atlantic biogeographic province (Spalding et al., 2007) was viewed as an opportunity to sell a differentiated foreign and a valuable product, instead of immediately intervening to eradicate the newcomer.

Failure to promptly respond to the arrival and subsequent spread of invasive species may delay management actions and result in significant crop losses with risk of species spreading to adjacent areas (Coutts and Forrest 2007). In many places, whether introduced by aquaculture intentionally or not, some NIMS like the Mediterranean mussel *M. galloprovincialis* and the Pacific oyster *Magallana gigas* (Thunberg, 1793) have invaded natural habitats and displaced native species (Robinson et al., 2007; Herbert et al., 2016).

Recently, a global risk assessment of introduction and spread of ascidians predicted that the species *Didemnum vexillum* and *Styela clava* have high risk in invading the Southern Hemisphere (Lins et al., 2018). Both species can growth in extreme densities and are considered a serious threat to shellfish industry. *Styela clava* can reach densities of 500-1500 individuals per square meter when fouling marine farms structures in Prince Edward Island (Locke et al., 2007). Coutts and Forrest (2007) documented the incursion response that failed to manage *D. vexillum* invasion in New Zealand. Lessons from those experiences allowed those authors to list particular requirements for eradication success, that should be adopted to other regions. They suggest the following actions: the acquisition of baseline knowledge and an effective surveillance regime of new arrivals; the establishment of clear lines of authority and rapid decision-making; and effective quarantine to prevent the spread of newcomers. As management becomes increasingly difficult inherently to the development of the mussel culture, preventive measures such as those must be effectively implemented to reduce additional impacts. For example, information on the relative susceptibilities of the substrates available at bivalve aquaculture (buoys, long-lines and mussel socks) to fouling NIMS can provide insights to develop a cost effective, customized management strategy with the participation of stakeholders to contain the impact of invasive species while monitoring for NIMS new introductions (Sievers et al., 2014).

To our knowledge, this kind of information and how local conditions influence fouling by NIMS remain largely unexplored. In this study we focused on eight sessile NIMS associated with the most productive region of mussel aquaculture in Brazil and tested whether localities and farm substrates have different susceptibility to fouling. We incorporated the size of each farm, farm´s distance to the nearest international port and recruitment (as a proxy for species realized propagule pressure) as factors into models to better understand their importance as drivers of NIMS in the region. We hypothesized that the probability of species occurrence will a) increase with farm size because bigger farms also provide more substrates susceptible to fouling, b) decrease with port distance given that ports represent the primary source of introduced species, c) increase with higher recruitment, because higher propagule pressure benefit the establishment of species.

We used the framework for biological invasions of Blackburn et al. (2011) to determine the stage of invasion of each the target species (established or invasive) in the region based of the frequency of the species in each locality and recruitment on experimental plates, as well literature information. We also assigned the impact concern of each species based on the SEICAT framework (Bacher et al., 2018), according to the level of deleterious impacts that we observed or that have been reported in the region.

Furthermore, we comment on policy options, management and regulation enforcement considering the distribution and relative abundance of the targeted species and their impacts in the region.

Methods

Study area

This study is focused on the Southwestern Atlantic Ocean, in a subtropical zone in the Brazilian state of Santa Catarina (Fig. 1). Among the study localities Palhoça leads the bivalve production with 8000 tons a year, followed by Florianópolis (900 t), Penha (600 t), and Governador Celso Ramos (250 t, Santos and Della-Giustina 2017). Eight mussel farms of variable sizes were accessed, two per locality (Fig. 1; Table 1). Marine farms are clustered in fairly protected bays near the coast, but can extend to over 600 m offshore. Depending on the magnitude of the ventures their infrastructure may vary, but all of them basically consist on main long-line cables arranged in parallel lines to the coast, from which vertical socks (filed with mussel spats) are kept hanging during growth. Buoys keep long-lines close to the surface and socks bottoms may reach as low as 3 m depth, and mooring on the extremities of the long-lines prevent their displacement by currents (Supplementary Fig. S1).

Target species

Target non-native species were selected based on their known occurrence in the coast of Santa Catarina (Kremer and Rocha 2011; 2016), and the matching of at least two of three different criteria: 1) history of introduction 2) history of invasion 3) easily recognized in the field.

Among the colonial ascidians, *Botrylloides giganteus* (Pérès, 1949) and *Didemnum perlucidum* Monniot F., 1983 are considered introduced (Dias et al., 2016 ; Rocha et al., 2019) while *Aplidium accarense* (Millar, 1953) is cryptogenic in the South West Atlantic. *Didemnum perlucidum* was already common in Penha mussel farms in a previous study (Kremer et al., 2010); It is also known to spread from artificial structures to seagrass beds (*Halophila ovalis*) in Western Australia (Simpson et al., 2016) and to overgrow sponges, corals, bryozoans, hydroids and mollusks in the Gulf of Mexico (Culbertson and Harper 2000). *Aplidium accarense* occurs in a few localities in both sides of the Atlantic Ocean,

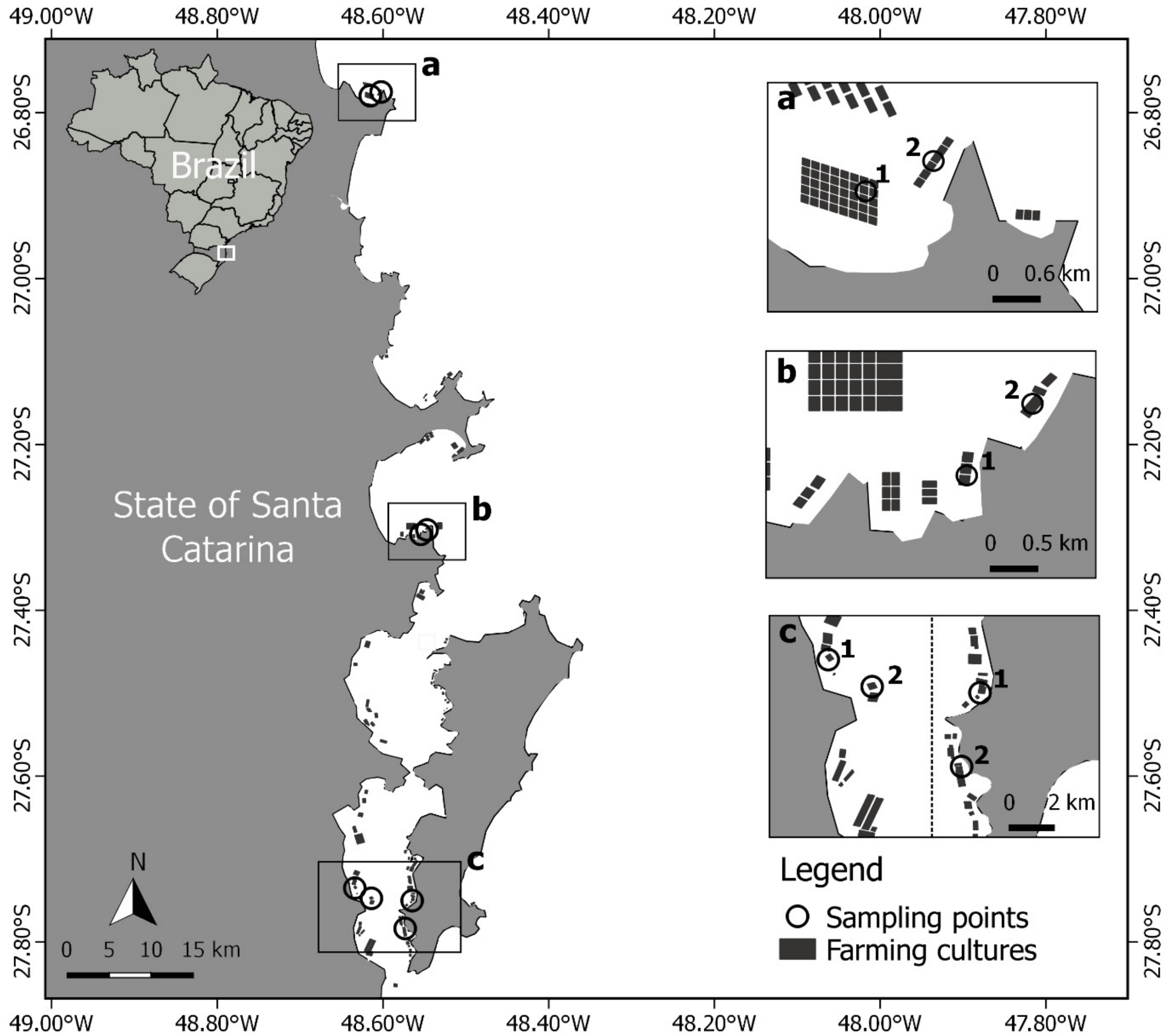


Fig. 1. Detail of the coast of Santa Catarina including Florianópolis Island and study localities: a) Penha; b) Gov. Celso Ramos; c) South Bay of Santa Catarina Island, Palhoça on the left panel and Ribeirão da Ilha – Florianópolis on the right panel. Areas that are licensed to bivalve farming are represented with dark polygons and circles represent the studied areas (see Table 1 for the geographic coordinates and site description).

and due to this disjunct distribution and unknown origin it is considered cryptogenic. The species *A. accarense* has been more recently introduced to the Mediterranean Sea where it is widespread in many harbors (López-Legentil et al., 2015). It has been found in Santa Catarina natural environments (Rocha et al., 2005), and is very common fouling mussel farms in Penha (Rocha et al., 2009). *Botrylloides giganteus* was recently reported introduced at the Baltra Navy dock in Galápagos (Lambert 2019). Invaded regions include southern California (Lambert and Lambert 2003), Italy, New Zealand and Australia (Rocha et al., 2019).

Table 1. Geographic coordinates of study sites, size of farmed areas and distance from the nearest international ports located to the north (Port of Itajaí) and to the south (Port of Imbituba) of the state of Santa Catarina, Southern Brazil.

Study sites	Coordinates	Farm Area (ha)	Distance to Port (Km)
Penha 1	26°46'50.6"S 48°36'42.3"W	5.27	14.06
Penha 2	26°46'40.0"S 48°36'14.1"W	0.72	15.03
Gov. Celso Ramos 1	27°18'27.1"S 48°33'12.0"W	0.91	46.68
Gov. Celso Ramos 2	27°18'07.2"S 48°32'44.1"W	0.5	46.27
Florianópolis 1	27°43'42.2"S 48°34'03.8"W	0.56	53.49
Florianópolis 2	27°47'02.5"S 48°34'21.8"W	2.9	49.66
Palhoça 1	27°44'07.8"S 48°38'01.6"W	2	54.49
Palhoça 2	27°44'49.3"S 48°36'48.6"W	6.9	53.24

The solitary ascidian *Styela plicata* and the cirripedia *Megabalanus coccopoma* (Darwin, 1854) are the only fouling species targeted in this study that are currently considered invasive by local Santa Catarina authorities (IMA-SC, 2016, but see Lins et al., 2020 for the impacts of *M. coccopoma*, *D. perlucidum* and *S. errata* on mussel yield). *Styela plicata* has been found in Brazil since late-19th and early-20th centuries, rarely occur in natural environments but is invasive on bivalve farms (Rocha and Kremer 2005) whereas *M. coccopoma* has rapid spread into natural environments despite its much more recent introduction, which has been reported in the country from the 70's (Lacombe and Monteiro 1974; Young 1994). Both species have successfully dispersed around the world encrusting ships, buoys and other man-made structures (Van Name 1945; Pérès 1951; Tokioka 1967; Newman and McConnaughey 1987; Kerckhof and Cattrijsse 2001; Ashton et al., 2016).

Such dispersal have resulted in the cosmopolitan distribution of both species (Barros et al., 2009; Yamaguchi et al., 2009; Pineda et al., 2011).

The bryozoan *Schizoporella errata* (Waters, 1878) was first described from the Mediterranean Sea where it is considered native, but its introduced range is poorly understood because of taxonomic confusion with related *Schizoporella* species (Tompsett et al., 2009). The taxonomic confusion also exists in a similar species, *Watersipora subtorquata* (d'Orbigny, 1852), particularly mistaken for *W. cucullata* (Busk, 1854) in the Mediterranean. For those bryozoans global morphological and molecular studies are still much needed (Mackie et al., 2006; Vieira et al., 2014). Introductions of *S. errata* and *W. subtorquata* have been related to the oyster aquaculture (*Magallana gigas*) (Ryland et al., 2009) and also confirmed to regions as far as the Pacific in Hawaii and in and Shark Bay, Australia (Vieira et al., 2014). In Brazil these bryozoans were first recorded in mid-19th century by d'Orbigny (1841 – 1847), *S. errata* is considered exotic and *W. subtorquata* is currently considered cryptogenic after applying Chapman and Carlton (1991) local and global criteria (Xavier et al., 2021). Both bryozoan species rank among the most hazardous target species in Australia based on overall impact (economic and environmental) (Haynes et al., 2005).

The fan worm polychaeta *Branchiomma luctuosum* (Grube, 1870) was introduced in several localities in the Mediterranean Sea where it ranks among the 100 'Worst Invasive species' (Streftaris and Zenetos 2006; Servello et al., 2019). In the Mediterranean reproductive season is between June and October (Mastrotoraro et al., 2014) and the species can be found from shallow to 30 meters depths, in a wide range of habitats and salinities (Haddad et al., 2007). In Brazil was first reported as *B. nigromaculatum* (Baird, 1865) and is currently widespread from Pernambuco to Santa Catarina been very common in the habitats of ports and recreational marinas (Nogueira et al., 2006; Oricchio et al., 2019).

We have added to our list of target species the Mediterranean mussel *Mytilus galloprovincialis*, only properly identified and reported to the region in 2020 (Belz et al., 2020). More recently the probable origin of the introduced populations that currently occur in Santa Catarina was assigned to the Mediterranean Sea (Lins et al., 2021). Farmers in the region have already raised concerns regarding the impacts of this species by competing with *Perna perna* for space on seed collectors (Santos et al., 2019). Competition with native species has also been observed in rocky shores of South Africa (Robinson et al., 2007). Additionally, there is the possibility of hybridization with native species, such was the case with *Mytilus platensis* in Puerto Madryn, Argentina (Zbawicka et al. 2018).

Spatial and Substrate Distribution

We actively searched for the target species on buoys, long-lines and mussel socks in the eight mussel farms, two farms per locality (Table 1). In order to determine the distribution of the target NIMS, we recorded their presence on approximately 15 buoys, 15 two-meter stretches of the main long-lines and 15 mussel socks per farm, between October and December of 2017. Due to variation in the amount of space availability to fouling among those substrates, abundance of the species was not estimated. The search was done in different regions of each farm area, considering long-lines near the coast, in the center of the farms and farther from the coast, whenever possible. Search time intervals were not determined *a priori* but search stopped after the realization that focal species could not be further found. Thus, search time varied due to the nature and spatial arrangement of structures in each farm. Segments of the long-lines could be analyzed usually in up to 1 minute, while search on buoys varied between 1 and 3 minutes depending on their size and also the degree of fouling and sediments making it difficult to find the target species. Long-lines and buoys were always assessed from boats, while mussel socks were often observed on land or sorting stations after harvest but before dismantling and the search time took between 3 to 5 minutes.

Drivers of species distribution

The recruitment rate of each focal species was measured as a proxy of the propagule pressure of the species in each farm. We submerged black polyethylene plates (12 x 12 cm) joined in pairs (sample unit), maintaining a 1.0 cm gap between plates with the aid of a pair of plastic hose as spacers. This methodology reduce the effects of predation on incrustation of the internal faces of the plates (Kremer and Rocha, 2016). Each unit was fixed horizontally at approximately 1.5 m deep, parallel to the ocean floor, to a rope hanging from the main long-lines of the mussel cultures and weighted with a bottled filled with sand and water to avoid flotation and too much movement caused by water hydrodynamism (Fig. S1). Twelve sample units were distributed in each of the eight sites, where they remained submerged for approximately 30 days. After this period the plates were removed from the water, labeled and fixed in 4% formalin solution and a new set of plates was fixed in the same ropes. The experiment was continuous between October 2017 and February 2018 and sampled monthly with a total of 384 submerged units at four recruitment intervals deployed to sample species settlement during spring and summer in the Southern Hemisphere. The relatively short interval (30 days) was long enough to have the interior of the plates fully covered by new recruits and not too long to allow competition for space and loss of information on the realized propagule supply.

In the laboratory the two internal faces of each unit were photographed and the number of recruits of the target species counted under a dissecting scope and summed to compose one sample unit. Photographs are available upon request. Ascidians were deposited in the scientific collections at the Zoological Department of UFPR. We used the sum of recruitment from the four months period monitoring plates of each target species at each farm as a proxy of species propagule pressure potential in the GLMM models together with the landscape variables describing the distribution of each species as described in the following method sections.

The landscape variables chosen to feed the GLMM models were 1. distance from the nearest international port, measured in kilometers as a straight line from each farm to the nearest port, and 2. total farm area, measured in hectares after drawing a polygon over the total farmed area close to which the farm we have accessed is located. Both measures were made with the georeferenced SIG online tool of the Local Development Plan for the Mariculture of Santa Catarina (PLDM).

Statistical analyses

Permutational analyses of variance (PERMANOVA) were applied to the presence – absence dataset in the four substrates analyzed to assess the significance of the following drivers: geographic location, which corresponded to the four localities (Penha, Governador Celso Ramos, Florianópolis and Palhoça) and substrate type (long-lines, buoys, mussel-socks and experimental recruitment units). In the case of significant factors, we ran permutational pairwise tests on levels of the factors. Similarly, we analyzed differences in relative dispersion among the groups determined by levels of significant factors using PERMDISP to verify whether the significant outcome in PERMANOVA was due to differences in the multivariate space, and not to differences in dispersion among the groups. Results were visualized with a non-metric multidimensional scaling (nMDS) plot. Analyses were carried out using Primer v. 6.1.13 statistical package (Clarke and Gorley 2006) with the PERMANOVA+ incorporated and using the Jaccard index. Recruits of *Watersipora subtorquata* were identified only on experimental plates under a magnifying dissecting scope and were not included in the analyses.

We also fitted general linear mix models (GLMM; McCullagh and Nelder 1989) to our binary (presence/absence) data describing the distribution of each species using a logistic regression (logit regression with logarithmic link function) and used the eight farms as a random factor with the explanatory variables: distance to the nearest port, farm area and recruitment. Overdispersion did not occur in any case. Presence/absence data were asymmetric (>absences) and so a cloglog link was used (Zuur et al., 2007). This approach provides a powerful statistical tool in the form of a ´regression type´ model to derive the simplest statistically significant model with the maximum explanatory power of the species response to the landscape and recruitment variables summed in this study. The best fitted model was determined by the sequential deletion of the least significant explanatory factors (or interaction) from the complete model including all fixed effects and interactions. Logarithmic transformation of variables was preferred when Akaike´s information criterion (AIC) indicated model improvements (Johnson and Omland 2004). The most influential factors on the distribution (presence/absence) of each species was defined as the percentage of null deviance explained by the model with that variable, and its significance was evaluated with chi-square tests on a deviance table (Zuur et al., 2007). The probability of occurrence of each species as a function of the most adequate factors revealed by the most parsimonious model was plotted using binomial logistic regression. Models were graphically validated by plotting each predictor against models' residuals. All tests were performed using R vers. 4 (R Core Team 2019).

Results

Seven species of the eight target species were present in all sites. The exception was *Mytilus galloprovincialis* which did not occur in Penha, locality further north where 158 artificial structures (recruitment plates and farm substrates pooled) where checked. *Styela plicata* is the most widely distributed fouling from 59% of all farm structures in site GCR 1 and up to 100% of the structures evaluated in Palhoça 1 (Table 2). The barnacle *Megabalanus coccopoma* is also widespread in the region but more prevalent in Penha (~80% of all substrates) and less common in Palhoça 1 where its frequency was as low as 14% (Table 2). *Schizoporella errata* occurrences were mainly concentrated in GCR (> 65%) but also common in Penha 1 fouling 58% of all the farm structures (Table 2). Other very frequent species (> 50%) are the ascidians *Aplidium accarense* in site Penha 2 and *Didemnum perlucidum* in Penha 1, the polychaeta *Branchiomma luctuosum* in both Palhoça sites and the mussel *M. galloprovincialis* in Palhoça 1 (Table 2). The ascidian *Botrylloides giganteus* was the least frequent species, only present in 45% of the structures analyzed in Palhoça 1 and 20% in Palhoça 2 (Table 2).

Table 2. Percentage of structures with eight target species in each of two farms analyzed per locality in Santa Catarina, southern Brazil.

Species	Penha 1 (53) *	Penha 2 (30)	GCR 1 (46)	GCR 2 (52)	Flor 1 (47)	Flor 2 (47)	Palh 1 (44)	Palh 2 (46)
Aplidium accarense (Millar, 1953)	45	63	0	13	0	19	2	13
Botrylloides giganteus (Pérès, 1949)	16	7	13	0	2	13	45	20
Branchiomma luctuosum (Grube, 1870)	30	13	2	8	36	40	52	67
Didemnum perlucidum Monniot F., 1983	79	43	0	8	17	38	2	24
Megabalanus coccopoma (Darwin, 1854)	79	80	33	60	70	74	14	24
Mytilus galloprovincialis Lamarck, 1819	0	0	22	15	13	23	61	17
Schizoporella errata (Waters, 1878)	58	20	65	67	0	26	27	7
Styela plicata (Lesueur, 1823)	70	90	59	85	74	85	100	89

* Sum of all mussel farms structures (long-lines, buoys and socks) analyzed in each farm. GCR – Governador Celso Ramos, Flor – Florianópolis and Palh – Palhoça (see detailed map at Fig. 1)

All the target species occurred in the substrates analyzed. Pooling data from 123 long-lines, 129 buoys and 113 mussel socks across all sites revealed that the least invaded structure in the region is the long-line (< 30%), although *S. plicata* reached 77% frequency on this substrate (Fig. 2). *Megabalanus coccopoma* is present in 80% of the buoys, followed by *S. plicata* (70%), *S. errata* (~ 40%), *D. perlucidum* (30%), and *A. accarense* and *B. giganteus* (20%). Mussel socks were mainly used by *S. plicata* (98%), *B. luctuosum*, *M. coccopoma*, *S. errata* (> 50%), *D. perlucidum* (40%), while *A. accarense* and *M. galloprovincialis* were present in 20% and *B. giganteus* in ~ 10%.

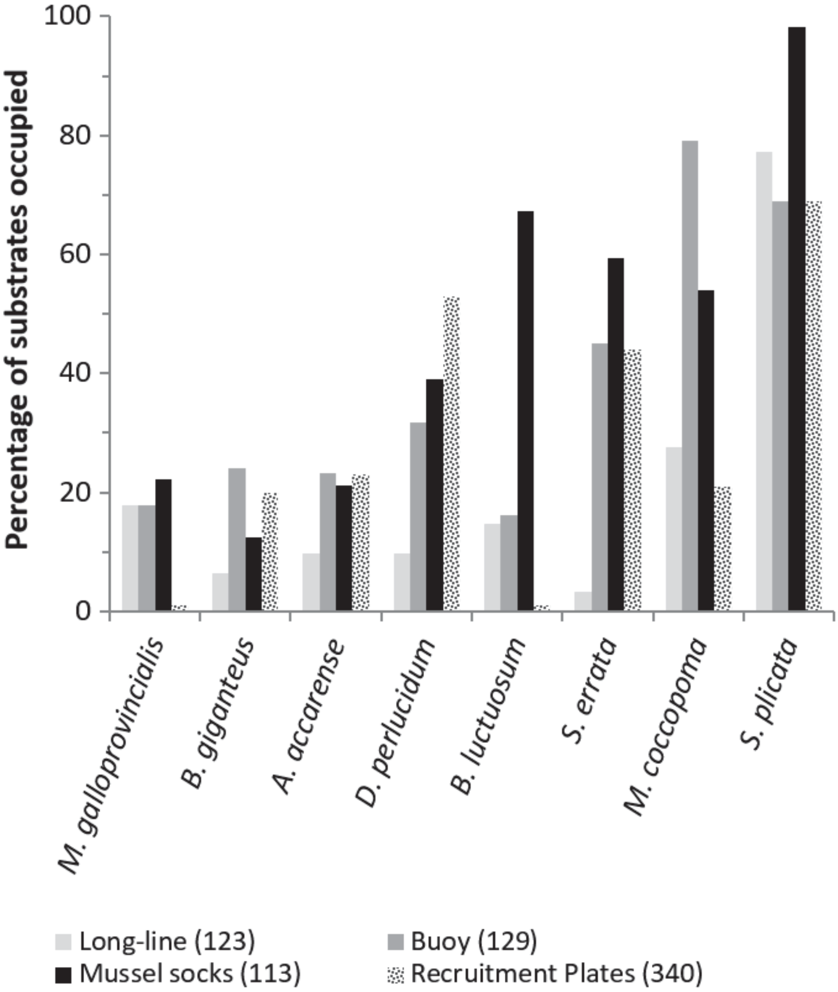


Fig. 2. Percentage of substrates occupied by each species, considering all four localities in Santa Catarina pooled. In parentheses is the total number of observations of each substrate (long-line, buoys, mussel socks and recruitment plates). Complete names of species as in Table 2.

Recruitment was not uniform across locations and species, and only five of the target species had an average abundance greater than 10 recruits in at least one of the localities and are reported here. In Penha *M. coccopoma*, *W. subtorquata* and *D. perlucidum* had the highest number of recruits (Fig. 3). In GCR, *S. errata* sustained the average recruitment of over thirty-five colonies per sample unit in the spring and increased towards summer, when it reached more than 50 colonies in every recruitment plate. In Florianópolis and Palhoça, the solitary ascidian *S. plicata* was the most abundant species to settle. Also in this region, the colonial ascidians *A. accarense* and *B. giganteus* had settled in higher numbers if compared to the localities north but with average of less than 10 colonies in recruitment plates. The species *B. luctuosum* and *M. galloprovincialis* rarely recruited during the study period.

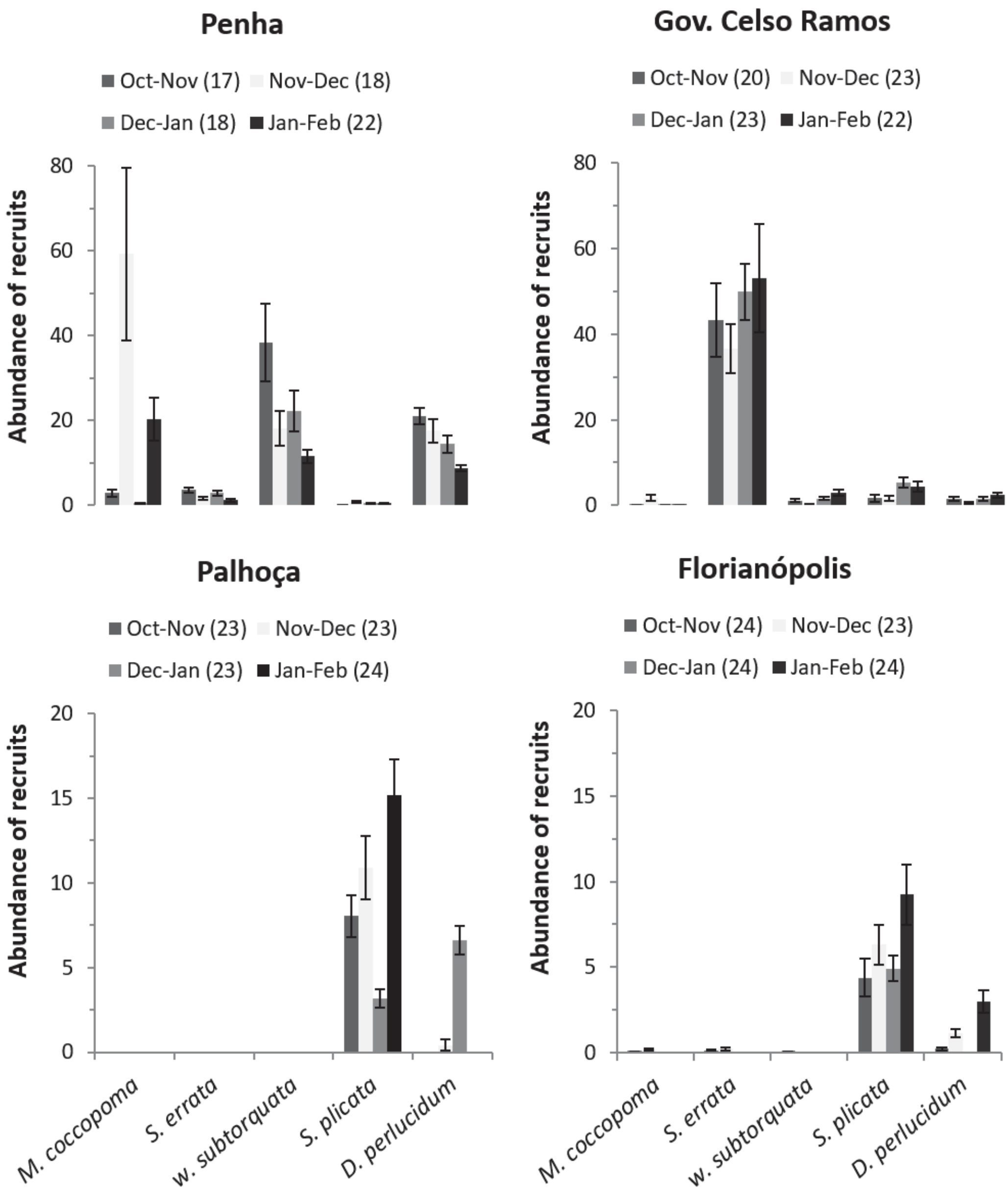


Fig. 3. Average number of recruits per unit (paired plates) deployed for approximately 30 days in mussel farms in the state of Santa Catarina, southern Brazil. In parentheses is the total number of units analyzed in each time series and the vertical lines are standard deviations. Only the five species with an average abundance greater than 10 recruits in at least one of the localities are represented. Note the y axis scales differ from up and down panels.

Based on the frequency of the target species on the localities and their presence on recruitment plates, we classified all species as established and invasive in the region, with the exception of *A. accarense* which is cryptogenic. They are already well spread in the region and recruited in most of the sites, showing self-sustainable populations, although recruitment of *M. galloprovincialis, B. luctuosum, A. accarense and B. giganteus* was very low on the plates. *Mytilus galloprovincialis* ranked higher in the SEICAT (Bacher et al., 2018) impact classification (MO- moderate concern) compared to the other target species which were classified as MN (minor concern, Table 3). It is the only species that had already caused reduction in the number of farmers since its introduction because of economic loss. Permutational analyses showed significant effect of localities and substrate types on target species community structure, which explained respectively 66 and 51% of the variation found. The effect of these two variables was not independent as demonstrated by the interaction. The PERMDISP analyses were also significant (Table 4), indicating heterogeneity in data dispersion across levels of the factors considered. Pairwise comparisons of the levels revealed that, there were significant differences between localities within substrate type with the exception of long-lines, when comparing Florianópolis and GCR or Florianópolis and Penha (Table S1). Pairwise comparisons between substrate types revealed significant differences within all the localities (Table S2).

The non-metric MDS plot based on presence-absence data (Jaccard index) showed Florianópolis and Palhoça points grouping while the other localities were more dispersed from each other. While buoys and mussel socks grouped withing each locality, long-lines of different localities grouped together and presented a distinct centroid (Fig. 4).

The best fit of GLMM models of most species was achieved with the variables distance to the port and recruitment, the exception being *B. luctuosum* for which the most parsimonious model was achieved with the variable Log (area cultivated) (Table 5; Fig. S4). The occurrence probability of *M. coccopoma*, *A. accarense* and *S. errata* was higher in the sites closer to the international ports and decreased as they move farther away, while this trend was opposite to *M. galloprovincialis*. Recruitment increased occurrence probabilities of the ascidians *B. giganteus*, *D. perlucidum* and the bryozoan *S. errata* but, to the widespread ascidian *S. plicata* recruitment had a strong opposite effect. The *S. plicata* GLMM model best fit included also the variables log (Area) and the interaction of recruitment and log (Area) (Table 5).

Table 3. Summary of results of the socio-economic (SEICAT) impact assessment of target non-indigenous marine species in Santa Catarina, Southern Brazil.

	SEICAT impact [1]	Confidence	Focal Area [2]	Description of SEICAT classification (adapted from Bacher et al., 2018)
Mytilus galloprovincialis	MO	High	Palhoça, Bombinhas[3]	Competition with *Perna perna* for space on recruitment ropes leading to a reduction in level of activity and partial abandonment of the business with no increase in opportunities due to the NIMS introduction because *M. galloprovincialis* detach from ropes before reaching commercial size and also during harvest.
Styela plicata	MN	High	Penha, Gov. Celso Ramos, Florianópolis, Palhoça	Synergy with other fouling species increase investment in cleaning (machinery and time) - reduces the profit; increase weight of crops causing occupational diseases or increase investment in machinery for lifting aquaculture gear.
Megabalanus coccopoma	MN	High	Penha, Gov. Celso Ramos, Palhoça	Synergy with other fouling species increase investment in cleaning (machinery and time) - reduces the profit; affects health due to injuries during cleaning and increase weight of crops causing occupational diseases or need to invest in PPE and tools; reduction of *Perna Perna* mussel yield.
Didemnum perlucidum	MN	High	Penha, Gov. Celso Ramos, Florianópolis	Synergy with other fouling species increase investment in cleaning (machinery and time) - reduces the profit; reduction of *Perna Perna* mussel yield.
Schizoporella errata	MN	High	Penha, Gov. Celso Ramos	Synergy with other fouling species increase investment in cleaning (machinery and time) - reduces the profit; reduction of *Perna Perna* mussel yield.
Branchiomma luctuosum	MN	Low	Penha, Florianópolis, Palhoça	Synergy with other fouling species increase investment in cleaning (machinery and time) - reduces the profit; no data on the impact on the growth of *Perna perna*.
Aplidium accarense	MN	Low	Penha, Palhoça	Synergy with other fouling species increase investment in cleaning (machinery and time) - reduces the profit; no data on the impact on the growth of *Perna perna*.
Botrylloides giganteus	MN	Low	Palhoça	Synergy with other fouling species increase investment in cleaning (machinery and time) - reduces the profit; no data on the impact on the growth of *Perna perna*.

[1] Abbreviation for impact classes: MN - Minor, MO - Moderate.
[2] Focal Area: the region in which the target species frequency in at least one substrate is above 40%
[3] Abundance assessed by Santos and Della Giustina 2017 and Santos et al., 2019 (see text for details).

Table 4. Permutational statistical analyses (PERMANOVA) of target species (list in table 1) among localities (Penha; Gov. Celso Ramos; Florianópolis; Palhoça) and substrates (long-lines, buoys, mussel-socks and recruitment plates)

	df	SS	pseudo-F	*p* value	*Permdisp*
Localities	3	276580.00	63.511	0.001	0.001
Substrates	3	239380.00	54.97	0.001	0.001
Interaction	9	164300.00	12.576	0.001	0.001
Residual	673	976920.00			

Analyses were performed for presence-absence (Jaccard index) data. PERMDISP probabilities of homogeneity of dispersion were also given.

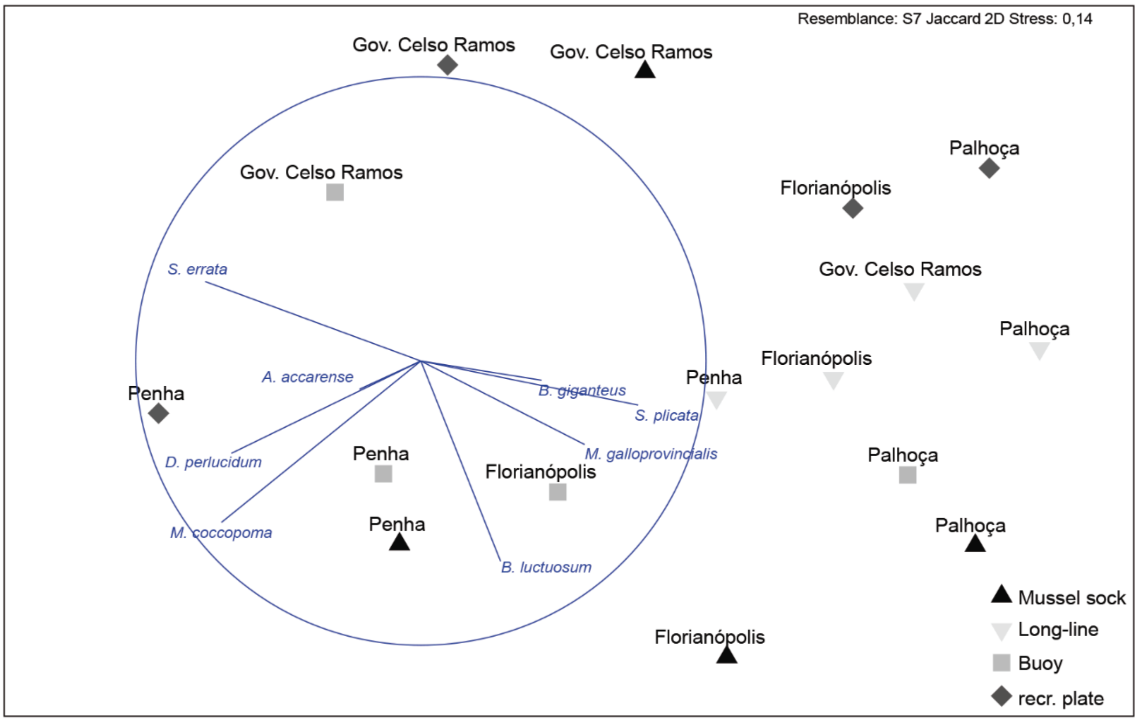


Fig. 4. Non-metric multidimensional scaling analysis (NMDS) plots based on fouling species presence-absence data (Jaccard index) shows the differentiation between localities and between mussel farms substrates, southern Brazil. Lines represent the strength and direction of the effects of each species rescaled for illustration (Species list in Table 2).

Table 5. Results of most parsimonious General Linear Mix Models (GLMM, family = binomial) to explain the presence of eight non-native target species in mussel aquaculture at 8 farms in south Brazil

	Estimate	Std. Error	Z value	p-value
A. accarense				
Intercept	0.7713	0.8335	0.925	0.3547
Port Distance	-0.07106	0.0205	-3.456	<0.001
B. giganteus				
Intercept	-2.7212	0.3801	-7.159	<0.001
(log) Recruitment	0.02425	0.0086	2.799	0.005
D. perlucidum				
Intercept	-5.6893	1.3637	-4.172	<0.001
(log) Recruitment	0.8834	0.2753	3.089	0.001
S. plicata				
Intercept	5.3079	2.096	2.532	0.0113
Recruitment	-0.0365	0.0111	-3.262	0.001
(log) Area	-0.4366	0.2195	-1.989	0.0467
Recruitment: (log) Area	0.004	0.0012	3.388	<0.001
B. luctuosum				
Intercept	-6.7397	2.1048	-3.202	<0.001
(log) Area	0.5787	0.2166	2.672	0.0075
M. coccopoma				
Intercept	2.1441	0.9225	2.324	0.0201
Port Distance	-0.047	0.0206	-2.284	0.0224
M. galloprovincialis				
Intercept	-7.9688	2.4827	-3.21	<0.001
Port Distance	0.1302	0.0489	2.66	0.0078
S. errata				
Intercept	1.8088	1.9223	0.941	0.3467
Recruitment	0.0009	0.0003	2.993	<0.005
(log) Port Distance	-0.9842	0.5441	-1.809	0.0747

Discussion

The widespread distribution of target species across all localities and substrates demonstrates well established populations fouling Santa Catarina mussel farms (Figs. S2 and S3). The region that concentrates the largest cultivated area is the South Bay of Florianópolis Island (Table 1), where Palhoça and Florianópolis are responsible for the production of approximately 9000 ton of mussels, which represented over 80% of the total production of the state in 2017 and over 95% of the national production (Santos and Della Giustina 2017). Given that these localities are practically at the same latitude only on opposite sides of the southern bay, environmental conditions are similar and the large differences in the extent of the cultivated areas between these localities could explain the higher frequencies in Palhoça of the species *B. giganteus*, *B. luctuosum* and *M. galloprovincialis* (Table 2). However, only *B. luctuosum* had the size in cultivated area as best predictor of the species occurrence, while the best predictors of *B. giganteus* is recruitment and *M. galloprovincialis* is port distance (Fig. S4). Both, Palhoça and Florianópolis receive ocean currents that enter the bay from the south and could potentially carry *M. galloprovincialis* larvae from ships that operate in the Imbituba port which is located 50 km away. Meanwhile, the species *M. coccopoma*, *A. accarense* and *S. errata* were inversely correlated with port distance. They were more frequent in Penha, that is only 15 km away from the Itajaí Port Complex in the northern zone of the region (Fig. S4). Palhoça, Florianópolis and Penha were the most invaded localities and also the most productive and should be prioritized for the monitoring and management of fouling NIMS.

The heterogeneity of species frequencies across the region indicates different stages in the process of invasion at each location. Bacher et al. (2018) proposed a framework to address socio-economic impacts (SEICAT) caused by non-indigenous species that use changes to human activities and/or well-being as currency to measure impact. Among the species studied here, *M. galloprovincialis* had the highest socio-economic impact. This species recruits on the ropes used to gather *Perna perna* seeds and reduce the total amount of seeds to be cultivated. When the farmers tried to cultivate *Mytillus* in the socks they lost most of the crop during storms and report that this species is not well accepted by the public. As a consequence, this NIMS has been able to produce negative effects to the entire community of farmers of Bombinhas, another municipality not included in this study, with reports of lower production and fewer people participating in the activity than before the appearance of this NIMS (Santos and Della Giustina 2017; Santos et al., 2019). The remaining species produced negative impacts, but no change in the size of the bivalve aquaculture is reported, and this is why they have been classified as of minor concern, although there are recognized social impacts. In general, the impact of the other target species included economic loss and health reduction. An example of the first is the cost to buy individual protection equipment and machinery to clean the fouling (Novaes et al., 2019), and an example of the second is the increase in weight of ropes and mussel socks that cause increase of labor effort resulting in impact on human health, such as back and joint injuries (Novaes et al., 2017). In addition, the species *M. coccopoma*, *S. errata* and *D.* perlucidum are also related to income loss as a result of reduced productivity and yield (Lins and Rocha, 2020). Considering the size and thickness of their colonies, the ascidians *A. accarense* and *B. giganteus* most likely affect mussel grow in the same way, but we conservatively applied low confidence to their negative impacts because these species occur in low frequencies and their specific impacts have not been studied. Such is the case of *B. luctuosum* that settles on the socks among the cultivate mussels (90% of the mussel socks cultivated in Florianópolis, Palhoça and Penha) but with less than 20% frequency in buoys and long lines (Fig. S3), but assessments of direct effects on mussel growth is still lacking.

Some flagship species are born out of necessity to raise awareness of authorities and stakeholders on important issues, and here we propose the NIMS *Mytilus galloprovincialis* to be such species because of its impact on shellfish production in Santa Catarina and potential impact on natural communities as well. After a sudden increase in the densities of this invasive mussel that was followed by reports of extensive profit losses (Santos and Della Giustina 2017), stakeholders have promoted public hearings, meetings and also created a technical panel to discuss management strategies. In this sense, the species became an ambassador for all the fouling invasive species that threat the business sustainability. There are extensive scientific records on historical introductions of the Mediterranean mussel and the species ranks among the 100 most invasive species worldwide (Lowe et al., 2000). So far, *M. galloprovincialis* occurrences were not reported from natural environments and to our knowledge the species is restricted to man-made structures in the region. *Mytilus galloprovincialis* competes for space with *Perna perna* in collectors of spats because in SC settlement (effort and timing) for both species is the same along the year, which makes it impossible to avoid the recruitment of the invasive species on collectors of *P. perna* spats (Lins et al., 2021). Molecular data of individuals from Santa Catarina indicate possible single introduction event at Bombinhas and similarity with the eastern Mediterranean and Black Sea populations (Lins et al., 2021). This does not mean that the species have been transported directly from this region because there is the possibility of the transport from intermediate regions used as “stepping stones" (Apte et al., 2000).

Santa Catarina is among the most important destinations for sailing and yachting worldwide and also one of the top nautical hubs for container ships nationwide (ANTAQ, 2021), with wide variety of possible vectors for the introduction and spread of NIMS. Even so, our survey in 2017-2018 failed to register the presence of *M. galloprovincialis* either on fouling farm structures or on recruitment plates at the northernmost locality (Penha), but in 2019, while conducting other surveys, we have assessed a few mussel socks harvested at Penha with fully grown *M. galloprovincialis.* The shells were attached to the inner ropes of the mussel socks, compatible with the hypotheses that their seeds were mixed with the Brown mussel seeds. Later we discovered that most of the mussel socks grown in Penha are seeded with juvenile Brown mussels recruited from other localities (e.g., Palhoça, producer collaborator *pers. communication*) or acquired from natural stocks on rocky shores (Marenzi 2004) and juveniles of *M. galloprovincialis* could have been introduced in this locality together with *Perna perna* seeds. Therefore, we encourage future studies to address all possible local vectors of *M. galloprovincialis* to Penha and management efforts to avoid the establishment of the species in this locality.

To our knowledge Bombinhas is the north limit where the species is well established. The municipality of Bombinhas was not in our survey, but one year after we started this study, we learned that it is also the epicenter of *Mytilus* invasion where the species dominance over *Perna* in recruitment ropes and the gregarious behavior have scaled to tremendous proportions which resulted in production lost under the effect of winds and waves making part of the producers abandoned the activity. Santos et al. (2019) evaluated 20 farms at Canto Grande and Zimbros beaches and found dominance of this invasive species in 14 of the farms sampled, and proportion of 69% of *Mytilus* to 31% of *Perna* that accounted for 646 ton of the invasive species from the 936 ton of the total mussels produced in this locality in 2017.

Based on the historical impacts in other regions, the species should be flagged for immediate management and actions should be taken to prevent further introductions at Penha, GCR and Florianópolis and contain the species at Bombinhas and Palhoça, which could be important sources of propagules due to the high prevalence of the species (Table 2).

The community structure is different between the localities in all but one substrate, therefore movement of mussel socks and buoys between localities should be done only after the removal of fouling species. On the other hand, the similarities of long-lines at Florianópolis and GCR and also Florianópolis and Penha (Table S1), suggest that the exchange of surface collectors between these localities would have little impact shifting species composition. The practice of exchanging equipment among localities must be approached with caution. It is used to mitigate for shortage of Brown mussel settlement but protocols should be improved to avoid increased propagule pressure of NIMS and risk of further biological invasions. To transport stocks producers are required to dispatch a document that must accompany mussel shipments and describe the animals being transported (*Guia de Transporte Animal* – GTA), but currently these documents are issue without warning the presence of *M. galloprovincialis* or other fouling NIMS. In compliance with best practices, socks should be completely cleaned of epibionts before introduction in other localities. If this practice proves to be impossible to enforce, then the transport document, should include a declaration that socks are free of any species within a list of target pests. In this case we suggest that the species that we target in the present study should be in the list used, but additional vagile species should be considered too.

Once NIMS spread to natural environments and become invasive, attempts to reduce the populations are extremely costly and eradication rarely successful (Forrest and Hopkins 2013), and for this reason farmers and authorities are strongly advised to promptly respond upon detection of invasive NIMS introductions. We suggest a task force to clean within a short time span once a year all fouling substrates including submerged and floating structures like boat hulls and moorings in the localities most affected by invasions. In Penha due to the prevalence of *M. coccopoma* and seasonality of the species reproductive cycle and lifespan in the water column (Raymond 1983), structures should be cleaned at least one month after the species spawning, to avoid the risk of making clear substrates available for the barnacle settlement. Furthermore, authorities and stakeholders should allocate resources to support the incursions aimed to mitigate the impacts of *Mytilus* before they scale to the point of causing Major impacts (sensu, Bacher et al., 2018) and spread to other regions currently less affected.

Little is known on actual cost of mussel production per kg/area/year in Santa Catarina, since most of the family-owned business and also producers who hire day-today help do not keep financial balances in check. Costs are roughly estimated between R$ 0.82 – 1.47/kg of mussel in the artisanal system (Suplicy 2019). Activities linked to fouling species management account for 42% of variable costs of production and many farmers labor diseases are linked to those activities (Rodrigues et al., 2007; Guertler et al., 2016).

Mariculture production is still rudimentary and with low levels of productivity in comparison with other countries such as Chile that became hundred-tons producers with the use of mechanized systems (FAO 2014; Novaes et al., 2019).

Fouling control can be labor intensive and time consuming. Simple and environmentally friendly treatments like exposition to air, mechanical and water pressured removal and immersion in water-based solutions depend on minimal technological improvements. Currently only the farm studied at the locality Palhoça 2, which has a system of cultivation based on continuous long-lines (instead of socks) and already has machinery to lift buoys and lines of mussels, could possibly implement those treatments with minor changes to their current operation. The most common management strategy for removing fouling is to turn buoys upside down to expose the species to the air and subsequently scrape them often discarding the by-products in the water. This may happen after a crop cycle (once a year), but many farmers perform the task only and if biofouling mass cause the sinking of the long-lines making excessively labor intensive to harvest mussels. By-products discards comprise living individuals, fragments or propagules that can re-infest the cultures, and should not be disposed off directly in the sea. Their survivor is lower in high turbidity and sedimentary environments with high predation rates and when fragments are small (Hopkins et al., 2011). Thus, cleaning of the structures and testing fouling control treatments should consider the local environmental conditions and prioritize areas and seasons ensuring highest propagule mortality of eventual releases.

Recruitment was a good predictor of some of the species occurrence such as *D. perlucidum* and *S. errata* (Fig. S4). Therefore, a secondary effect of cleaning the buoys and other structures of the farms is the reduction of adult population and consequently the settlement of those species on mussel socks. While *D. perlucidum* recruitment was low in our experiment, recruitment of *S. errata* was high during the four months of the experiment in Governador Celso Ramos (Fig. 3), locality that should be particularly benefited by cleaning the structures.

Prevention is usually less costly than remediation, and one alternative to reduce fouling settlement is the use of aeration (Bullard et al., 2010). Although some investment will be necessary to implement pumps and hoses, this method does not require the heavy machinery necessary to suspend and treat the socks and could be implemented even in systems of cultivation using continuous long-lines. There are a variety of aerators used to produce ‘bubble curtains’ and protect aquaculture nets that could be adapted and tested in areas of high density of farms, like Penha that is a high productive locality and is one of the most affected by invasions. Testing this treatment efficiency in the field is necessary, given that it has been tested in a small experimental scale (Bullard et al., 2010) and that disturbance during mussel growth may lower livestock yield and can cause reverse reactions due to over management making new, clear substrate available for fouling species (Clark and Johnston 2011; Sievers et al., 2017; Viola et al., 2018).

Authorities began to regulate the sector two decades after first farms implementation and even now aquaculture farmers in Santa Catarina are not well organized in regional cooperatives and associations (Suplicy 2019). In order to have good aquaculture governance and to ensure the sustainability of the sector, stakeholders should participate in the development and implementation of standards of environmental compliance (FAO 2017). For example, the adoption of continuous programs to monitor and mitigate environmental impacts in aquaculture facilities and adjacent regions is complementary for eco-labeling acquisition, which allows the business to growth and reach international markets as well. Given the great task of providing sustainable food security in a changing environment, stakeholders should create in each locality a technical committee and engage the shellfish community providing means to secure ecological awareness, to actively participate in the traceability, best handling practices, sanitary and task forces, much needed to monitor and control fouling invasive species.

ACKNOWLEDGMENTS

We would like to express our deepest gratitude to all the mussel farmers who have supported us in the field. This research was partially funded by a research grant to RMR provided by the National Council for the Scientific and Technological Development – Brazil (CNPq) and a scholarship to DML provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES, finance Code 001).

References

Alves TP, Mafra LL (2018) Diel variations in cell abundance and trophic transfer of diarrheic toxins during a massive dinophysis bloom in southern Brazil. Toxins 10:1–19. doi: 10.3390/toxins10060232

Apte S, Holland BS, Godwin LS, Gardner JPA (2000) Jumping ship: a stepping stone event mediating transfer of non-indigenous species via a potentially unsuitable environment. Biol. Invasions 2, 75–79.

Ashton G V, Davidson IC, Geller J, Ruiz GM (2016) Disentangling the biogeography of ship biofouling: barnacles in the Northeast Pacific. Glob Ecol Biogeogr 25:739–750. doi: 10.1111/geb.12450

Bacher S, Blackbur, TM, Essl F, et al (2018) Socio-economic impact classification of alien taxa (SEICAT). Methods Ecol. Evol. 9, 159–168. doi: 10.1111/2041-210X.12844

Blackburn TM, Pys P, Bacher S, et al (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339. doi: 10.1016/j.tree.2011.03.023

Blackburn TM, Lockwood JL, Cassey P (2015) The influence of numbers on invasion success. Mol. Ecol. 24, 1942–1953.

Barros RC, Rocha RM, Pie MR (2009) Human-mediated global dispersion of S*tyela plicata* (Tunicata, Ascidiacea). Aquat Invasions 4:45–57. doi: 10.3391/ai.2009.4.1.4

Bullard SG, Shumway SE, Davis C V (2010) The use of aeration as a simple and environmentally sound means to prevent biofouling. Biofouling 26, 587–593 doi: 10.1080/08927014.2010.496038

Chapman JW, and Carlton JT (1991) A Test of Criteria for Introduced Species: The global invasion by the Isopod *Synidotea Laevidorsalis* (Miers, 1881). Journal of Crustacean Biology, 11(3), 386–400. doi:10.2307/1548465

Clarke KR, Gorley RN (2006) PRIMER v6: user manual/tutorial. Plymouth: Primer-E

Clark GF, Johnston EL (2009) Propagule pressure and disturbance interact to overcome biotic resistance of marine invertebrate communities. Oikos 118:1679–1686. doi: 10.1111/j.1600-0706.2009.17564.x

Clark GF, Johnston EL (2011) Temporal change in the diversity – invasibility relationship in the presence of a disturbance regime. Ecol Lett 14:52–57. doi: 10.1111/j.1461-0248.2010.01550.x

Coles SL, Reath PR, Skelton PA, et al (2003) Introduced marine species in Pago Pago Harbor, Fagatele Bay and The National Park Coast, American Samoa. Bish Museum Tech Rep No 26

Cohen AN, and Carlton JT (1995) Nonindigenous aquatic species in a United States estuary: a case study of the biological invasions of the San Francisco Bay and Delta. A report for the United States Fish and Wildlife Service, Washington, DC and the National Sea Grant College Program Connecticut Sea Grant, 246 pp

Courchamp F, Fournier A, Bellard C, Bertelsmeier C, Bonnaud E, Jeschke JM, Russell JC (2017) Invasion Biology: Specific Problems and Possible Solutions. Trends in Ecology & Evolution 32: 13–22. https://doi.org/10.1016/j.tree.2016.11.001

Coutts ADM, Dodgshun TJ (2007) The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. Mar Pollut Bull 54:875–886. doi: 10.1016/j.marpolbul.2007.03.011

Coutts ADM, Forrest BM (2007) Development and application of tools for incursion response: Lessons learned from the management of the fouling pest Didemnum vexillum. J. Exp. Mar. Bio. Ecol. 342, 154–162. https://doi.org/10.1016/j.jembe.2006.10.042

Coutts ADM, Valentine JP, Edgar GJ, Davey A, Burgess-Wilson B (2010) Removing vessels from the water for biofouling treatment has the potential to introduce mobile non-indigenous marine species. Mar. Pollut. Bull. 60, 1533–1540. https://doi.org/10.1016/j.marpolbul.2010.04.015

Culbertson J, Harper D (2000) Settlement of a colonial ascidian on an artificial reef in the Gulf of Mexico. In: Mckay M, Nides J, Vigil D (eds) Proceedings of Gulf of Mexico fish and fisheries: Bringing together new and recent research. OCS Reports, US Minerals Management Service, no 2002-2004, New 503 Orleans, LA, pp 614-630

Darbyson E, Locke A, Hanson JM, Martin Willison JH (2009) Marine boating habits and the potential for spread of invasive species in the Gulf of St. Lawrence. Aquat Invasions 4:87–94. doi: 10.3391/ai.2009.4.1.9

Davidson IC, Zabin CJ, Chang AL, et al (2010) Recreational boats as potential vectors of marine organisms at an invasion hotspot. Aquat Biol 11:179–191. doi: 10.3354/ab00302

Dias PJ, Rocha R, Godwin S, et al (2016) Investigating the cryptogenic status of the sea squirt *Didemnum perlucidum* (Tunicata, ascidiacea) in Australia based on a molecular study of its global distribution. Aquat Invasions 11:239–245. doi: 10.3391/ai.2016.11.3.02

FAO (2014) The State of World Fisheries and Aquaculture. Rome

FAO (2017) Aquaculture development. 7. Aquaculture governance and sector development. FAO Technical Guidelines for Responsible Fisheries. No. 5. Suppl. 7. Rome, Italy. 50 pp.

Fitridge I, Dempster T, Guenther J, de Nys R (2012) The impact and control of biofouling in marine aquaculture: A review. Biofouling 28, 649–669. https://doi.org/10.1080/08927014.2012.700478

Forrest Barrie M, and Hopkins GA (2013) Population control to mitigate the spread of marine pests: insights from management of the Asian kelp *Undaria pinnatifida* and colonial ascidian *Didemnum vexillum*. Management of Biological Invasions, vol. 4, No. 4, pp. 317–326

Geller J, Mackie JA, Schroeder G, and Gerhinger D (2008) Distribution of highly invasive bryozoans belonging to a cryptic species complex in the genus *Watersipora* determined by DNA sequences. Moss Landing Marine Laboratories, Moss Landing, 40 pp.

Guertler C, Mari G, Mannrich G, et al (2016) Aquacultural Engineering Occupational health and safety management in Oyster culture. Aquac Eng 70:63–72. doi: 10.1016/j.aquaeng.2015.11.002

Haddad M, Azzati RC, García-Carrascosa AM (2007) *Branchiomma luctuosum* (Polychaeta: Sabellidae): a non-indigenous species at Valencia Port (western Mediterranean Sea, Spain). Marine Biodiversity Records, Spain, p. 1-8, 2007.

Halpern BS, Frazier M, Potapenko J, et al (2015) Spatial and temporal changes in cumulative human impacts on the world's ocean. Nat Commun 6:1–7. doi: 10.1038/ncomms8615

Hayes K, Sliwa C, Migus S, McEnnulty F, Dunstan P (2005) National priority pests: Part II Ranking of Australian marine pests. An independent report undertaken for the Department of Environment and Heritage by CSIRO Marine Research.http://www.marine.csiro.au/crimp/reports/PriorityPestsFinalreport.pdf

Hayward PJ, Ryland JS (1999). Cheilostomatous Bryozoa Part 2 Hippothooideae Celleporoidea, second ed. In: Synopses of the British Fauna (New Series) No. 14. Published for the Linnean Society of London and The Estuarine and Coastal Sciences Association by The Field Studies Council, Shrewsbury

Herbert JH, et al. (2016) Ecological impacts of non-native Pacific oysters (*Crassostrea gigas*) and management measures for protected areas in Europe. Biodiversity Conservation, vol. 25, pp. 2835–2865.

Hishamunda N, Ridler N, Martone E (2014) Policy and Governance in Aquaculture: Lessons learned and way forward. FAO Fisheries and Technical Paper No. 577, 59 pp. FAO, Rome.

Hopkins GA, Forrest BM, Piola RF, Gardner JP (2011). Factors affecting survivorship of defouled communities and the effect of fragmentation on establishment success. J. Exp. Mar. Bio. Ecol. 396, 233–243. https://doi.org/10.1016/j.jembe.2010.10.027

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. J Appl Ecol 46:10–18. doi: 10.1111/j.1365-2664.2008.01600.x

IMA-SC (2016) Lista comentada de espécies exóticas invasoras do estado de Santa Catarina: espécies que ameaçam a diversidade biológica. Instituto de Meio Ambiente – IMA, Florianópolis, 88 pp.

Johnson JB and Omland KS (2004) Model selection in ecology and evolution. Trends Ecol. Evol., 19, 101–108

Keough MJ, Ross J (1999) Introduced fouling species in Port Phillip Bay., In: Hewitt, C. L.; Campbell; M.;Thresher, R.; Martin,(Eds.). Marine Biological Invasions of Port Phillip Bay, Victoria. Center for Research on Introduced Marine Pests, CSIRO Marine Research. Hobart, Tasmania. Pp. 193-225

Kerckhof F, Cattrijsse A (2001) Exotic Cirripedia (Balanomorpha) from Buoys off the Belgian Coast. Senckenbergiana maritima 31:245–254

Kloh AS, Farrapeira CR, Rigo A, Rocha RM (2013) Intertidal native and introduced barnacles in Brazil: distribution and abundance. Marine Biodiversity Records doi:10.1017/S1755267213000766; Vol. 6; e102; 2013

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204. doi: 10.1016/S0169-5347(01)02101-2

Kremer LP, da Rocha RM (2016) The biotic resistance role of fish predation in fouling communities. Biol Invasions 18:3223–3237. doi: 10.1007/s10530-016-1210-6

Kremer LP, Rocha RM (2011) The role of *Didemnum perlucidum* F. Monniot, 1983 (Tunicata, Ascidiacea) in a marine fouling community. Aquat Invasions 6:441–449. doi: 10.3391/ai.2011.6.4.09

Kremer LP, Rocha RM, Roper JJ (2010) An experimental test of colonization ability in the potentially invasive *Didemnum perlucidum* (Tunicata, Ascidiacea). Biol Invasions 12:1581–1590. doi: 10.1007/s10530-009-9571-8

Lambert CC, Lambert G (2003) Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. 259:145–161

Lins DM, de Marco P, Andrade AFA, Rocha RM (2018) Predicting global ascidian invasions. Divers. Distrib. 24, 692–704. https://doi.org/10.1111/ddi.12711

Lins DM, and Rocha RM (2020) Cultivated brown mussel (*Perna perna*) size is reduced through the impact of three invasive fouling species in southern Brazil. Aquat Invasions 15, 114–126. https://doi.org/10.3391/ai.2020.15.1.08

Lins DM, Zbawicka M, Wenne R, Poćwierz-Kotus A, Molina JR, Alves LP, Rocha RM (2021) Ecology and genetics of *Mytilus galloprovincialis*: A threat to bivalve aquaculture in southern Brazil 540. https://doi.org/10.1016/j.aquaculture.2021.736753

Locke A, Hanson JM, Ellis KM, et al. (2007) Invasion of the southern Gulf of St. Lawrence by the clubbed tunicate (*Styela clava* Herdman): potential mechanisms for invasions of Prince Edward Island estuaries. Journal of Experimental Marine Biology and Ecology [Proceedings of the 1st International Invasive Sea Squirt Conference, Woods Hole, Massachusetts, USA, April 21-22, 2005.], 342(1):69-77. http://www.sciencedirect.com/science/journal/00220981

Lord JP (2016) Impact of seawater temperature on growth and recruitment of invasive fouling species at the global scale. Mar Ecol 1–10. doi: 10.1111/maec.12404

Lord JP (2017) Temperature, space availability, and species assemblages impact competition in global fouling communities. Biol Invasions 19:43–55. doi: 10.1007/s10530-016-1262-7

Lord JP, Calini JM, Whitlatch RB (2015) Influence of seawater temperature and shipping on the spread and establishment of marine fouling species. Mar Biol 162:2481–2492. doi: 10.1007/s00227-015-2737-2

Lowe S, Browne M, Boudjelais S, De Poorter M (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. Published by The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN). Aliens. Invas. Spec. Bull. 12, 1–12.

Mackie JA, Keough MJ, Christidis L (2006) Invasion patterns inferred from cytochrome oxidase I sequences in three bryozoans, Bugula neritina, *Watersipora subtorquata*, and Watersipora arcuata. 285–295. doi: 10.1007/s00227-005-0196-x

Marenzi AWC (2004) Development of mussels *Perna perna* (Linnaeus, 1758) (Mollusca-Bivalvia) in culture in south Brazil. J Coast Res II:1102–1105

Mastrototaro F, Chimienti G, Matarrese A, Gambi MC and Giangrande A (2015) Growth and population dynamics of the non-indigenous species *Branchiomma luctuosum* Grube (Annelida, Sabellidae) in the Ionian Sea (Mediterranean Sea). Mar Ecol, 36: 517-529. Doi: 10.1111/maec.12160

McCullagh P and Nelder JA (1989) Generalized Linear Models. Chapman and Hall, London

McGeoch MA, Butchart SHM, Spear D, et al (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. Divers Distrib 16:95–108. doi: 10.1111/j.1472-4642.2009.00633.x

McKenzie LA, Brooks R, Johnston EL (2011) Heritable pollution tolerance in a marine invader. Environ Res 111:926–932. doi: 10.1016/j.envres.2010.12.007

Mckindsey CW, Landry T, Beirn FXO, Davies IM (2007) Bivalve Aquaculture and Exotic Species: a Review of Ecological Considerations and Management Issues Bivalve Aquaculture and Exotic Species: a Review of Ecological Considerations and Management Issues. J Shellfish Res 26:281–294

McKinney FK, McKinney MJ (2002) Contrasting marine larval settlement patterns imply habitat-seeking behaviours in a fouling and a cryptic species (phylum Bryozoa). J Nat Hist 36:487–500. doi: 10.1080/00222930010013755

Mesel I De, Kerckhof F, Norro A, et al (2015) Succession and seasonal dynamics of the epifauna community on offshore wind farm foundations and their role as stepping stones for non-indigenous species. Hydrobiologia 756:37–50. doi: 10.1007/s10750-014-2157-1

Mineur F, Cottier-cook EJ, Minchin D, et al (2012) Changing coasts: Marine aliens and artificial structures. Oceanogr Mar Biol An Annu Rev 50:189–234. doi: 10.1201/b12157-5

Miranda AA, Almeida ACS, Vieira LM (2018) Non-native marine bryozoans (Bryozoa: Gymnolaemata) in Brazilian waters: Assessment, dispersal and impacts. Mar Pollut Bull 130:184–191. doi: 10.1016/j.marpolbul.2018.03.023

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Front Ecol Environ 6:485–492. doi: 10.1890/070064

Newman WA, McConnaughey RR (1987) A Tropical Eastern Pacific Barnacle, *Megahalanus coccopoma* (Darwin), in Southern California, following EI Nino 1982-83. Pacific Sci 41:31–36

Nogueira JM, ROSSI MCS, LÓPEZ E (2006) Intertidal species of Branchiomma Kölliker, 1858 and Pseudobranchiomma Jones 1962 (Polychaeta: Sabellidae: Sabellinae) occurring on rocky shores along the state of São Paulo, southeastern Brazil. Zoological Studies, 45(4): 586–610

Novaes ALT, Andrade GJPO, Alonço AS, Magalhães ARM (2019) Operational performance in aquaculture: A case study of the manual harvesting of cultivated mussels. Aquac. Eng. 84, 67–79. https://doi.org/10.1016/j.aquaeng.2018.12.006

Olden JD, Poff NL, Douglas MR, et al (2004) Ecological and evolutionary consequences of biotic homogenization. Trends Ecol Evol 19:18–24. doi: 10.1016/j.tree.2003.09.010

Oricchio FT, Marques AC, Hajdu E, et al., (2019) Exotic species dominate marinas between the two most populated regions in the southwestern Atlantic Ocean. Marine Pollution Bulletim 146:884–892. Doi: 10.1016/j.marpolbul.2019.07.013

Paetzold SC, and Davidson J (2010) Viability of golden star tunicate fragments after high-pressure water treatment. Aquaculture 303, 105–107. https://doi.org/10.1016/j.aquaculture.2010.03.004

Penchaszadeh PE (1980) Ecologia larvaria y reclutamiento del mejillon del atlantico suroccidental, mytilus platensis d'orbigny (1). Cah Biol Mar XXI: 169–179

Pineda MC, López-Legentil S, Turon X (2011) The whereabouts of an ancient wanderer: Global phylogeography of the solitary ascidian *Styela plicata*. PLoS One 6: doi: 10.1371/journal.pone.0025495

Pineda MC, Turon X, López-legentil S (2012) Stress levels over time in the introduced ascidian *Styela plicata*: the effects of temperature and salinity variations on hsp70 gene expression. Cell Stress Chaperones 17:435–444. doi: 10.1007/s12192-012-0321-y

Piola RF, Johnston EL (2008) Pollution reduces native diversity and increases invader dominance in marine hard-substrate communities. Divers Distrib 14:329–342. doi: 10.1111/j.1472-4642.2007.00430.x

Probert AF, Volery L, Vimercati G, et al. (2020) Understanding uncertainty in the Impact Classification for Alien Taxa (ICAT) assessments. NeoBiota 62, 387–405.

Raymond JEG (1983) Plankton and productivity in the oceans. 2a Ed. Vol.2. Zooplankton. Pergamon Press.Oxford. 382pp.

Robinson TB, Branch GM, Griffiths CL, et al (2007) Changes in South African rocky intertidal invertebrate community structure associated with the invasion of the mussel *Mytilus galloprovincialis*. doi: 10.3354/meps340163

Robinson TB, Griffiths CL, McQuaid CD, Rius M (2005) Marine alien species of South Africa — status and impacts. African J Mar Sci 27:297–306

Rocha RM Da, Moreno TR, Metri R (2005) Ascídias (Tunicata, Ascidiacea) da Reserva Biológica Marinha do Arvoredo, Santa Catarina, Brasil. Rev Bras Zool 22:461–476. doi: 10.1590/S0101-81752005000200024

Rocha RM, Costa LVG (2005) Ascidians (Urochordata : Ascidiacea) from Arraial do Cabo, Rio de. 95:57–64

Rocha RM, Kremer LP (2005) Introduced Ascidians in Paranagua Bay, Parana, southern Brazil. Rev Bras Zool 22:1170–1184

Rocha RM, Kremer LP, Baptista MS, Metri R (2009) Bivalve cultures provide habitat for exotic tunicates in Southern Brazil. Aquat Invasions 4:195–205. doi: 10.3391/ai.2009.4.1.20

Rodrigues P de TR, Frasson Z, Kroth LT (2007) Viabilidade econômica do cultivo de ostras na região da grande Florianópolis. Panor. da Aquicultura

Rodrigues S a., Rocha RM (1993) Littoral compound ascidians (Tunicata) from São Sebastião, Estado de São Paulo, Brazil. Proc Biol Soc Washingt 106:728–739

Ruiz GM, Freestone AL, Fofonoff PW, Simkanin C (2009) Habitat distribution and heterogeneity in marine invasion dynamics: the importance of hard substrate and artificial structure. Wahl M Mar Hard Bottom Communities Patterns, Dyn Divers Chang Berlin, Springer 321–332. doi: 10.1007/978-3-540-92704-4

Ryland JS, (1965) Catalogue of main marine fouling organisms (found on ships coming into European waters). Organisation for Economic Co-operation and Development 2, 64–65

Ryland JS, Blauwe H De, Mackie JA (2009) Recent discoveries of alien Watersipora (Bryozoa) in Western Europe, with redescriptions of species. Zootaxa 2093:43–59

Santos AA, Della-Giustina EG (2017) Síntese informativa da maricultura 2017, Florianópolis. Available at: https://cedap.epagri.sc.gov.br/ (accessed 20/11/2020)

Santos AA, Mondardo M, Marchiori NC (2019) Presença de *Mytilus cf. edulis platensis* em fazendas marinhas de Bombinhas, Santa Catarina, Brasil. Agropecuária Catarinense 32, 53–55. https://doi.org/10.22491/rac.2019.v32n3.6

Sakai AK, Allendorf FW, Holt JS, et al (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Schröder V, and De Leaniz CG (2011) Discrimination between farmed and free-living invasive salmonids in Chilean Patagonia using stable isotope analysis. Biological Invasions 13, 203–213

Servello G, Andaloro F, Azzurro E, et al., (2019) Marine alien species in Italy: A contribution to the implementation of descriptor D2 of the marine strategy framework directive. Mediterranean Marine Science, 20(1), 1-48. doi: 10.12681/mms.18711

Severino A, Resgalla CJ (2005) Descrição dos estágios larvais de *Megabalanus coccopoma* (Darwin, 1854) e sua variação temporal na enseada de itapocoroy (Santa Catarina, Brasil). Atlântica 27:5–16

Sievers M, Dempster T, Fitridge I, Keough MJ (2014) Monitoring biofouling communities could reduce impacts to mussel aquaculture by allowing synchronisation of husbandry techniques with peaks in settlement. Biofouling J Bioadhesion Biofilm Res 1–10. doi: 10.1080/08927014.2013.856888

Sievers M, Fitridge I, Bui S, Dempster T (2017) To treat or not to treat: a quantitative review of the effect of biofouling and control methods in shellfish aquaculture to evaluate the necessity of removal. Biofouling 33, 755–767. https://doi.org/10.1080/08927014.2017.1361937

Simberloff D (2009) The Role of Propagule Pressure in Biological Invasions. Annu Rev Ecol Evol Syst 40:81–102. doi: 10.1146/annurev.ecolsys.110308.120304

Simberloff D, Martin J, Genovesi P, et al (2013) Impacts of biological invasions: what' s what and the way forward. Trends Ecol Evol 28:58–66. doi: 10.1016/j.tree.2012.07.013

Simpson TS, Wernberg T, Mcdonald JI (2016) Distribution and Localised Effects of the Invasive Ascidian *Didemnum perlucidum* (Monniot 1983) in an Urban Estuary. 1–17. doi: 10.1371/journal.pone.0154201

Sorte CJB, Stachowicz JJ (2011) Patterns and processes of compositional change in a California epibenthic community. Mar Ecol Prog Ser 435:63–74. doi: 10.3354/meps09234

Streftaris N, and Zenetos A (2006) Alien Marine Species in the Mediterranean - the 100 'Worst Invasives' and their Impact. Mediterranean Marine Science, 7(1), 87-118. doi:10.12681/mms.180

Suplicy FM (2019) Plano Estratégico para o Desenvolvimento Sustentável da Maricultura Catarinense (2018-2028). Florianópolis: Epagri 290, 76p. accessed set 2021 at: www.agricultura.sc.gov.br/index.php/arquivos/cederural/camara-setorial-da-maricultura/409-plano-estrategico-da-maricultura-catarinense-2018-2028/file

Sutherland JP (1978) Functional Roles of Schizoporella and Styela in the Fouling Community at Beaufort, North Carolina Author (s): John P. Sutherland Published by: Wiley on behalf of the Ecological Society of America Stable URL: https://www.jstor.org/stable/1936371 REFE. Ecology 59:257–264

Sutherland JP, Karlson RH (1977) Development and stability of the souling community at beaufort. North Carolina. Ecol Monogr 47:425–446

Thiyagarajan V, Qian P (2003) Effect of temperature, salinity and delayed attachment on development of the solitary ascidian *Styela plicata* Lesueur). J Exp Mar Bio Ecol 290:133–146. doi: 10.1016/S0022-0981(03)00071-6

Tompsett S, Porter JS, Taylor PD (2009) Taxonomy of the fouling cheilostome bryozoans *Schizoporella unicornis* (Johnston) and *Schizoporella errata* (Waters). J Nat Hist 43:2227–2243. doi: 10.1080/00222930903090140

Van Name WG (1945) The North and South American Ascidians. Bull Am Museum Nat Hist 84:1–476

Vieira LM, Jones MS, Taylor PD (2014) The identity of the invasive fouling bryozoan *Watersipora subtorquata* (d'Orbigny) and some other congeneric species. 3857:151–182

Viola SM, Page HM, Zaleski SF, et al (2018) Anthropogenic disturbance facilitates a non-native species on offshore oil platforms. J Appl Ecol 55:1583–1593. doi: 10.1111/1365-2664.13104

Wasson K, Zabin CJ, Bedinger L, et al (2001) Biological invasions of estuaries without international shipping: the importance of intraregional transport. Biol Conserv 102:143–153

Yamaguchi T, Prabowo RE, Ohshiro Y, et al (2009) The introduction to Japan of the Titan barnacle *Megabalanus coccopoma* (Darwin, 1854) (Cirripedia: Balanomorpha) and the role of shipping in its translocation. Biofouling J Bioadhesion Biofilm 25:325–333. doi: 10.1080/08927010902738048

Zbawicka M, Trucco MI, Wenne R (2018) Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels: hybridization with invasive European *Mytilus galloprovincialis*. Genet Sel Evol 1–14. Doi:10.1186/s12711-018-0376-z

Zhao Y. and Li J. (2016) Ascidian bioresources: common and variant chemical compositions and exploitation strategy – examples of *Halocynthia roretzi*, *Styela plicata*, Ascidia sp. and *Ciona intestinalis*" Zeitschrift für Naturforschung C, vol. 71, no. 5-6, 2016, pp. 165-180. https://doi.org/10.1515/znc-2016-0012

Zuur AF, Ieno EN and Smith GM (2007) Analysing ecological data. Springer, New York

MATERIAL SUPLEMENTAR

CAPÍTULO 1

Table S1. Permutational pairwise comparisons of target species similarity between localities, by substrate type.

	t	p value
Long-lines		
Florianópolis, Gov. Celso Ramos	1.573	0.052
Florianópolis, Palhoça	24.599	0.002
Florianópolis, Penha	12.543	0.18
Gov. Celso Ramos, Palhoça	16.485	0.033
Gov. Celso Ramos, Penha	23.471	0.002
Palhoça, Penha	31.986	0.001
Buoys		
Florianópolis, Gov. Celso Ramos	40.837	0.001
Florianópolis, Palhoça	39.548	0.001
Florianópolis, Penha	2.946	0.001
Gov. Celso Ramos, Palhoça	64.995	0.001
Gov. Celso Ramos, Penha	61.297	0.001
Palhoça, Penha	48.371	0.001
Mussel socks		
Florianópolis, Gov. Celso Ramos	72.115	0.001
Florianópolis, Palhoça	3.575	0.001
Florianópolis, Penha	60.235	0.001
Gov. Celso Ramos, Palhoça	52.178	0.001
Gov. Celso Ramos, Penha	62.122	0.001
Palhoça, Penha	6.406	0.001
Recruitment plates		
Florianópolis, Gov. Celso Ramos	80.878	0.001
Florianópolis, Palhoça	25.842	0.001
Florianópolis, Penha	10.246	0.001
Gov. Celso Ramos, Palhoça	10.454	0.001
Gov. Celso Ramos, Penha	78.521	0.001
Palhoça, Penha	13.464	0.001

Table S2. Permutational pairwise comparisons of target species similarity between substrate type, by localities.

	t	p value
Florianópolis		
socks, long-lines	42.136	0.001
socks, buoys	39.001	0.001
socks, recruitment plates	6.402	0.001
long-lines, buoys	28.365	0.002
long-lines, recruitment plates	33.143	0.001
buoys, recruitment plates	52.556	0.001
Gov. Celso Ramos		
socks, long-lines	37.585	0.001
socks, buoys	57.888	0.001
socks, recruitment plates	28.209	0.001
long-lines, buoys	58.246	0.001
long-lines, recruitment plates	59.235	0.001
buoys, recruitment plates	62.213	0.001
Palhoça		
socks, long-lines	31.952	0.001
socks, buoys	2.467	0.002
socks, recruitment plates	63.215	0.001
long-lines, buoys	26.517	0.001
long-lines, recruitment plates	40.157	0.001
buoys, recruitment plates	46.042	0.001
Penha		
socks, long-lines	41.224	0.001
socks, buoys	32.787	0.001
socks, recruitment plates	60.416	0.001
long-lines, buoys	32.994	0.001
long-lines, recruitment plates	67.446	0.001
buoys, recruitment plates	50.992	0.001

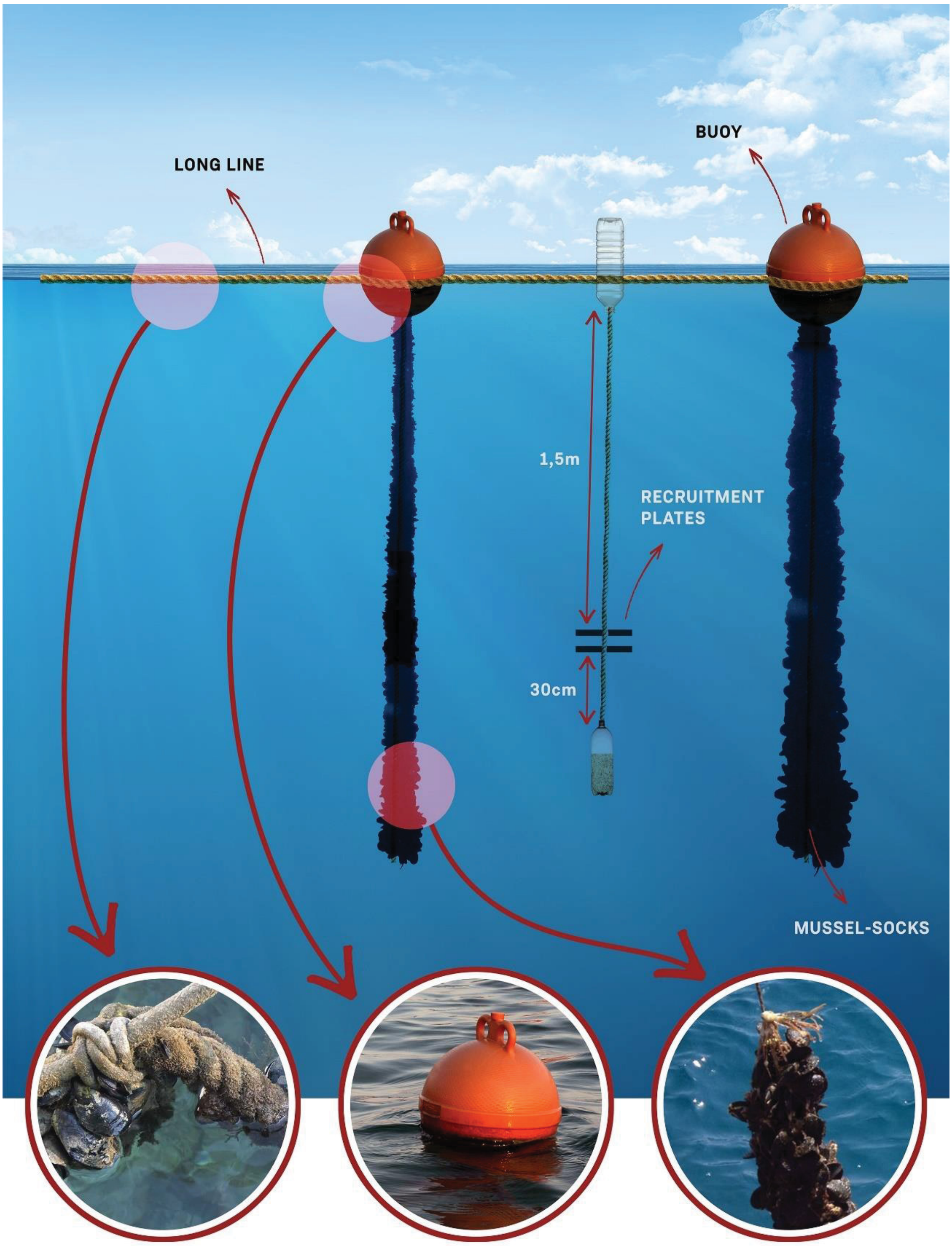


Fig. S1. Schematic drawing of the structures sampled in the farms and the sample unit for recruitment.

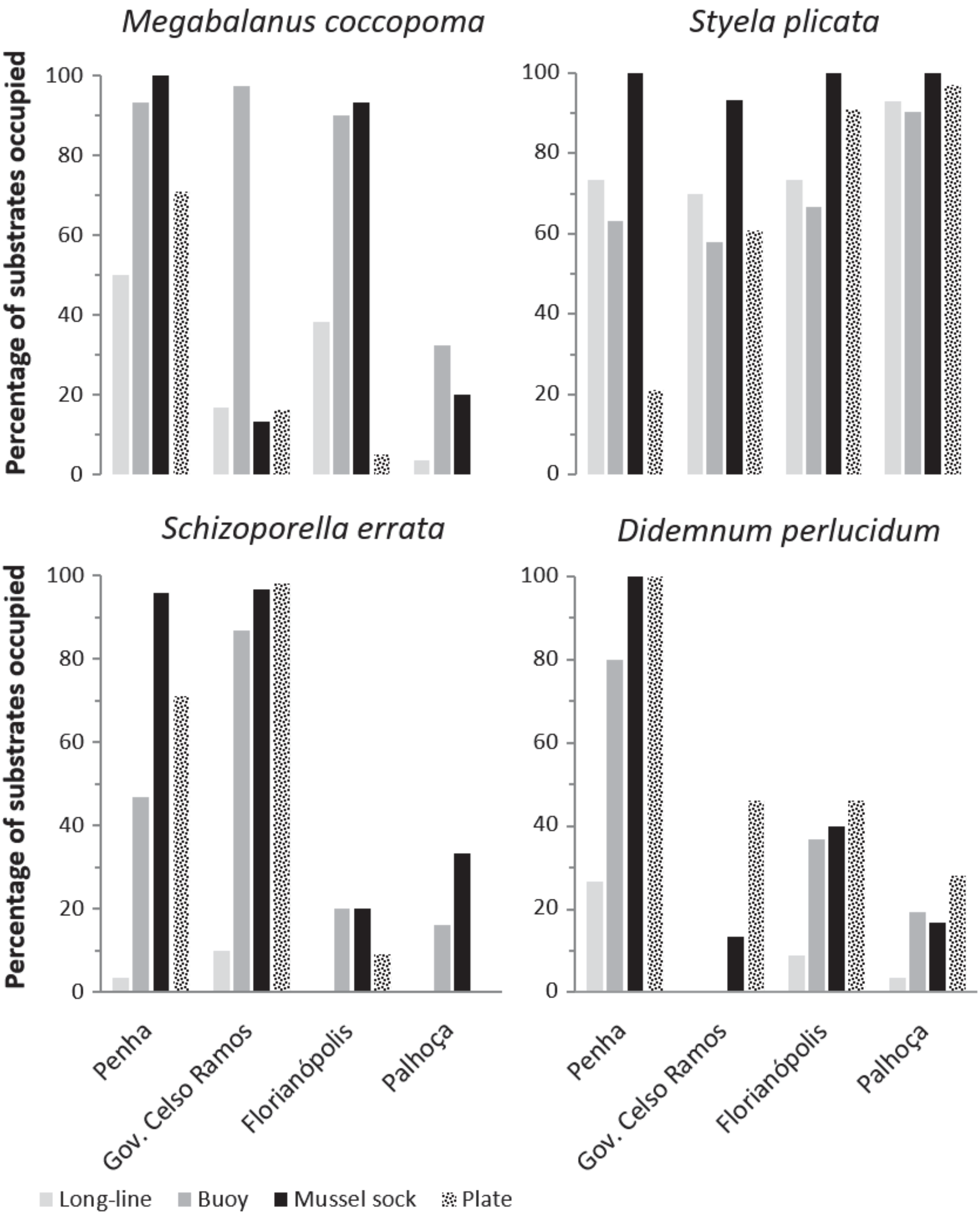


Fig. S2. Percentage of artificial substrates occupied by each species in bivalve farms in Santa Catarina, Southern Brazil.

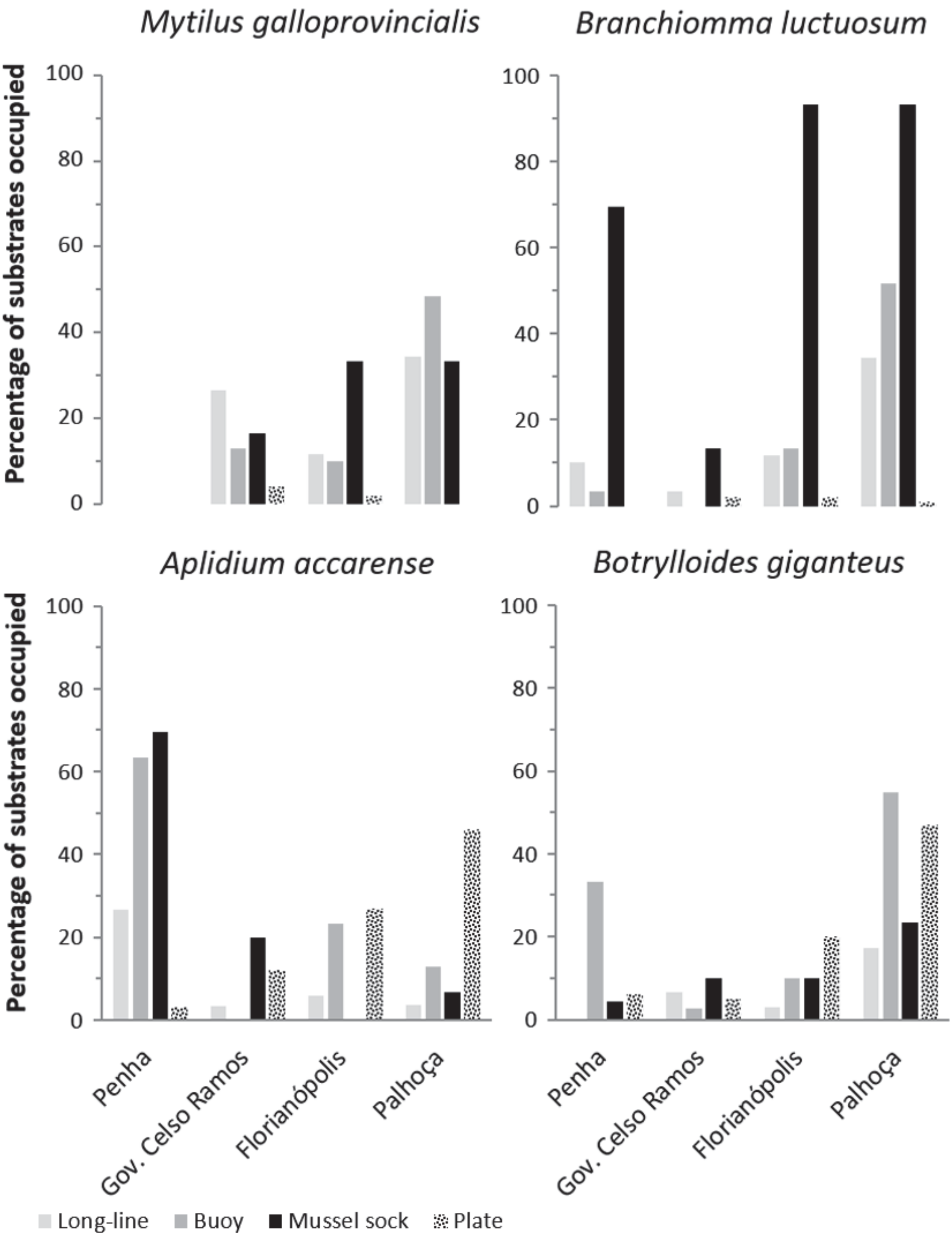


Fig. S3. Percentage of artificial substrates occupied by each species in bivalve farms in Santa Catarina, Southern Brazil.

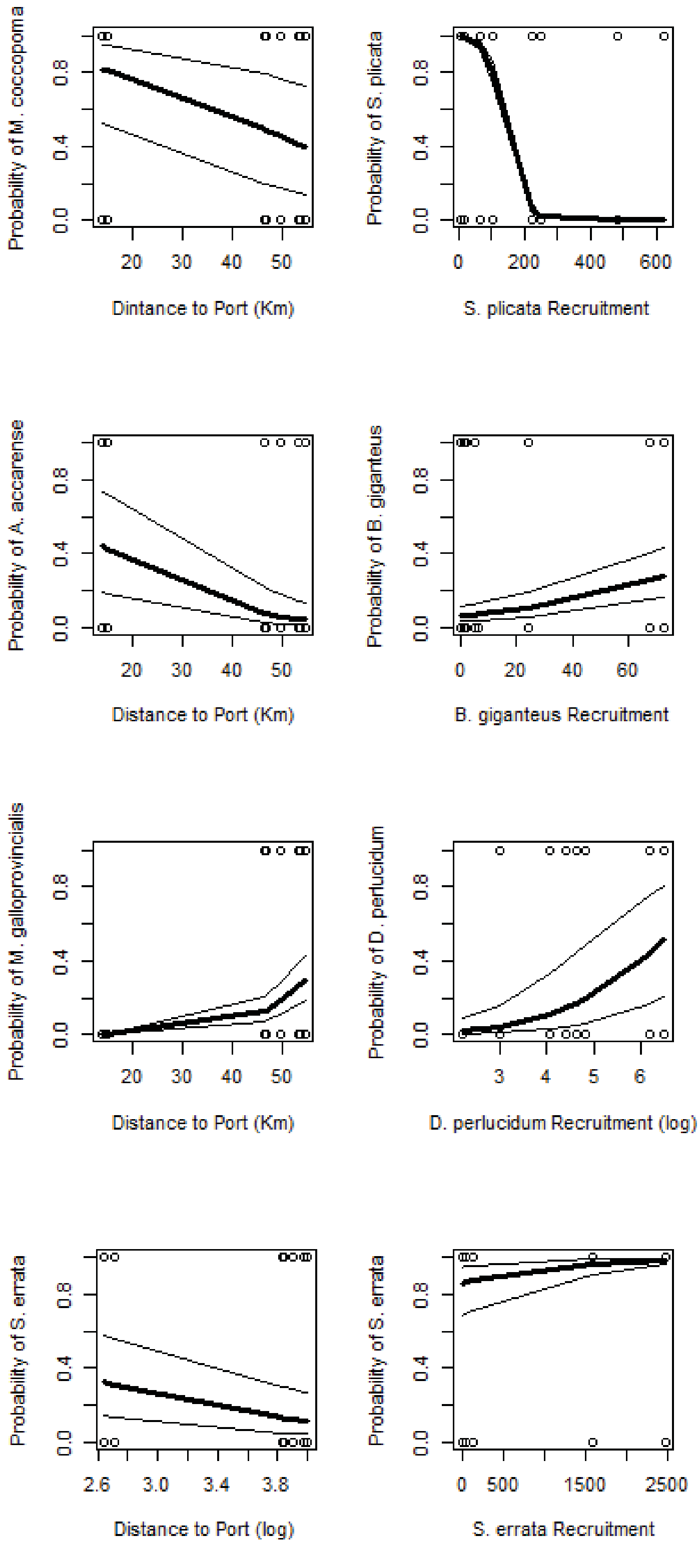


Fig. S4. Probability of occurrence of each species as a function of the explanatory variables in the most parsimonious GLMM model using binomial logistic regression and site as a random factor.

CAPÍTULO 2

CULTIVATED BROWN MUSSEL (*PERNA PERNA*) SIZE IS REDUCED THROUGH THE IMPACT OF THREE INVASIVE FOULING SPECIES IN SOUTHERN BRAZIL

Publicado: 4 novembro 2019 na revista *Aquatic Invasions* https://doi.org/10.3391/ai.2020.15.1.08

Cultivated brown mussel (*Perna perna*) size is reduced through the impact of three invasive fouling species in southern Brazil

Daniel M. Lins[1] and Rosana M. Rocha[2]
[1]Ecology and Conservation Graduate Program, Universidade Federal do Paraná, Curitiba, PR, Brazil
[2]Zoology Dept., Universidade Federal do Paraná, Curitiba, PR, Brazil

Abstract

Invasive species reduce the productivity of shellfish mariculture worldwide. Brown mussel culture harvests were examined for invasive species in the state of Santa Catarina – the most important region for shellfish mariculture in Brazil. Here, we describe the impact of the three most abundant invasive species on harvested *Perna perna* for the first time. The ascidian *Didemnum perlucidum*, the barnacle *Megabalanus coccopoma*, and the bryozoan *Schizoporella errata* were all associated with smaller mussel size. Fouled mussels were 19–36% smaller and weighed ~ 60% less than non-fouled mussels. Reductions in mussel size were greatest for shell weight and size when associated with *D. perlucidum* and tissue dry weight for *M. coccopoma*. This large reduction in productivity indicates that management of these fouling species should be prioritized to protect the mussel fishery.

Keywords: condition index, shellfish mariculture

Introduction

Mariculture often faces financial challenges due to fouling species because they reduce productivity and add costs associated with cleaning gear and maintaining equipment. A large number of fouling species are introduced outside their native distribution and many are also invasive (Rocha et al. 2009; Fitridge et al. 2012). Species become invasive if the barriers that previously limited population growth are removed, with individuals widespread at multiple sites across the extent of occurrence (Blackburn et al. 2011). In many regions, several invasive fouling species may be present. Risk assessment, therefore, must examine the regional fouling community to determine the importance of each species to guide targeted management plans where invasive species are common or likely to become a problem (Fitridge et al. 2012).

An example of an exotic fouling species (i.e. epibiont) with devastating impacts on mariculture is the ascidian *Ciona intestinalis* in Canada, which can become extremely abundant and increase mortality in a native mussel *Mytilus edulis* (Daigle and Herbinger

2009), thereby reducing crop size and increasing production costs. Costs due to fouling vary geographically because they are a consequence of the composition of the epibiont community, abundance and recruitment regimes of the species involved and their temporal dynamics, all of which vary regionally (Lacoste and Gaertner-Mazouni 2015). To date, estimates on the impacts of fouling species, often including ascidians, suggest that up to 30% of production costs go towards combating fouling organisms (Watson et al. 2009; Dürr and Watson 2010; Adams et al. 2011; Fletcher et al. 2013).

The brown mussel *Perna perna* is regulated in Brazil as a native commercial species (*Instrução normativa IBAMA No. 105, 20/7/2006*). For more than 20 years, the southern state of Santa Catarina has led Brazilian brown mussel production. The market of over 20 thousand tons of mollusks provides a substantial source of revenue for the ~ 550 independent mussel producers (Santos and Della-Giustina 2017). Most producers are family-owned artisanal businesses, and impacts due to fouling species may challenge the sustainability and growth of this important commodity. One of the most productive regions in the state is in the Itajaí river basin, the largest watershed flowing into the Atlantic Ocean. Here, in the city of Penha, productivity is negatively affected by fouling species that include many exotic ascidians (Rocha et al. 2009). This is also likely to be an important recipient region for the introduction and spread of additional species due to its proximity to the international port of Itajaí, one of the largest ports in Brazil (ANTAQ 2018).

Three species, the colonial ascidian *Didemnum perlucidum* Monniot, 1983, the barnacle *Megabalanus coccopoma* (Darwin, 1854), and the cheilostome bryozoan *Schizoporella errata* (Walters, 1878), are among the most common fouling species in suspended mussel farms in Penha (*personal observation*). They are also common in many ports, harbors, marinas and on associated artificial structures (Hedge and Johnston 2012); transport by shipping is considered the most common pathway to introduction and spread of these species. The ascidian *D. perlucidum* is widespread globally, and locally it has been introduced in Santa Catarina mussel farms for at least ten years (Kremer et al. 2010; Dias et al. 2016). *Didemnum* colonies overgrow mussels and can completely envelope the valves. Colonies can also grow over barnacles and other sessile organisms (Culbertson and Harper 2000), even though it might be a weak competitor for primary substrate (Kremer and Rocha 2011). The barnacle *M. coccopoma* recruits rapidly on disturbed as well as primary substrates (Newman and McConnaughey 1987). Because the barnacle has sharp edges, infestation can degrade ropes and injure farmers if not handled with great care. Removal of barnacles by scraping from harvested mussels can be extremely time-consuming. The barnacle was first reported in Brazil in the 1970s (Lacombe and Monteiro 1974; Young 1994) and is the only fouling species on the Santa Catarina exotic invasive species list (FATMA 2016). The bryozoan *Schizoporella errata* is a widespread, subtropical, shallow water fouling species that grows vigorously on sea-farm structures (McKinney and McKinney 2002). In Penha, it fouls mussel shells, ropes and buoys with heavily calcified purple/brown colonies (called “rust” by the fishermen) that are up to 20 cm in diameter when growing on buoys (personal observation). Although listed in the official Brazilian list of invasive exotic species (Lopes 2009), recently *S. errata* has been conservatively considered cryptogenic because of taxonomic issues in which the name comprises a complex of species (Miranda et al. 2018).

To better understand the impact of these fouling species on mussel culture, we tested how each of the three focal taxa affects mussel yield and predicted that fouled mussels would exhibit reduced size and/or weight when compared to unfouled mussels in traditional artisanal shellfish cultivation in Brazil.

Methods

Mussel farming

Mussels were sampled at mussel farms in the Armação do Itapocoroy bay in Penha, Santa Catarina (26°46′30″S; 48°36′34″W). Here, mussel farming begins with brown mussel “seed” that is collected from natural banks on rocky shores. Mussel seed is graded using a 3 cm grid and then placed in production. A 2 m rope with filamentous loops for mussel attachment is inserted into a 2 m long tubular cotton cloth bag (the cultivation sock). About 800 mussel seeds are poured into each sock and then placed in the cultivation grid. Harvest occurs eight to ten months after socking and, when harvested, socks weigh ~ 40 kg. Mussel seed density does not affect production (i.e. shell size and flesh weight are constant over normal densities in the socks, Suplicy 2018). Our sampling was carried out as the mussels were being harvested and thus are representative of typical sizes at harvest time.

Sampling methods

We sampled mussels during the austral summer months of November–February of 2016–2018, when surface water temperature varied from 23–27 °C and salinity from 32 to 35 PSU. We first examined several mussel farms in Santa Catarina to determine which fouling species were important and amenable to study. We examined mussel socks to find individual mussels clear of fouling invertebrates and algae and with no evidence of prior encrustation (hereafter referred to as the control) as well as mussels fouled by at least one of the focal invasive species (at least one valve covered > 70%): *Didemnum perlucidum, Megabalanus coccopoma*, or *Schizoporella errata* (hereafter referred to as "fouled"). We rarely found all three target species in the same sock; therefore, we collected all the fouled mussels covered by only one of the targets and respective controls in each sock. Controls were selected from the same sock as the fouled individuals and so all samples by sock contain fouled and control individuals of the same age. However, because fouling is intense, the minimum number of controls of two was often less than the number of fouled individuals. If we were unable to find controls in a sock, we did not use that sock in any analysis. Occasionally, small individuals were found in socks, but they clearly do not belong to the same cohort as that of the majority of mussels in each sock, and those individuals were not included in analyses. Each sampled mussel was individually placed in a sealed bag, labeled and frozen prior to processing.

In the laboratory, shell length was measured to the nearest 0.5 mm using a digital caliper. Besides shell length, the weight of mussels is also important to the market and so, fouled mussel-shells were scraped clean, heated for 5 min at 91–96 °C and dissected. Then, shell and tissue of each sample were desiccated for 48 h at 60 °C and then weighed to 0.001 g precision on a digital balance. A condition index that indicates the tissue percentage of total individual dry weight was calculated as dry $\text{weight}_{\text{tissue}}/(\text{dry weight}_{\text{tissue}} + \text{dry weight}_{\text{shell}}) \times 100$ and is the percentage of total mussel weight due to tissue (Davenport and Chen 1987).

Statistical analysis

The effects of fouling on mussel yield were tested using one-sided t-tests predicting that mussel size was reduced by fouling. The following four variables of mussel size were tested: shell length, dry shell weight, dry tissue weight and condition index. Because all foul-control samples of socks comprised one fouling species in each sock, we analyzed each fouling species separately. Our sampling did not permit testing for interactions due to fouling by more than one species. If an effect due to fouling was established, we compared the effect size of each fouling species as the difference in the variables of the fouled individuals subtracted from the mean values of those variables from their respective controls, using analysis of variance. Because all socks were measured at the time they were harvested, we assumed that socks were independent of the effect of fouling, but examined the potential of that effect in scatterplots. All tests were considered significant at $\alpha = 0.05$.

Statistical analyses were carried out in R 3.5.3 (R Core Team 2019).

Results

Most mussels in each sock had several fouling species and few had none at all. Thus, while difficult to find mussels in each sock fulfilling our criteria, we collected 21 mussels fouled by *D. perlucidum* with 15 controls in five socks, 24 mussels fouled by *M. coccopoma* with 22 controls in eight socks, and 41 mussels fouled by *S. errata* and 31 controls in nine socks.

Scatter plots of mussel measurements demonstrated that socks did not influence mussel size, as we assumed, given that mussels from different socks were not grouped together (Figure 1). Thus, we did not include sock as a covariate in any of the subsequent

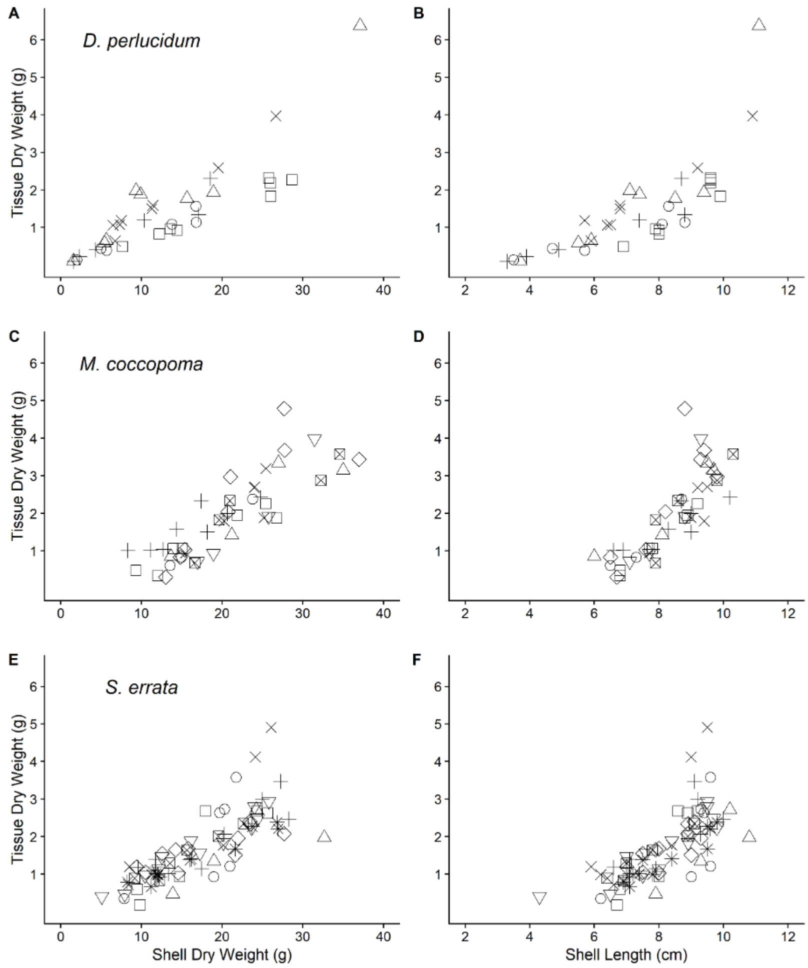


Figure 1. Scatterplots of mussel dry tissue weight as a function of two size measurements (dry shell weight, shell length) of fouled samples to illustrate that there is no apparent effect of sock of origin (symbols only indicate different socks, so symbols are independent of species – that is, the same symbol for a different species indicates a different sock). A) and B) – Didemnum perlucidum, n = 5 socks), C) and D) – Megabalanus coccopoma, n = 8 socks, E) and F) – Schizoporella errata, n = 9 socks.

analyses. Fouled mussels tended to be smaller, often substantially so, in all measurements and for all fouling species (with the exception of the condition index in *D. perlucidum*, Table 1, Figure 2). Fouled mussels weighed less than half as much as control mussels (*S. errata*, 42%) to just more than one third as much (*D. perlucidum*, 36%, and *M. coccopoma*, 37%). There was no evidence for allometric influences during growth or fouling of any of the target species, nor did sock influence any of the measurements (Figure 2). Thus it was clear that fouled individuals simply tended to be smaller. The effect size (the reduction in growth due to fouling) was greatest in *D. perlucidum* on shell weight and length, and on tissue dry weight for *M. coccopoma* (Table 2, Figure 3).

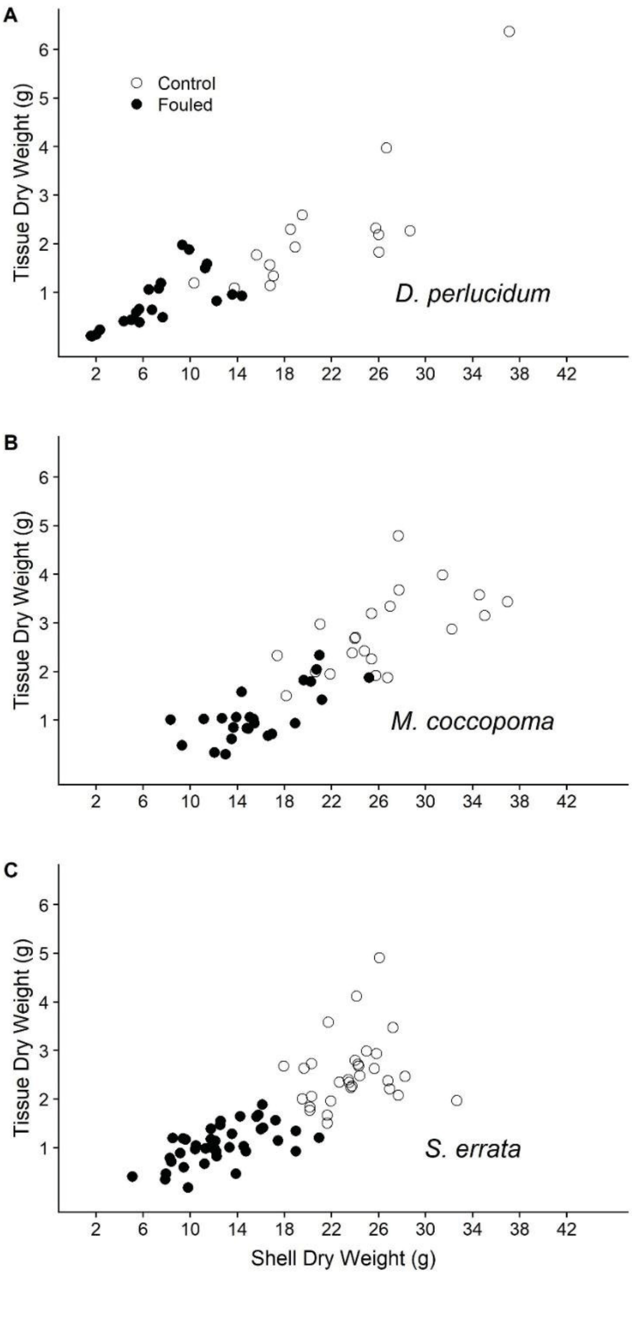


Figure 2. The impact due to fouling was mainly due to reduced growth (filled circles are fouled samples) as shown in scatter plots of tissue dry weight by shell dry weight, by fouling species (filled circles). A) Didemnum perlucidum, B) Megabalanus coccopoma, C) Shizoporella errata.

Table 1. Summary of *t*-tests comparing size and quality measurements of harvested brown mussels (*Perna perna*) between control and fouled treatments. All *t*-tests were statistically significant, with Control > Fouled, at P < 0.001, except Condition index, in *D. perlucidum*, where P = 0.627.

Variable	Control	Fouled	*t*
Didemnum perlucidum (df = 34)			
Length	9.19	5.93	7.42
Dry tissue weight	2.25	0.82	4.39
Condition index[1]	9.31	9.66	0.33
Megabalanus coccopoma (df = 44)			
Length	9.32	7.54	8.76
Dry tissue weight	3.00	1.11	6.95
Condition index	10.10	6.44	4.80
Schizoporella errata (df = 70)			
Length	9.34	7.33	10.82
Dry tissue weight	2.54	1.07	11.24
Condition index	9.71	7.84	3.42

[1] Condition index = dry $weight_{tissue}$/(dry $weight_{tissue}$ + dry $weight_{shell}$) x 100

Table 2. Comparison of the effect size (control minus fouled) among fouling species on three variables of size and quality in the brown mussel (*Perna perna*). Underlined values indicate those that are statistically different from the others (Tukey's post hoc test). See Figure 3.

Variable	*Didemnum perlucidum* (N = 21)	*Megabalanus coccopoma* (N = 24)	*Schizoporella errata* (N = 41)	*F*	*P*
Tissue Weight (g)	1.43 (0.56)	<u>1.89 (0.54)</u>	1.47 (0.39)	6.93	0.001
Shell Weight (g)	<u>13.90 (3.87)</u>	10.60 (4.08)	11.1 (3.48)	5.17	0.007
Length (cm)	<u>3.26 (1.48)</u>	1.78 (0.84)	2.00 (0.94)	12.6	<0.001

Discussion

Fouling by the ascidian *D. perlucidum*, the barnacle *M. coccopoma* and the bryozoan *S. errata* all clearly impacted the size of the brown mussel. These mussels were all collected from socks at the time of harvesting; if they had not yet reached general commercial standards (length > 9 cm) at this point in time, it was likely due to fouling. Fouled mussels were as much as 36% smaller and weighed ~ 60% less than expected at harvest. Consequently, it was clear that not only will fouled mussels take longer to reach marketable size, but many may never reach marketable standards. Once harvestable size has been reached in a sock, the harvesting practice is to simply collect all individuals because after that time, growth rate slows, mortality and fouling increases, and parasitism may also increase (Baird 1966; Holt et al. 1998). Thus, simply leaving the sock out for longer to allow fouled mussels to increase in yield will not compensate for the losses. Controlled experimental studies have also demonstrated that mussel yield declines after variable periods of growth and circumstances due to fouling. Managed ten-month-old mussel socks that were cleaned monthly produced brown mussels that were 7% longer and 15% heavier in flesh weight than socks that were not cleaned (Sá et al. 2007). The mussel *Mytilus galloprovincialis* fouled by the ascidian *Ciona robusta* for only two months were shorter (by 4%) and smaller (flesh weight reduction of 21%) than unfouled mussels (Sievers et al. 2013). *Mytilus galloprovincialis* fouled by the hydroid *Eudendrium crocea* were also shorter (4%) and lighter (23%) after six months (Fitridge and Keough 2013). Our data also demonstrated a substantial decline in size and weight of fouled mussels.

Mussels can respond to stress and, when faced with environmental challenges such as fouling, can invest less energy in growth and reproduction (Petes et al. 2008). Surprisingly, the condition index of mussels fouled by *D. perlucidum* was similar to that of controls, yet fouled mussels were much smaller (Table 1). This indicates a problem with using the condition index as a measure of mussel quality and the impact from fouling. The condition index is widely used as a tool to determine if mussels are at their best cost-benefit relationship for consumers (Okumuş and Stirling 1998; Peharda et al. 2007).

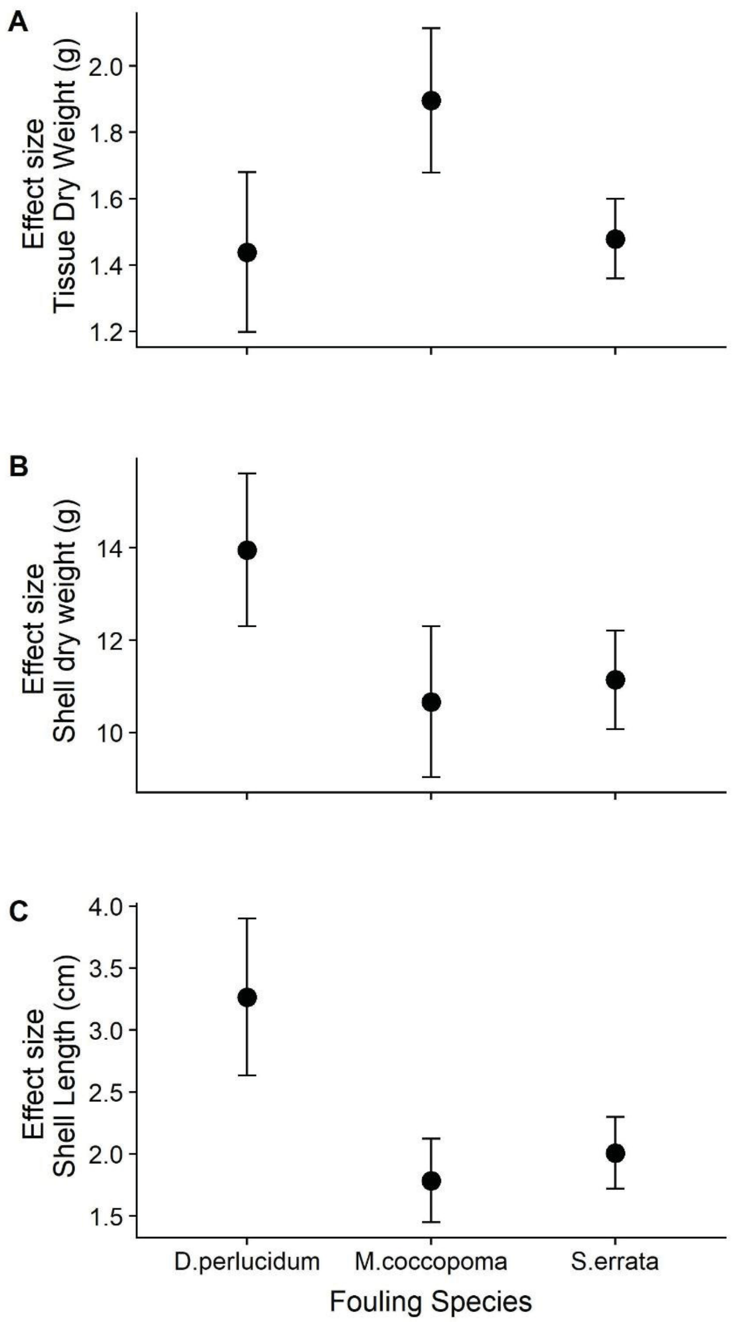


Figure 3. Comparison of effect size (mean control value minus the individual fouled sample values) among fouling species in three size and quality variables in the brown mussel (Perna perna). A) Tissue dry weight (g), B) Shell weight (g) and C) Shell length (cm). Statistical results in Table 2.

However, to be effective, this index requires that mussels be of the same size or that the index is used to compare those growing at the same allometric rates. If those conditions are not met, small shelled mussels may have relatively large flesh and so the condition index will be a large number, while large shelled mussels may have proportionally smaller flesh and hence a lower index value. However, the large, low-index value mussel can have a much larger flesh than the small, large-index value mussel. Because the goal is to cultivate larger quantities of marketable flesh, using the condition index during mussel cultivation can result in misleading conclusions when small mussels have large condition values.

Mussels seem to be impacted by fouling species because they compete for food (Woods et al. 2012; Sievers et al. 2013) or grow over the valves such that they hinder typical water flow in some way, perhaps by impeding shell opening (Lodeiros and Himmelman 1996, 2000). The impact of *D. perlucidum* was greater than that of the other two species on shell weight and size. This is surprising because *D. perlucidum* is less calcified and more flexible than the other fouling species, which suggests that it should interfere less with valve growth. For instance, size and condition were not reduced in the green-lipped mussel *P. canaliculus* fouled by another invasive co-generic colonial ascidian, *Didemnum vexillum,* after 15 months of exposure (Fletcher et al. 2013). *Megabalanus coccopoma* caused a greater reduction in tissue dry weight, suggesting that its greater weight might interfere with shell movement and either decrease feeding or increase energy use by mussels when opening and closing heavier shells (Lodeiros and Himmelman 1996, 2000). The mechanisms causing detrimental consequences of mussel fouling should be further studied to better understand what possible control mechanisms, and thus management options, might be feasible. For instance, competition for food suggests that regular cleaning of infrastructures should be carried out along with cleaning the mussels themselves.

Several methods are used to clean fouling species during production, including exposure to air, immersion in a variety of water-based treatments, and pressure washing. All of these may influence mussel growth as well (Sievers et al. 2017), and none has been adequately tested to determine the cost-benefit relationship with the three fouling species examined in this study. Management of fouling species is time consuming and expensive, especially for artisanal farmers that do not have the machinery and employees required. Handling costs (in time, effort and money) may also differ depending upon the fouling species. For example, *D. perlucidum* and *S. errata* can be removed with very small risk of damaging mussels, while the barnacle *M. coccopoma* must be manually scraped from each mussel. Also, by-products of cleaning fouling animals themselves must be disposed of appropriately. For instance, colonial tunicates, if returned to the water, are likely to invade additional locations or simply reinfect the farm where they were cleaned (Paetzold and Davidson 2010). Hard shells of some animals, such as barnacles, may then become substrate for attachment of exotic species if shells accumulate on the soft bottom beneath the cultures. Therefore, until studies specifically address the costs, benefits and environmental consequences of cleaning, it is suggested that farmers do not attempt to remove fouling species from shells during mussel growth (Metri et al. 2002; Lodeiros et al. 2007) and limit efforts to cleaning the finished product. For example, only clean when the external shell appearance matters, such as in local markets, as opposed to commercially shelled and cooked mussels.

Rather than *in situ* cleaning, a better management plan would be to adjust timing of harvesting to avoid the time during which fouling is at its peak. Reproduction by *D. perlucidum* and *M. coccopoma* may occur throughout the year at this location, but *M. coccopoma* tends to appear in early summer and reach its greatest settlement during the summer (Severino and Resgalla 2005), while *D. perlucidum* reaches its greatest abundance (biomass) in the summer and settlement rates are greatest in March (Kremer et al. 2010), after mussels are harvested. The bryozoan *S. errata*, similarly, reproduces most during warmer months in another subtropical region (Sutherland and Karlson 1977). Given that most farmers prepare (seed) mussel socks within a brief time interval (weeks to a couple months) starting in March, harvesting of eight to nine-month-old mussels between November and December would avoid the time of greatest abundance of these fouling species. Following this recommendation would result in mussels that could be marketed as shelled and cooked to be preserved for export. In the Armação do Itapocoroy bay, optimum brown mussel commercial standards are usually reached at about seven months, with a maximum increase of > 3.5 g in flesh weight from October to November (Marenzi and Branco 2005). However, mussels are harvested throughout the summer (December–February) because it is the most important tourist season, and larger shelled mussels tend to be preferred when bought fresh on site, which is how they are typically served in Brazilian restaurants.

Here, we clearly show that three epizootic species that are dominant in mariculture fouling can cause a substantial reduction in mussel productivity. The list of Brazilian invasive exotic species does not mention *D. perlucidum* and defines both *M. coccopoma* and *S. errata* only as established (Lopes 2009), and thus should be updated. The status of *S. errata* is still in dispute, and in a more conservative view is recently considered cryptogenic because of the lack of information about its geographical origin (Miranda et al. 2018). On the other hand, being exotic is moot because, if we use the definition of Valéry et al. (2009), the species is indeed invasive to the cultures, and its impact on mussels suggests the necessity of management. Because of similar environmental conditions, the ~ 100 ha of the Armação do Itapocoroy bay mussel farms is probably already invaded by all three fouling species. These three invasive species are also found in other mariculture operations in the state of Santa Catarina (unpublished data). To reduce the likelihood of transport of non-native species, stakeholders must be aware that invasive species can “hitch-hike” between regions that are producing bivalves. Current Brazilian law requires that a document must accompany mussel shipments and describe the animals being transported (*Guia de Transporte Animal* – GTA). However, this is insufficient because the document does not require information of the epizootic invasive species that may be present nor is the document checked by biologists during transport or at their destinations.

In conclusion, we emphasize the need for further study to examine the cost-effectiveness of fouling mitigation in Brazilian mariculture. Based on their impact, these three species should be considered as priorities for any management action. Recruitment of fouling species should be continually monitored so that cleaning socks and infrastructures can be undertaken at times that would be most effective. Harvesting may be carried out before invasive species reach high densities. Regional coordinated strategies to mitigate fouling species impacts should be implemented and are highly recommended to prevent the reinfection of structures and the transport of associated species between regions.

ACKNOWLEDGEMENTS

We are very grateful to the mussel farmers who contributed their time and animals to this study. Thanks to LabGeM at the Universidade Federal do Paraná for the loan of the precision equipment used in the mussel measurements and James J. Roper for constructive comments on early versions of the manuscript, statistical help and English revision. Finally, we want to thank the voluntary members of the Scientific Steering Committee and Local Organizing Committee of the ICMB-X, the editor and three anonymous referees. The Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil (CNPq) funded RMR (200914/2008-1, 305201/2014-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) provided a scholarship (finance Code 001) to DML.

References

ANTAQ (2018) Agência Nacional de Transportes Aquaviários. Statistical Panel. http://web.antaq.gov.br/anuario/ (accessed 4/2/2019)

Adams CM, Shumway SE, Whitlatch RB (2011) Biofouling in marine molluscan shellfish aquaculture: A survey assessing the business and economic implications of mitigation. *Journal of The World Aquaculture Society* 42(2): 242–252

Baird RH (1966) Factors affecting the growth and condition of mussels (*Mytilus edulis* L.). *Fisheries Investigations*, Volume 2, London, 33 pp

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. *Trends in Ecology and Evolution* 26: 333-339, doi: 10.1016/j.tree.2011.03.023

Culbertson J, Harper D (2000) Settlement of a colonial ascidian on an artificial reef in the Gulf of Mexico. In: Mckay M, Nides J, Vigil D (eds), Proc. Gulf of Mexico fish and fisheries: Bringing together new and recent research. OCS Reports, US Minerals Management Service, no 2002-2004, New Orleans, LA, pp 614-630

Daigle RM, Herbinger CM (2009) Ecological interactions between the vase tunicate (*Ciona intestinalis*) and the farmed blue mussel (*Mytilus edulis*) in Nova Scotia, Canada. *Aquatic Invasions* 4(1): 177–187, doi: 10.3391/ai.2009.4.1.18

Davenport J, Chen X (1987) A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). *Journal of Molluscan Studies* 53: 293–297

Dias PJ, Rocha R, Godwin S, Tovar-Hernandez MA, Delahoz MV, McKirdy S, Lestang P, McDonald JI, Snow M (2016) Investigating the cryptogenic status of the sea squirt *Didemnum perlucidum* (Tunicata, Ascidiacea) in Australia based on a molecular study of its global distribution. *Aquatic Invasions* 11(3): 239–245, doi: 10.3391/ai.2016.11.3.02

Dürr S, Watson DI (2010) Biofouling and antifouling in aquaculture. In: Dürr S, Thomason JC (eds), Biofouling. Wiley-Blackwell, Oxford, pp 267−287

FATMA (2016) Lista comentada de espécies exóticas invasoras do estado de Santa Catarina: espécies que ameaçam a diversidade biológica. Fundação do Meio Ambiente – FATMA, Florianópolis, 88 pp

Fitridge I, Keough M (2013) Ruinous resident: The hydroid *Ectopleura crocea* negatively affects suspended culture of the mussel *Mytilus galloprovincialis*. *Biofouling* 29(2): 119–131, doi: 10.1080/08927014.2012.752465

Fitridge I, Dempster T, Guenther J, Nys R (2012) The impact and control of biofouling in marine aquaculture: a review. *Biofouling* 28(7): 649–669, doi: 10.1080/08927014.2012.700478

Fletcher LM, Forrest BM, Bell JJ (2013) Impacts of the invasive ascidian *Didemnum vexillum* on green-lipped mussel *Perna canaliculus* aquaculture in New Zealand. *Aquaculture Environment Interactions* 4: 17–30, doi: 10.3354/aei00069

Hedge LH, Johnston EL (2012) Propagule pressure determines recruitment from a commercial shipping pier. *Biofouling* 28(1): 73–85, doi: 10.1080/08927014.2011.652622

Holt TJ, Rees EI, Hawkins SJ, Hartnoll RG (1998) An overview of dynamic and sensitivity characteristics for conservation management of marine SACs. *Biogenic reefs*, vol. 9, Liverpool, 170 pp

Kremer LP, Rocha RM (2011) The role of *Didemnum perlucidum* F. Monniot, 1983 (Tunicata, Ascidiacea) in a marine fouling community. *Aquatic Invasions* 6(4): 441–449, doi: 10.3391/ai.2011.6.4.09

Kremer LP, Rocha RM, Roper JJ (2010) An experimental test of colonization ability in the potentially invasive *Didemnum perlucidum* (Tunicata, Ascidiacea). *Biological Invasions* 12: 1581–1590, doi: 10.1007/s10530-009-9571-8

Lacombe D, Monteiro W (1974) Balanídeos como indicadores de poluição na Baía de Guanabara. *Brazilian Journal of Biology* 34(4): 633-644

Lacoste E, Gaertner-Mazouni N (2015) Biofouling impact on production and ecosystem functioning: a review for bivalve aquaculture. *Reviews in Aquaculture* 7(3): 187–196, doi: 10.1111/raq.12063

Lodeiros CJM, Himmelman JH (1996) Influence of fouling on the growth and survival of the tropical scallop, *Euvola* (*Pecten*) *ziczac* L. 1758) in suspended culture. *Aquaculture Research* 27: 749–756

Lodeiros CJM, Himmelman JH (2000) Identification of factors affecting growth and survival of the tropical scallop *Euvola* (*Pecten*) *ziczac* in the Golfo de Cariaco, Venezuela. *Aquaculture* 182: 91–114

Lodeiros CJM, Galindo L, Buitrago E, Himmelman JH (2007) Effects of mass and position of artificial fouling added to the upper valve of the mangrove oyster *Crassostrea rhizophorae* on its growth and survival. *Aquaculture* 262: 168–171, doi: 10.1016/j.aquaculture.2006.10.002

Lopes RM (ed) (2009) Informe sobre as espécies exóticas invasoras marinha no Brasil. Ministério do Meio Ambiente – MMA, Biodiversidade 33, Brasília, 440 pp

Marenzi AWC, Branco JO (2005) O mexilhão *Perna perna* (Linnaeus) (Bivalvia / Mytilidae) em cultivo na Armação do Itapocoroy, Santa Catarina, Brasil. *Revista Brasileira de Zoologia* 22(2): 394–399

McKinney FK, McKinney MJ (2002) Contrasting marine larval settlement patterns imply habitat-seeking behaviours in a fouling and a cryptic species (phylum Bryozoa). *Journal of Natural History* 36: 487–500, doi: 10.1080/00222930010013755

Metri R, Rocha RM, Marenzi A (2002) Epibiosis reduction on productivity in a mussel culture of *Perna perna* (Linné, 1758). *Brazilian Archives of Biology and Technology* 45(3): 325–331

Miranda AA, Almeida ACS, Vieira LM (2018) Non-native marine bryozoans (Bryozoa: Gymnolaemata) in Brazilian waters: Assessment, dispersal and impacts. *Marine Pollution Bulletin* 130: 184–191, doi: 10.1016/j.marpolbul.2018.03.023

Newman WA, McConnaughey RR (1987) A tropical eastern Pacific barnacle, *Megabalanus coccopoma* (Darwin), in Southern California, following EI Nino 1982-83. *Pacific Science* 41: 31–36

Okumuş I, Stirling HP (1998) Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. *Aquaculture* 159: 249–261, doi: 10.1016/S0044-8486(97)00206-8

Paetzold SC, Davidson J (2010) Viability of golden star tunicate fragments after high-pressure water treatment. *Aquaculture* 303: 105–107, doi: 10.1016/j.aquaculture.2010.03.004

Peharda M, Župan I, Bavčević L, Frankić A, Klanjšček T (2007) Growth and condition index of mussel *Mytilus galloprovincialis* in experimental integrated aquaculture. *Aquaculture Research* 38: 1714–1720, doi: 10.1111/j.1365-2109.2007.01840.x

Petes LE, Menge BA, Harris L (2008) Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. *Ecological Monographs* 78(3): 387–402

R Core Team (2019) R: A language and environment for statistical computing. http://www.R-project.org

Rocha RM, Kremer LP, Baptista MS, Metri R (2009) Bivalve cultures provide habitat for exotic tunicates in Southern Brazil. *Aquatic Invasions* 4: 195–205, doi: 10.3391/ai.2009.4.1.20

Sá FS, Nalesso RC, Paresque K (2007) Fouling organisms on *Perna perna* mussels: is it worth removing them? *Brazilian Journal of Oceanography* 55(2): 155–161

Santos AA, Della Giustina EG (2017) Síntese informativa da maricultura 2017.

https://cedap.epagri.sc.gov.br/ (accessed 10/7/2018)

Severino A, Resgalla CJ (2005) Descrição dos estágios larvais de *Megabalanus coccopoma* (Drawin, 1854) e sua variação temporal na Enseada de Itapocoroy (Santa Catarina, Brasil). *Atlântica* 27(1): 5–16

Sievers M, Fitridge I, Dempster T, Keough MJ (2013) Biofouling leads to reduced shell growth and flesh weight in the cultured mussel *Mytilus galloprovincialis*. *Biofouling* 29: 97–107, doi: 10.1080/08927014.2012.749869

Sievers M, Fitridge I, Bui S, Dempster T (2017) To treat or not to treat: a quantitative review of the effect of biofouling and control methods in shellfish aquaculture to evaluate the necessity of removal. *Biofouling* 33: 755–767, doi: 10.1080/08927014.2017.1361937

Suplicy FM (2018) Efeito da densidade inicial de cultivo sobre a produtividade de mexilhões *Perna perna* em Santa Catarina. *Agropecuária Catarinense* 31: 77–81, doi: 10.22491/RAC.2018.v31n1.11

Sutherland JP, Karlson RH (1977) Development and stability of the fouling community at Beaufort, North Carolina. *Ecological Monographs* 47(4): 425–446

Valéry L, Fritz H, Lefeuvre JC, Simberloff D (2009) Invasive species can also be native. *Trends in Ecology and Evolution* 24(11): 585

Watson D, Shumway S, Whitlach RB (2009) Biofouling and the shellfish industry. In: Shumway SE, Rodrick GE (eds), Shellfish Safety and Quality. Woodhead Publishing, Cambridge, UK, pp 317–336

Woods C, Floerl O, Hayden BJ (2012) Biofouling on Greenshell™ mussel (*Perna canaliculus*) farms: A preliminary assessment and potential implications for sustainable aquaculture practices. *Aquaculture International* 20: 537–557, doi: 10.1007/s10499-011-9484-2

Young PS (1994) Superfamily Balanoidea Leach (Cirripedia, Balanomorpha) from the Brazilian coast. *Boletim do Museu Nacional* 356:1–36

CAPÍTULO 3

ECOLOGY AND GENETICS OF *MYTILUS GALLOPROVINCIALIS*: A THREAT TO BIVALVE AQUACULTURE IN SOUTHERN BRAZIL

Publicado: 10 abril 2021 na revista Aquaculture https://doi.org/10.1016/j.aquaculture.2021.736753

Ecology and genetics of *Mytilus galloprovincialis*: a threat to bivalve aquaculture in southern Brazil

Daniel M. Lins[1], Małgorzata Zbawicka[2], Roman Wenne[2], Anita Poćwierz-Kotus[2], Jonathan R A Molina[3], Luis Paulo Alves[1], Rosana M. Rocha[4]

1. Ecology and Conservation Graduate Program, Universidade Federal do Paraná, Brazil
2. Institute of Oceanology Polish Academy of Sciences, Powstańców Warszawy 55, 81-712 Sopot, Poland
3. Biology undergraduate student, Universidade Federal do Paraná, Brazil
4. Zoology Department, Universidade Federal do Paraná, Brazil

Abstract

The commercial mussel *Mytilus galloprovincialis* is invasive in the Southern Hemisphere having a large impact on rocky shore communities. It recently appeared in the state of Santa Catarina (SC) which is the most important shellfish aquacultural region in Brazil. Whether this introduction was intentional or accidental is unclear. We used single nucleotide polymorphisms (SNPs) to study population genetics of four introduced populations within at most 70 km from one another in SC; strong similarity among these populations suggests a single introduction event. The Mediterranean Sea is the most probable origin. We monitored recruitment of the invasive *Mytilus* in the site most affected by the invasion and compare it with *Perna perna*, the local commercial species. Results reveal that both species have similar seasonal recruitment trends along the year, which makes it impossible to control the invasive species by recruitment management. We recommended the suppression of *Perna perna* production in the most affected site for at least one year, beginning prior to the reproductive season, followed by cleaning all mussel fouling on submerged and floating structures. This study provides baseline data of an invasive process at its beginning and adds ecological and genetic information for one of the 100 most invasive species worldwide. This information, and continued study will help us understand the evolutionary process of invasion, and the rate of and degree to which *M. galloprovincialis* adapts to a new, warm environment in the southern hemisphere.

Keywords: shellfish management, bioinvasion, aquaculture, Mediterranean mussel, biofouling, genetic variability

1. Introduction

The Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 has become invasive in most of its introduced range and is classified as one of the top 100 invasive species in the world (Lowe et al., 2000). This mussel can be transported unintentionally across oceans as it fouls artificial structures, such as it was recently found on mobile oil rigs that traveled between South Africa, Australia, and New Zealand (Gardner et al. 2016). In South Africa *M. galloprovincialis* is now the most dominant, invasive species of the intertidal and mid-zone natural habitats (Mead et al. 2011; Skein et al. 2018) where it is very widespread and covers rocky shores of the western and southern coasts (Robinson et al. 2005; Assis et al. 2015). Mussel communities that became established in the 1980s were invasive by 2001, and in 2012 some populations on the high shore were replaced by another invader, the barnacle *Balanus glandula* Darwin, 1854 (Sadchatheeswaran et al. 2015). Consequently, the indigenous *Choromytilus meridionalis* (F. Kraus, 1848) was replaced by the invasive Mediterranean mussel in less than 20 years after it first invaded the west coast of South Africa. On the southern coast, *Chthamalus dentatus* Krauss, 1848 populations declined (Hanekom 2008) and the brown mussel *Perna perna* (Linnaeus, 1758) has almost disappeared with remaining populations displaced to the lower intertidal zone of high hydrodynamic areas (Bownes and McQuaid 2006; 2010; Robinson et al. 2007).

Recently, the Mediterranean mussel overcame the tropical barrier and once again became introduced into the Southern Hemisphere. This invasion took place in shellfish farms (*Perna perna* (Linnaeus, 1758) in southern Brazil where its impacts are challenging local production since 2016. Although it is commonly cultivated in Europe, South Africa and Asia (Figueras 2004), in Brazil most producers who attempted to farm this invasive mussel failed. According to local producers, the invasive mussel does not tolerate high summer temperatures and are not well-accepted by the public. While *Perna perna* withstands hydrodynamic stress very well, the much lower attachment strength of *M. galloprovincialis* (Zardi et al. 2006), when it is mixed with *Perna perna*, makes the mussel-socks much less resistant. Thus, large numbers of mussels are often lost during harvesting and during rough seas. This loss is due to the longlines used in artisanal farms in Brazil where mussel socks hang loose from supports, and during storms can whiplash, move and batter against each other and floaters. This farming method is very different from rafts and racks or mechanized systems that are used for *M. galloprovincialis* farming in other countries (Suplicy 2017).

Additionally, one of the greatest impacts of *M. galloprovincialis* on local *P. perna* aquaculture is due to space occupation on special recruitment ropes that are used for collecting spats (juvenile mussels, < 3 cm) in nature, greatly decreasing the number of *P. perna* spats collected. These specially designed ropes are used due to increasing demand for mussel seeds for production. With limited banks of *P. perna* on rocky shores, natural stocks started to be regulated (Normative Instruction n° 105, June 2006 and ordinance nº4, 9 March 2009 - IBAMA) and only sustainable exploitation is allowed, for which a license is required. Currently, the most mussel producers in southern Brazil rely on natural settlement of the native mussel on these recruitment ropes and so any reduction of spat supply will cascade down-stream to production and its profitability.

The recruitment pattern of *P. perna* is locally known, and recruitment occurs twice yearly: following the first, brief, small autumn spawning (late March to the first quarter of May), and following the larger and longer spring spawning that is responsible for most of the recruitment (late August to early November) (Ferreira et al. 2007). In contrast, local recruitment patterns for *M. galloprovincialis* are not known, but this information is crucial to help mussel farmers to better target only *P. perna* when collecting spats. Also, time of seeding has a strong effect on growth of cultivated mussels. Producers in Chile were able to decrease harvesting time by three months when they began seeding in winter (Díaz et al. 2014). In Morocco, both *P. perna* and *M. galloprovincialis* mussels that were seeded in summer grew faster, and mussels reached commercial size two months earlier than those seeded in the spring (IdHalla et al. 2017). Monitoring recruitment at a fine scale is needed and will provide important information to benefit management of invaded areas.

The first goal of our study was to correctly identify the new comer, because of a local claim that it might be *Mytilus platensis* d'Orbigny 1842, native to Argentina and Uruguay and which was reported from southern Brazil (Rio Grande do Sul) since the end of the nineteenth century. However, a review of Brazilian scientific collections in the 1960's did not find any in Santa Catarina (Klappenbach 1965). More recently, a study affirmed that the black mussels collected in Santa Catarina were *M. galloprovincialis*, based on the genetic marker cytochrome oxidase (COI) (Belz et al. 2020). Yet, the occurrence of doubly uniparental inheritance of mitochondrial DNA (mtDNA) introgression of *Mytilus* taxa might make this identification uncertain (Filipowicz et al. 2008; Smietanka et al. 2013; Zbawicka et al. 2003; 2014), so we used a different approach here to make identification certain. *Mytilus* species are morphologically very similar and can hybridize, such as in Puerto Madryn, Argentina, where *M. platensis* and *M. gallopoviciallis* coexist (Zbawicka et al.

2018). By using nuclear DNA markers including genome-wide single nucleotide polymorphisms (SNPs), we can better identify the species because we can also find aspects of individual species' genetics, reveal hybridization with South American species, and find the geographic origins of interbreeding populations (Zbawicka et al. 2018; 2019). Information that helps us find the probable origin of the new exotic population is important to reveal possible vectors and routes of transport as well as to find biological traits of the species in its original habitat that may provide useful information for management.

We also compare the recruitment periods of the invasive *M. galloprovincialis* (*Mytilus,* hereafter) with the commercial species *P. perna* (*Perna,* hereafter) to understand how spat collection might be managed to avoid the invasive species. The main goal is to help the sustainability of this important commodity by finding feasible management strategies to mitigate this latest invasion by *M. galloprovincialis*.

2. Methods

2.1. Study area

Santa Catarina, in southern Brazil, is very important for the study of marine bioinvasion because it is among the most important summer destinations for sailing and yachting. The region is home to many yacht clubs, nautical garages, hotels, real estate developments and high-end marinas. The region is also notable for having the second largest concentration of shipyards in Brazil (SEBRAE 2012). In addition to this wide variety of possible vectors for the introduction and spread of invasive marine species, the state is the largest national producer of mollusks, accounting for over 95% of national production (Suplicy et al. 2017). The shellfish industry infrastructure provides many opportunities for the establishment of introduced fouling species on its floating structures (Rocha et al. 2009).

Extensive mariculture began in the 1990's and has provided income for artisanal fishermen ever since. Today, around 500 mussel producers are spread over 12 coastal municipalities (Fig. 1) with production between 10 and 20 thousand tons/year (Santos and Giustina 2017). In this region, monthly average minimum seawater temperature is 15.7 °C, maximum is 27.5 °, and peaks of surface temperature can reach 29-30 °C, that occur in late February to March. Although most farms are exposed to full saline marine conditions (salinity > 34 $^0/_0$), most are in small bays that are protected from strong waves (Suplicy 2017).

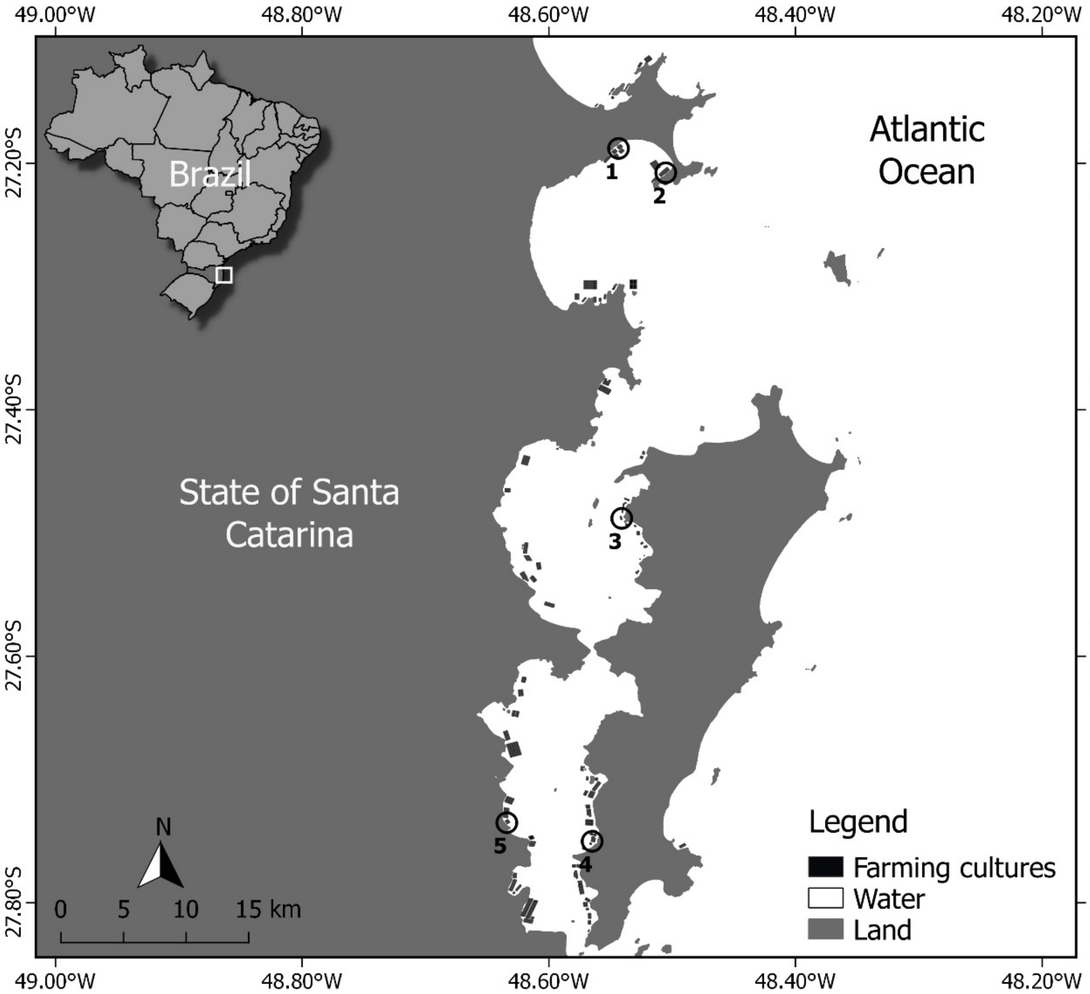


Fig. 1 Detail of the coast of Santa Catarina including Florianópolis Island and study sites: 1. Zimbros and 2. Canto Grande (CGB), both in Bombinhas; 3. Sambaqui (SAF) and 4. Ribeirão da Ilha (RIF), Florianópolis north and south bays; 5. Palhoça (PAL).

In November 2018 we collected mussels for molecular study from four localities in Santa Catarina, including two at Bombinhas municipality (samples for sites 1 and 2 pooled - Fig. 1) where we also carried out year-round ecological assessment. This location is the most affected by *Mytilus* and is the northernmost known extent of the regional invasion. It is an enclosed and fairly protected bay with calm waters and approximately 100 ha of farms divided between Canto Grande (78%) and Zimbros (22%) beaches. The bay provides a safe harbor for fishing and recreational boating and is 30 km south of the international Itajaí port complex. It is also just 12 km from Arvoredo Marine Biological Reserve, the most important protected marine area in the region. The other sampling sites are in the municipalities of Florianópolis (sites 3 and 4) and Palhoça (site 5), and are the most productive studied locations, but so far, the invasive mussel has had lower impact here (Fig. 1).

Table 1. Site name, location, country, and origin information for mussel samples, along with basic genetic diversity measures for each sample.

65SNP

Name	No ind	Location	Country	Region	[illegible]	% P_O	MAF	H_O	H_E	HW	Av. gene diversity over loci	Av. no. of pairwise differences within population	F_{IS}
CGB	27	Canto Grande - Bombinhas	Brazil	Atlantic	29	44,6	0.076	0,247	0,265		0,110	6,73	0,05
PAL	28	Palhoça	Brazil	Atlantic	27	41,5	0.072	0,253	0,266		0,103	6,29	0,01
SAF	27	Sambaqui - Florianópolis north bay	Brazil	Atlantic	33	50,8	0.083	0,227	0,260	4	0,111	7,06	0,07
RIF	29	Ribeirão da Ilha - Florianópolis south bay	Brazil	Atlantic	33	50,8	0.079	0,228	0,250		0,113	7,13	0,06
Reference													
M. trossulus	30	Canada, Denmark (Greenland)	North America	Atlantic	38	58,5	0,093	0,187	0,226	3	0,116	6,91	0,09
M. edulis	25	Iceland, France, North Ireland, USA	Europe, North America	Atlantic	25	38,5	0,056	0,281	0,306		0,110	7,31	0,06
M. galloprovincialis	21	Italy (Oristano, Lido)	South Europe	Mediterranean	33	50,8	0,092	0,245	0,273	2	0,132	7,79	0,04
M. chilensis	27	Chile (Chiloe, Punta Arenas)	South America	Pacific	26	40	0.078	0,265	0,279		0,109	6,98	0,04
M. platensis	30	Argentina (Bahia Camarones, Comodoro Rivadavia, Mar del Plata)	South America	Atlantic	25	38,5	0.065	0,233	0,250	1	0,095	6,05	0,05

53SNP

Name	No ind	Localisation	Country	Water area	P_O	% P_O	MAF	H_O	H_E	HW	Av. gene diversity over loci	Av. no. of pairwise differences within population	F_{IS}
CGB	27	Canto Grande - Bombinhas	Brazil	Atlantic	25	47,2	0.082	0,267	0,279		0,120	6,12	0,04
PAL	28	Palhoça	Brazil	Atlantic	25	47,2	0.084	0,259	0,271		0,119	5,90	0,01
SAF	27	Sambaqui - Florianópolis north bay	Brazil	Atlantic	28	52,8	0.088	0,241	0,266	2	0,105	6,18	0,05
RIF	29	Ribeirão da Ilha - Florianópolis south bay	Brazil	Atlantic	29	54,7	0.088	0,238	0,258		0,124	6,36	0,05
Reference													

M. galloprovincialis Atlantic	29	Camarinal (CAM)	Spain	Atlantic	24	45,3	0.115	0,319	0,342	2	0,139	7,23	0,05
M. galloprovincialis North-central Mediterranean Sea	81	Lido (LID), Torre Grande port (IMC), Oristano (ORI)	Italy	Mediterranean	29	54,7	0.103	0,252	0,2813	2	0,132	6,69	**0,08**
M. galloprovincialis East Mediterranean Sea	18	Izmir (TURK)	Turkey	Aegean Sea	23	43,4	0.089	0,307	0,323		0,126	6,11	0,06
M. galloprovincialis Black Sea	30	Black Sea (Azo)	Ukraine	Black Sea	26	49,1	0.081	0,273	0,270	1	0,130	6,43	-0,04
LID	32	Lido	Italy	Adriatic Sea	28	52,8	0.109	0,270	0,306	2	0,119	6,96	**0,12**
IMC	20	Torre Grande port	Italy	Mediterranean	29	54,7	0.101	0,249	0,279	1	0,148	6,82	0,09
ORI	29	Oristano	Italy	Mediterranean	25	47,2	0.097	0,283	0,307	1	0,118	6,23	0,03
													0.081

P_O, % of polymorphic loci; MAF, minor allele frequency; F_{IS}, inbreeding coefficient; H_O, observed heterozygosity; H_E, expected heterozygosity; values with P < 0.05 after Benjamini–Yekutieli correction are marked in bold

2.2. Molecular study

2.2.1. Sample collection and SNP genotyping

Mytilus spp. samples of mixed sizes (5 to 50 mm shell length) were collected from four localities at south Brazil in 2018 (Fig. 1, Table 1 – samples from sites 1 and 2 were pooled, N = 111 individuals). Whole specimens or tissue samples were stored in 96% ethanol. DNA was isolated from the mantle tissue using DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer´s protocol, and quantified on a NanoDrop device. Nine previously described reference samples taxa comprising 270 specimens of pure *Mytilus* from North Atlantic and South America were included: *M. trossulus* from Atlantic Canada (Halifax, Nova Scotia) and Greenland (Savissivik); *M. edulis* from the Atlantic coast of USA and Northern Ireland, UK; four samples of *M. galloprovincialis* from the Atlantic coast of Spain, the Italian and Turkish Mediterranean Sea coast, and Black Sea; *M. chilensis* from Chiloé and Punta Arenas Chile; *M. platensis* from Argentina (Bach et al. 2019; Gardner et al. 2016; Larraín et al. 2018; Wenne et al. 2016; 2020; Zbawicka et al. 2012; 2018; 2019). Seventy-nine SNPs differentiating amongst *Mytilus* taxa were used (Zbawicka et al. 2012; 2018; Gardner et al. 2016). All samples were genotyped using the Sequenom MassARRAY iPLEX genotyping platform (Gabriel et al. 2009).

2.2.2. Genetic diversity

Samples were analyzed for allele frequencies, proportion of polymorphic SNPs (P_O), minor allele frequency (MAF), genetic diversity, observed (H_O) and expected (H_E) heterozygosity, genetic differentiation (pairwise F_{ST}), and inbreeding coefficient (F_{IS}) and departures from Hardy-Weinberg equilibrium (HWE) using Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010). The false discovery rate test (FDR-BY) was used to correct significance values (P) after multiple testing (Benjamini and Yekutieli 2001; Narum 2006).

2.2.3. Population genetic differentiation and structure

F_{ST} distance measures in the Newick format, obtained in POPTREEW (Takezaki et al. 2014), were used to construct a neighbour-joining (NJ) tree to illustrate the genetic relationships among populations. First, a NJ tree was constructed for Brazilian samples and reference populations from the northern and southern hemispheres, second for Brazilian and *M. galloprovincialis* samples from elsewhere. Clustering and assignment testing were performed using three methods. First, the Bayesian-based method implemented in STRUCTURE v. 2.3.4 with no prior information about the origin of individuals (Pritchard et al. 2000, Falush et al. 2007), assuming admixture and allowing for the correlation of allele frequencies among clusters. The most appropriate number of genetic clusters was determined by a diagram-based comparison of log-likelihoods for values of *K* ranging from 1 to the study number of populations plus 1. At least 5 runs were used to determine each *K* value, following the method described by Evanno et al. (2005). At the plateau of the curve (plot of likelihood against K), the value of *K* captures the main structure of the populations. The length of burn-in period was 50,000 and the number of MCMC cycles after burn-in was 100,000 iterations each. Second, correspondence analysis (CA; Benzécri 1992), implemented in GENETIX (Belkhir et al. 2003), was used to visualize genetic substructure among Brazilian samples. Third, assignment tests were implemented in GeneClass2.0 (Piry 2004) based on frequency criteria on the basis of multilocus genotype data (Paetkau et al. 1995) and in BayesAss, the Bayesian method of Rannala and Mountain (1997). To validate classification results leave-one-out procedure (LOO) was used (Efron 1983). Individuals were considered to be correctly assigned to their location of origin if the assignment probability to that group was greater than their assignment probability to any other group (Larraín et al. 2018).

2.3. Recruitment study

An ongoing large-scale survey of bioinvasion at mussel farms in Santa Catarina indicated that Bombinhas municipality was the most affected by *M. galloprovincialis* and might be the epicenter of this invasion (unpublished data). Thus, we monitored recruitment of *Mytilus* and *Perna*, at mussel farms in Bombinhas. Experimental substrates were deployed in Zimbros and Canto Grande (sites 1 and 2, Fig. 1) suspended from several longlines. We used two different settlement structures (substrates). One of pairs of black polyethylene plates (12 x 12 cm), parallel to each other separated by a 1.5 cm gap, allowing recruits to be fairly protected within from fish predation. Settlement on both inner faces of one pair of plates was counted as one sample. The other settlement structure was a 30 cm-long piece of nylon rope made of refurbished fish nets with filamentous loops that should maximize recruitment, and which are extensively used by local mussel farmers to acquire mussel spats for production. Twelve plate structures and six rope pieces (per site) were attached individually to vertical ropes hanging from the production longlines at 1.5 m depth for a month (the actual number of days was determined by weather conditions, see Appendix Fig. A.1), after which substrates were recovered, individually preserved in 4% formaldehyde solution and replaced by a new set. This was repeated for one year from November 2018 to November 2019. Due to weather, intervals varied and the resultant year of study was divided into 11 rather than 12 units for analysis. We standardized the time unit to make all months comparable by dividing the recruitment data by the number of days exposed, then multiplying by 30.

In the laboratory, each plate or rope was viewed under a dissecting scope to count the total number of juvenile *Mytilus* and *Perna* recruited. The species are easily recognized, even when very young and small, because they have different color patterns: *M. galloprovincialis* have dark valves with a whitish umbo region, *P. perna* has brown shells with irregular white stripes parallel to the growth lines (Fig. 2). To the best of our knowledge, no other similar Mytilidae species is found in the region. We compared efficiency of settlement structures (plates vs ropes) using the non-parametric Wilcoxon test, by species, using the abundance of recruits per settlement unit, disregarding units without recruitment. Recruitment was compared between species and sites over time using repeated measures ANOVA. Normality of residuals was checked using Goodness-of-fit tests. We also tested whether temperature (maximum and minimum) was correlated with recruitment (summarized by site and month) by species (Spearman test). Environmental data was obtained from the website SeaTemperature.org, at Tijucas station - Santa Catarina and are satellite readings of month averages provided by NOAA. Data were analyzed using R-4.0.0 (R Core Team).

Fig. 2 *Perna perna* (brown) and *Mytilus galloprovincialis* (Mediterranean) mussels from ~30-day-old collectors from Santa Catarina, Brazil with scale.

3. Results

3.1. Genetic variation and differentiation among populations

Samples from southern Brazil (Florianópolis and Bombinhas) were analyzed for SNP variation along with reference populations from both hemispheres. Sixty-five SNPs were successfully genotyped for 246 mussels (111 from Brazil and 135 from six reference taxa; Table 1, and A.1). Additional analysis with reference samples of *M. galloprovincialis* from different regions were carried out with 53 SNPs.

The percentage of polymorphic loci (%P_O) for Brazilian samples ranged from 41.5 to 50.8% (65 SNPs), the lowest of which was observed for *M. edulis* and *M. platensis* (38.5%). Similar values were obtained (43.4 to 54.7%) (53 SNP) in comparisons of the samples from Brazil with *M. galloprovincialis* from different regions (Table 1). Most SNP loci were in Hardy-Weinberg equilibrium (HWE) in Brazilian samples. The observed heterozygosity (H_O) for all Brazilian samples was very similar (about 0.24) compared to the large variation among reference samples (Table 1). The most polymorphism and differences within population among Brazilian samples was observed in the sample from Ribeirão da Ilha and the fewest in the sample from Palhoça, across the Florianópolis south bay and close to Ribeirão da Ilha (Table 1, Fig. A.2). MAF in populations ranged from 0.06 to 0.083 (65 SNPs) and 0.08 to 0.11 (53 SNPs). The lowest values were observed for *M. edulis* and *M. platensis*, while the highest for *M. trossulus* and *M. galloprovincialis*. When, comparing MAF values for the samples from Brazil with *M. galloprovincialis* from different regions, values for all Brazilian samples were low, very homogenous, and similar to East Mediterranean Sea and Black Sea forms of *M. galloprovincialis* (Table 1).

A neighbour-joining (NJ) tree shows the genetic relationships between the four samples from Brazil and six reference samples from both hemispheres based on F_{ST} distance measures (Table 2, Fig. 3,). The NJ tree revealed five well-supported clades of *Mytilus* taxa: *M. trossulus*, *M. edulis*, *M. galloprovincialis*, *M. chilensis* and *M. platensis*. All Brazilian samples grouped together with the *M. galloprovincialis* clade. Within the Brazilian samples there was limited evidence of differentiation. Pairwise F_{ST} values among the four samples from Brazil were not statistically significant ($F_{ST} = 0$, Table 3), which indicated that Brazilian samples are homogenous. The Brazilian samples were most similar to *M. galloprovincialis* ($F_{ST} = 0.002$-0.013). In contrast, pairwise F_{ST} values between Brazilian and reference samples from the Northern and Southern Hemispheres, except for *M. galloprovincialis,* were significant after FDR-BY correction. STRUCTURE analysis showed that the LnP(D) increase was largest for K=2 and then K=3 and

Table 2. F_{ST} distance matrix for 65 SNP, among four Brazilian samples of *Mytilus* and reference samples from the southern and northern hemispheres.

	PAL[1]	CGB[1]	SAF[1]	RIF[1]	*M. trossulus*	*M. edulis*	*M. galloprovincialis*	*M. chilensis*
CGB	0.000							
SAF	0.002	-0.003						
RIF	-0.003	-0.002	-0.001					
M. trossulus	**0.823**	**0.818**	**0.809**	**0.811**				
M. edulis	**0.512**	**0.501**	**0.493**	**0.493**	**0.779**			
M. galloprovincialis	0.013	0.005	0.002	0.005	**0.803**	**0.469**		
M. chilensis	**0.578**	**0.566**	**0.552**	**0.554**	**0.784**	**0.496**	**0.522**	
M. platensis	**0.630**	**0.618**	**0.608**	**0.610**	**0.782**	**0.391**	**0.585**	**0.344**

Values with P < 0.05 following the Benjamini–Yekutieli correction are marked in bold
[1] abbreviations as in Table 1

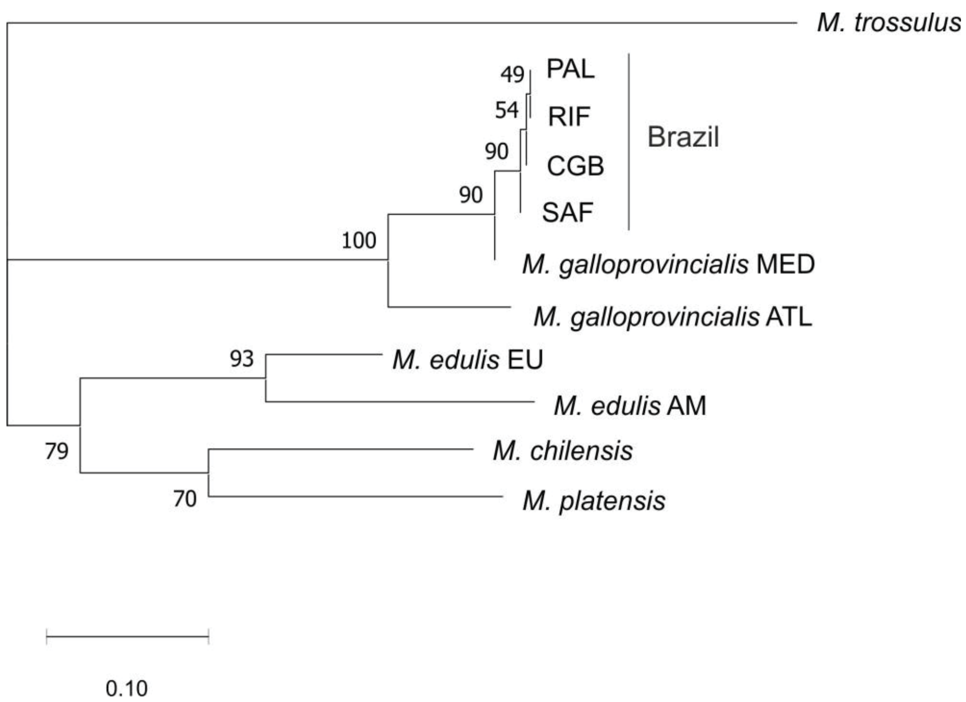


Fig. 3 Neighbour joining tree (NJ tree) of the four mussel populations from Brazil and reference populations of different species from the northern and southern hemispheres, based on the F_{ST} distance matrix from allele frequencies of the SNP loci. NJ tree obtained with POPTREEW and visualized with MEGA version 6. Population/sample codes as shown in Table 1 and Fig. 1. Bootstrap values are shown at nodes as percentages of 100000 replicates. Numbers at each node are well supported. Scale Bar represents 0.05 changes per nucleotide position.

K=5, where the curve reaches a plateau and the value of *K* captures the main structure of the populations (Fig. A.3). For *K=2 M. trossulus* was separated from other *Mytilus* taxa (not shown), whilst *K=3* clusters corresponded to *M. trossulus*, *M. galloprovincialis* with Brazilian samples and *M. edulis* together with South American *M. chilensis* and *M. platensis*. For *K*=5 all reference individuals (*M. edulis*, *M. trossulus*, *M. galloprovincialis*, *M. chilensis* and *M. platensis*) were assigned to their original samples (taxa) (Fig. 4). Again, all Brazilian individuals clustered together with *M. galloprovincialis* taxon.

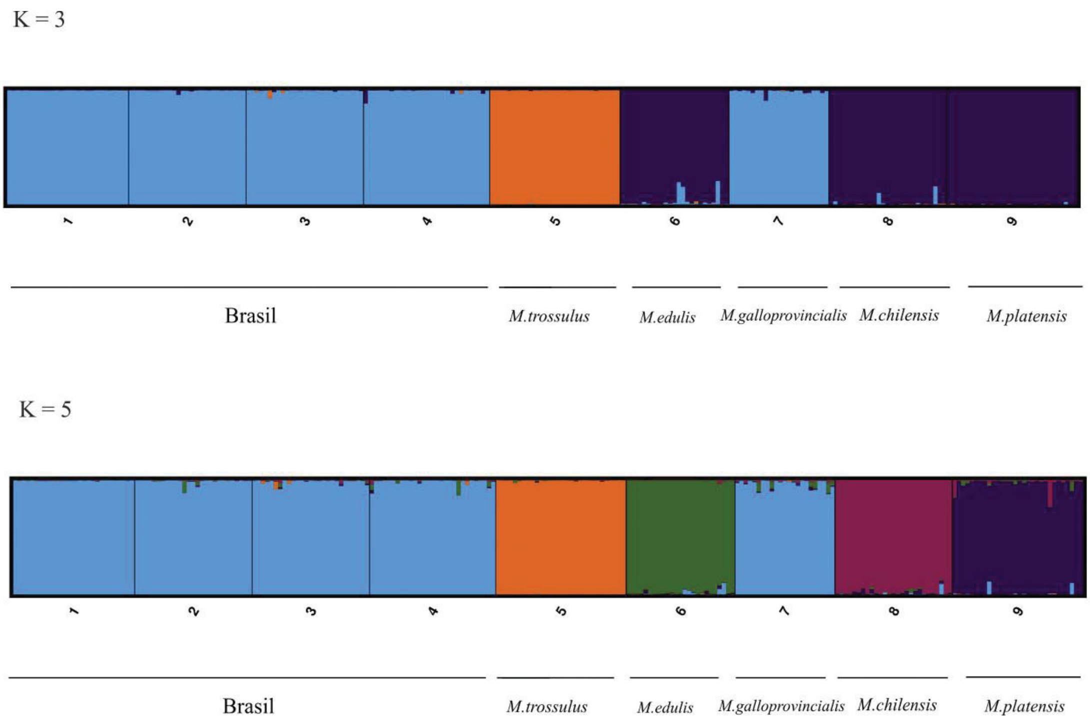


Fig. 4 Structure plot (*K* = 3, 5) for four Brazil mussel populations and reference populations of different species from the northern and southern hemispheres.

Additional analysis with reference samples of *M. galloprovincialis* from different regions were carried out with 53 SNPs. The NJ tree revealed four groups of *M. galloprovincialis* with evidence of geographic structure (Fig. 5): the most distinct and well supported group of the Atlantic form (introgressed with *M. edulis*), the second group was of *M. galloprovincialis* from the north-central Mediterranean Sea, and one group each (with moderate support) from the east Mediterranean Sea and from the Black Sea. All Brazilian samples were situated between the eastern Mediterranean Sea and Black Sea forms of *M. galloprovincialis*. The four Brazilian samples were relatively undifferentiated compared to the differentiation between *M. galloprovincialis* samples from different regions (Table 3). The greatest similarity was between Brazilian (especially, Canto Grande – Bombinhas) and *M. galloprovincialis* samples from the Black Sea (F_{ST} = 0.005-0.015). This similarity among Brazilian samples is likely to indicate a single origin.

Table 3. F_{ST} distance matrix for 53 SNP, among four Brazilian samples of *Mytilus* and reference samples of *M. galloprovincialis* from different regions.

	PAL[1]	CGB[1]	SAF[1]	RIF[1]	Atlantic	Mediterranean Sea (north-central)	Mediterranean Sea (eastern)
CGB	-0.009						
SAF	-0.008	-0.005					
RIF	-0.005	-0.006	-0.003				
Atlantic	**0.075**	**0.078**	**0.085**	**0.076**			
Mediterranean Sea (North-central)	**0.015**	**0.016**	0.010	**0.015**	**0.071**		
Mediterranean Sea (East)	**0.036**	0.029	0.008	**0.031**	**0.134**	**0.040**	
Black Sea	0.006	0.005	0.015	0.010	**0.063**	**0.012**	**0.045**

Values with P < 0.05 following Benjamini–Yekutieli correction are marked in bold.
[1] abbreviations as in Table 1

Correspondence analysis (CA) characterizing the structure of the population for 53 SNPs was carried out for Brazilian samples and six reference *M. galloprovincialis* samples from different locations (samples – Fig. 6a; individuals – Fig. 6b). The first two axes accounted for 60 – 68% of total variation. Axis 1 showed a clear separation between Atlantic *M. galloprovincialis* and other samples. Brazilian samples formed a very tight group in contrast to *M. galloprovincialis* from the Mediterranean Sea (Fig. 5a), and they lie between *M. galloprovincialis* from the eastern Mediterranean Sea and the Black Sea. CA analysis indicates that individuals from Brazil overlapped completely with *M. galloprovincialis* individuals from the eastern Mediterranean Sea and Black Sea. They partly overlapped with individuals with greater dispersion from the north-central Mediterranean Sea (Fig. 6b).

In assignment testing using GeneClass2, all reference individuals were correctly assigned to their taxa. Probabilities of assignation are estimated by frequency criteria on the basis of

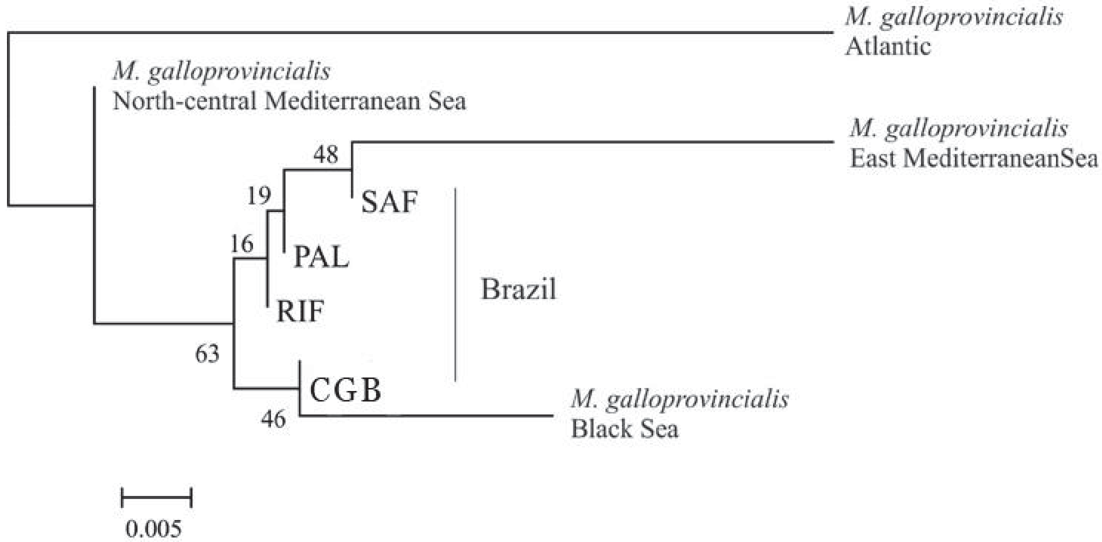


Fig. 5 Neighbour joining tree of the four mussel populations from Brazil and reference samples of *M. galloprovincialis* from different locations, based on the F_{ST} distance matrix from allele frequencies of the SNP loci. NJ tree obtained with POPTREEW and visualized with MEGA version 6. Population/sample codes as shown in Table 1 and Fig. 1. Bootstrap values are shown at nodes as percentages of 100000 replicates. Scale Bar indicates 0.005 changes per nucleotide position.

multilocus genotype data and Bayesian criteria and the results were comparable. All four Brazilian populations were assigned to *M. galloprovincialis* taxa (Table A.1). Assignment testing to region of origin shows that the three southern samples (PAL, SAF, RIF) were assigned to the north-central Mediterranean Sea, while the sample from Bombinhas (CGB) was most similar to Black Sea lineages, although analysis for individual mussels found that individuals from Brazil were most similar to the Black Sea and east Mediterranean Sea form of *M. galloprovincialis,* while many fewer were assigned to the north-central Mediterranean Sea or to the Atlantic lineage of *M. galloprovincialis* (results with probability above 50%). Most individuals from Palhoça (PAL), Ribeirão da Ilha (RIF) and Canto Grande (CGB) were assigned to the Black Sea form, while most individuals from Sambaqui (SAF) were similar to East Mediterranean Sea form of *M. galloprovincialis*.

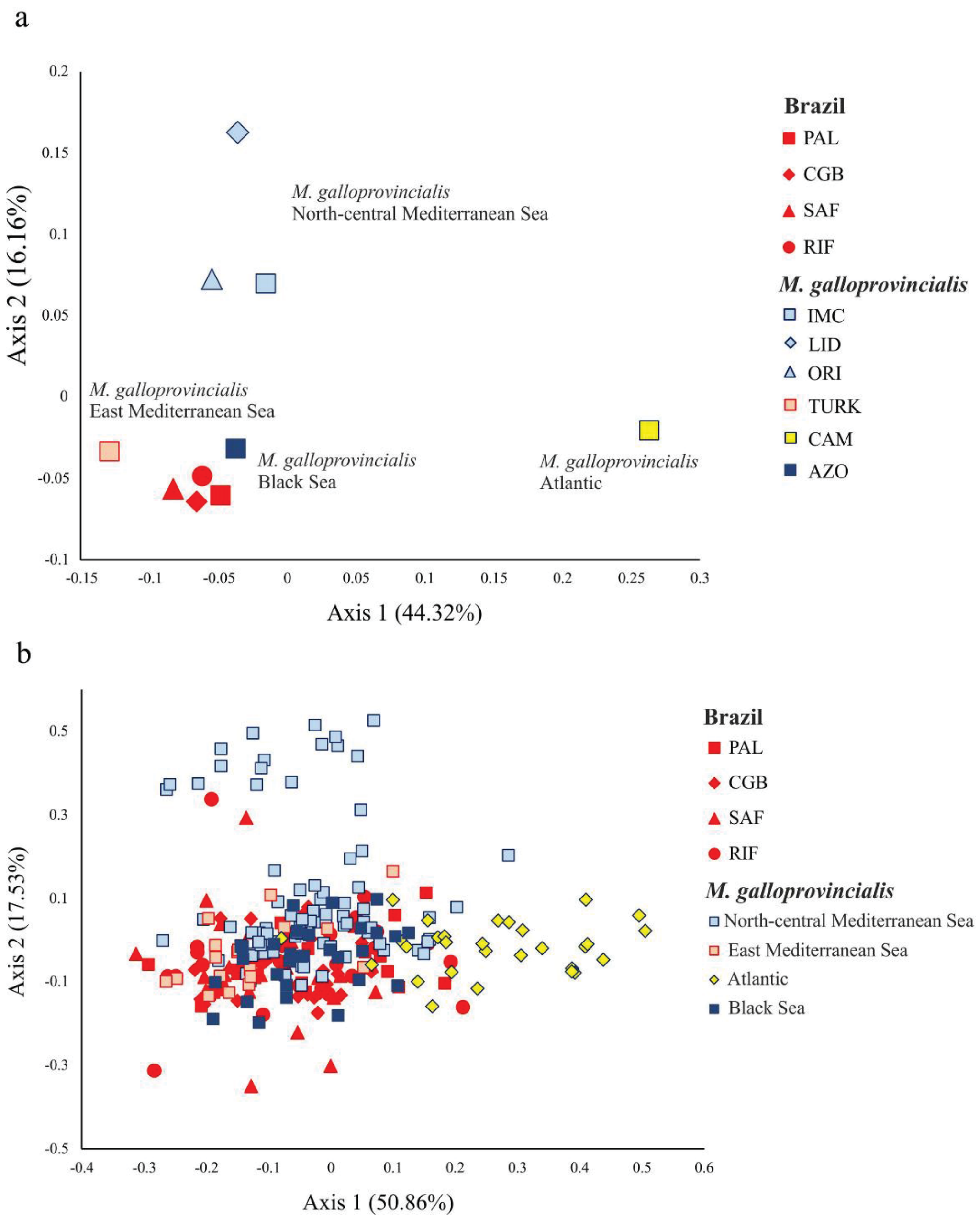


Fig. 6 Correspondence analysis of a) populations, and, b) individuals from four mussel samples from Brazil and reference samples of *M. galloprovincialis* from different locations. Population/sample codes as shown in Table 1.

3.2. Recruitment study

The seasonal pattern of recruitment on ropes, and of abundance per substrate, were similar to that on plates at both sites (*Mytilus*: z = 0.80, p = 0.423, n = 105; *Perna*: z = 1.13, p = 0.260, n = 120, Table A.2). This result demonstrates that recruitment is very similar on ropes and plates, but plates are much easier to work with and standardize with respect to total available surface for recruitment. For this reason and because sample size was larger, we report all results based on plates. Abundance of *Mytilus* was similar in both sites (repeated measures ANOVA $F_{1,1} = 0.079$, $p = 0.785$, n = 22, Table A.3) and abundance by species was also similar along the year (repeated measures ANOVA $F_{1,1} = 1.66$, $p = 0.200$, n = 44, Table A.4).

Recruitment was seasonal and followed similar trends for both species through the year, on experimental plates. No recruitment occurred from February to April (Fig. 7). Recruitment began in May, the southern autumn, and continued through the year. A small peak in recruitment occurred at the beginning of winter, and then recruitment of *Mytilus* declined in July and of *Perna* declined in August, followed by a strong increase that reached the main peak from September (*Mytilus)* to November (*Perna,* Fig. 7). During recruitment peaks of *Mytilus* most plates were colonized by new recruits, which indicated that larvae were spread across the entire culture (Fig. A.4).

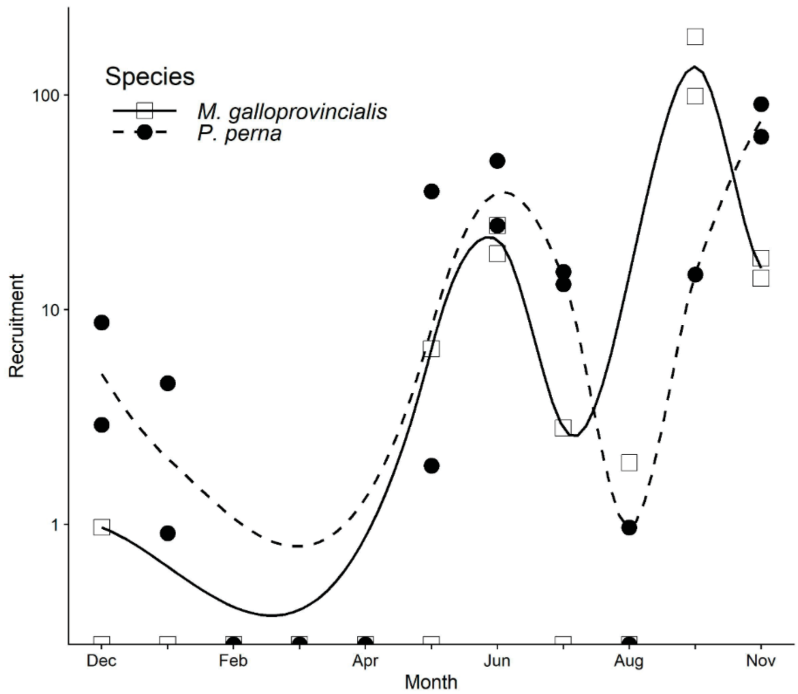


Fig. 7 Abundance of invasive (*Mytilus galloprovincialis*) and local (*Perna perna*) mussels recruited on experimental plates deployed at mussel farms from November 2018 until November 2019 in Zimbros and Canto Grande at Bombinhas municipality, south Brazil. Each point is the total count of recruits in 12 monthly replicates for each of the sites. Note the logarithmic scale of axis Y.

Recruitment in both species tended to decline with increasing seawater temperature (maximum temperature: *Mytilus:* $r_s = -0.649$, $p = 0.001$, *Perna:* $r_s = -516$, $p = 0.014$, $n = 22$, Fig. 8A; and minimum temperature: *Mytilus:* $r_s = -0.564$, $p = 0.006$, *Perna:* $r_s = -0.530$, $p = 0.011$, $n = 22$, Fig. 8b). While the correlation between recruitment abundance and temperature are similar and negative in both species, the peak in *Mytilus* recruitment (17 – 21 °C) is greater than, and at a lower temperature, than the peak in *Perna* (17 – 24 °C).

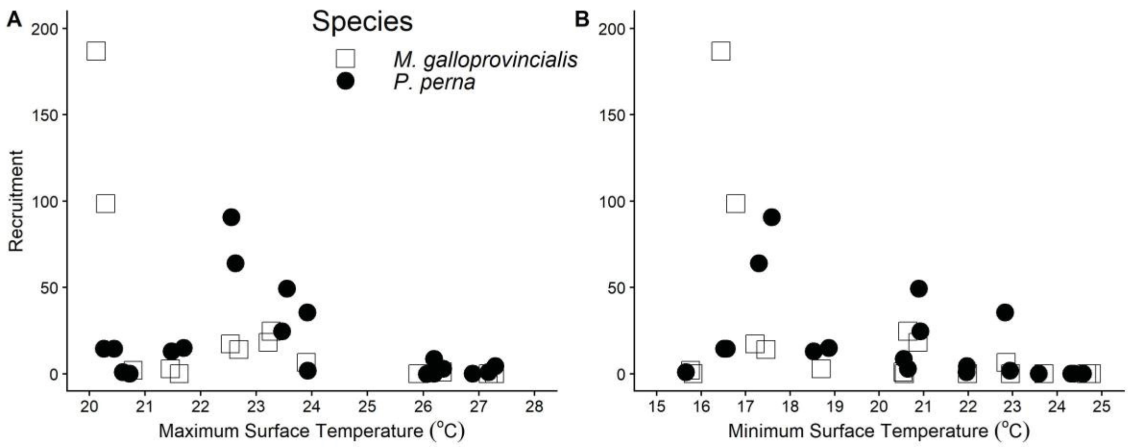


Fig. 8 Correlation of the abundance of recruits with maximum and minimum sea surface temperatures. Dots represent the sum of recruits on experimental plates deployed monthly at mussel farms from November 2018 until November 2019 in each of two sites (Zimbros and Canto Grande at Bombinhas municipality, south Brazil). Seawater temperature (monthly averages for each plate deployment period) obtained from https://SeaTemperature.org at Tijucas, Santa Catarina.

4. Discussion

We confirm the identity of the new invader in southern Brazil as *Mytilus galloprovincialis* through molecular analysis, and thus we ruled out natural expansion of *M. platensis* from Argentina. Further, this population is more similar to the Mediterranean than the Atlantic populations included in the comparison. The hypothesis of human mediated introduction of *M. galloprovincialis* either by ballast water, hull fouling or even aquaculture (Castro et al. 2017) provides the best explanation for possible vectors of introduction. Santa Catarina is the most important nautical hub in southern Brazil, and only follows Rio de Janeiro and São Paulo nationwide. Asia is the primary origin of imports into the region, followed by Europe (ANTAQ), and both have important mussel industries (Figueiras 2004).

The samples of *M. galloprovincialis* from Brazil were undifferentiated, which suggests a single introduction event followed by dispersal from the settled population. The candidate site is likely to be Ribeirão da Ilha, on the south side of Florianópolis, where the most genetic polymorphism was observed. These samples are not characterized by lower genetic polymorphism in comparison with the Mediterranean populations. The invasive populations are genetically most similar to the Black Sea and eastern Mediterranean populations, as demonstrated by the NJ tree and correspondence analysis (Figs 3 and 5), as well as by the MAF values (Table1). The CA for individuals confirmed that the majority of mussels sampled in Brazil form a common group with the reference specimens from the Black Sea and eastern Mediterranean. Very few individuals were similar to Atlantic and north-central Mediterranean mussels. Yet, despite finding genetic similarity with overseas populations, this does not allow precise identification of true geographic origin, because the eastern Mediterranean mussels may have been introduced to other geographic regions of the Pacific and then subsequently introduced in Brazil. Nevertheless, we did exclude some *M. galloprovincialis* source populations, including Argentina, South Africa, Australia and New Zealand. In South Africa, Atlantic *M. galloprovincialis* populations were first introduced in the 70s and lack inner Mediterranean genetic characteristics (Assis et al. 2015; Zardi et al. 2018). Mussels in Australia are mixed populations of Atlantic and Mediterranean *M. galloprovincialis* with native *M. planulatus* (Popović et al. 2020; Zbawicka et al. unpublished data). In Argentina, the population is mainly mixed (hybrid) *M. platensis* with Mediterranean *M. galloprovincialis,* as reported from Puerto Madryn (Zbawicka et al. 2018). We found no native Atlantic South American *M. platensis* alleles in Brazil. In Chile, an introduced *M. galloprovincialis* population in the Gulf of Arauco (Larraín et al. 2018) is similar to the north-central Mediterranean population.

Introduced *M. galloprovincialis* populations were found for the first time in China and Japan in the 1930s and, and which have expanded their geographic range since (Han et al. 2017). Based on mitochondrial gene COI sequences, their similarity to middle Mediterranean population was reported by Han et al., (2017). In contrast, Pickett and David (2018), using published and archived COI sequences, indicated isolation of Chinese populations. Subtle differences among populations of *M. galloprovincialis* from the Mediterranean, China and Bodega Bay in California were found using nuclear DNA SNPs (Han and Dong 2020). The results of our research indicate the origin of these mussels in Brazil was the eastern Mediterranean. Nonetheless, with the results of genotyping with different molecular markers, we cannot rule out the possibility that the population in Brazil may have originated from secondary introduction of mussels transported by ships from the Pacific Ocean through the Panama Canal.

At the epicenter of the Brazilian invasion, our results demonstrate that both mussels, *Pern*a and *Mytilus*, have overlapping reproductive periods and any attempt to maintain *Perna* productivity will require dealing with the invasion first. Seasonal patterns in recruitment for both mussel species demonstrated a smaller peak in the beginning of winter followed by a large peak in spring, with no recruitment of the Mediterranean mussel between December and April. In the southern hemisphere, patterns are seasonal with two pulses, as detected by long series of data in New Zealand where *Mytilus* consistently has a small recruitment in April and a large peak in October (Atalah et al. 2017). Likewise, in Saldanha Bay, South Africa, there is a recruitment peak in October and from November until February recruitment ceases (DML, personal observation). In contrast, in its native region in the northern hemisphere, *M. galloprovincialis* recruitment occurs all year long with major settlement during the summer and autumn. For instance, in Galicia, NW Spain, the main recruitment occurs from May to September (Figueras 2004) when seawater temperatures range from 15 – 18 °C (https://www.seatemperature.org), and in the Aegean Sea the main recruitment is in October and November, with seawater temperatures varying from 12 – 17 °C (Yildiz et al. 2010), and finally, in the Adriatic Sea spawning occurs before October (Pampanin et al. 2005), when seawater temperatures vary from 16 – 22 °C (https://www.seatemperature.org). In conclusion, *M. galloprovincialis* recruitment occurs in different seasons between northern and southern populations, probably driven by seawater temperatures (17 – 21°C in Santa Catarina, which are the minimum values in the region, Fig 8).

Larval mussel recruitment and mortality are affected by many factors (Brenner and Buck 2010), and here we found that lower water temperatures may trigger spawning of *M. galloprovincialis*. This is compatible with temperate origin of the species as well as the high mortality of *Mytilus* reported by Brazilian fishermen in late summer (March) when sea surface water temperatures can reach peaks of 29-30 °C in Santa Catarina (Mizuta et al. 2012). Although temperature seems to be controlling *Mytilus* in southern Brazil, thereby limiting its spread northward (into warmer waters), this mussel was successfully introduced into very different environments, and its large genetic variability has been associated with its high capacity to adapt (Han and Dong 2020). Even in the Mediterranean, higher monthly seawater temperatures can reach 26-27 °C in late summer, exposing native populations to a thermal challenge. The finding of some large (> 7 cm) individuals in longlines shows not only summer temperature tolerance, but also fast-growing individuals may be selected for in the near future in these warmer waters. Because those individuals were probably found 2 – 3 years after the establishment of the species, it is of concern that some individuals rapidly crossed the physiological barrier imposed by high sea water temperature in the region. Given that the amount of recruitment was similar between species (Table A.2), the possibility that *Mytilus* may adapt to higher temperatures (by a decrease in mortality) raises the concern that *Mytilus* populations may begin to quickly increase in the region. Still, there is an opportunity to use *Mytilus* as a model to examine the evolution of the genome with rising temperatures due to climate change (Bindoff et al. 2019).

Information from monitoring fouling is used to reduce the impacts of fouling to mussel aquaculture by synchronization of husbandry periods with peaks in recruitment (Sievers et al. 2014). Farmers in Bombinhas have been trying for the last few years to temporarily avoid *Mytilus* while targeting *Perna* settlement periods, but they have met with little success. Our results show that both invasive and local mussels have equally settled in the refurbished fish-net rope, most commonly used in the region, in the same period of time. Therefore, to achieve the goal of controlling *Mytilus* population, mussel producers at heavily invaded areas must cease the use of recruitment ropes, and replace them with *Perna* seeds recruited elsewhere.

The production of mussels in the current artisanal system was not planned for cultivation of *Mytilus,* and the investment for switching from simple long-lines (that often use improvised floats) to a culture sustained by rafts or racks, must first be considered before switching to the cultivation of the invasive species. Moreover, mussels decrease attachment strength when hydrodynamic stress is reduced (Zardi et al. 2006). Thus, replacing long-lines, that are more prone to hydrodynamic stress, for rafts that are much more stable, may further increase losses due to weakening byssal strength. Given the costs and losses to be expected by increasing *Mytilus* abundance and eventually replacement of *Perna*, immediate action must be taken to manage the invasion in Bombinhas to avoid spreading to other productive areas. Eradication of marine invasive species is almost impossible, and the few successful examples were achieved by early detection and rapid response in small closed regions or with very small populations (Giakoumi et al. 2019).

Although *Mytilus* is found in many farms between Penha in the north to Palhoça in the south of the state (Belz et al. 2020, our unpublished data), the worst invasion seems restricted to the bay where this study took place, and the natural rocky shores have not yet been invaded (LPA, *unpublished data*), and so the opportunity to control this population still exists. Failing to keep *Mytilus* from natural habitats may result in the depletion of natural stocks of *Perna* and, *Perna* stocks can be strongly reduced and displaced to hard-to-reach areas (as occurred in South Africa). In South Africa, *M. galloprovincialis* spread at a rate of over 100 km/year in natural habitats because of local maritime currents (Branch and Steffani 2004).

We suggest that a quarantine of one year should be established in the region with the removal of all artificial structures, including buoys, ropes, and any other floating devices. Boats must be dry docked and cleaned to prevent reinfection and further spreading. We think that it is likely that mussels have been accumulating beneath cultivation due to the frequent falls from long-lines, which is likely to be an important source of reproductive adults. If this is true, then dragging efforts should also be carried out, but with methods that do not compromise other forms of life beneath the aquicultures. Additionally, divers should manually remove mussels from permanent concrete blocks used for anchoring. The best time for cleaning would be the end of summer (February-March) and prior to the first recruitment of *Mytilus* in May. Farmers will still benefit from summer profits, but will need financial and technical support from the local, state and federal governments to carry out the tremendous effort to clean the region. This effort should also be supported by the regional community and associated with procedures such as substitution of long-lines and buoys at the end of summer with new, clean equipment, and once a year dry-dock cleaning of boats that anchor in the bay. These measures will benefit all stakeholders in the mussel production chain. Additionally, trade of live mussels among farmers from different bays along the coast should be inspected to avoid the spread of *Mytilus*.

Artificial hatcheries are growing in economic interest and importance and can provide alternative sources of pure *Perna* seeds for the producers engaged in invasion management. Another alternative is to find optimum nursery areas where *Mytilus* is absent, and to deploy recruitment ropes, and these will be used only for distribution to provide seeds to mussel farms elsewhere. Also, to guarantee sustainable use of natural stocks of *Perna*, licenses to remove mussels from rocky shores must be issued only after updated impact studies of current conditions are carried out.

We also suggest a research program comparing susceptibilities of *Mytilus* and *Perna* to procedures such as air-drying, fresh water cleaning, and other treatments that have been used to eliminate fouling from bivalve cultivation (Sievers et al. 2017). Permanent monitoring of *Mytilus* propagules and colonization pressures should be established in each productive bay by deployment of similar settlement plates as we used in this study, because they are inexpensive, easy to check for the mussels, permit standardization, and reflect recruitment just as the commercially used ropes that are less standardized and more time consuming to check for spats. The increase in *M. galloprovincialis* recruitment would alert managers to the necessity of broader measures.

Authors contribution

DML: Conceptualization, Investigation, Writing – original draft. **MZ, RW, AP:** Resources, Funding acquisition, Writing – Review & Editing. **JRAM, LPA:** Investigation. **RMR:** Conceptualization, Writing – Review & Editing, Funding acquisition and Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS

We thank the mussel farmers who allowed access to their farms and made their mussels available for research. We are very grateful to the Secretariat of Fisheries and Aquaculture from the Bombinhas municipality that promptly offered a boat, skipper and supported field excursions. We thank James J. Roper for statistics advice and language review. We also thank two anonymous reviewers for their suggestions that improved the quality of this manuscript.

Funding: This research was partially funded by the National Council for the Scientific and Technological Development - Brazil (CNPq) by a research grant to RMR (200914/2008-1, 305201/2014-0) and a scholarship to JRAM and by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) that provided a scholarship (finance Code 001) to DML and LPA. RW was funded by the 2011/01/B/NZ9/04352 NCN project and statutory task IV.1 in the IOPAS.

References

ANTAQ, 2018. Agência Nacional de Transportes Aquaviários. Statistical Panel. http://web.antaq.gov.br/anuario/ [accessed 20/4/2019]

Assis, J., Zupan, M., Nicastro, K.R., Zardi, G.I., McQuaid, C.D., Serrão, E.A., 2015. Oceanographic conditions limit the spread of a marine invader along southern African shores. PLoS ONE 10, e0128124. https://doi.org/10.1371/journal.pone.0128124

Atalah, J., Rabel, H., Forrest, B.M., 2017. Modelling long-term recruitment patterns of blue mussels *Mytilus galloprovincialis*: A biofouling pest of green-lipped mussel aquaculture in New Zealand. Aquac Environ Interact 9, 103–114. https://doi.org/10.3354/aei00216

Bach, L., Zbawicka, M., Strand, J., Wenne, R., 2019. *Mytilus trossulus* in NW Greenland is genetically more similar to North Pacific than NW Atlantic populations of the species. Marine Biodiversity 49, 1053–1059. https://doi.org/10.1007/s12526-018-0870-0

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 2003. GENETIX version 4.04, logiciel sous Windows™ pour la genetique des populations. Laboratoire Genome, Populations, Interactions: CNRS UMR 5000, Université de Montpellier II, Montpellier.

Belz, C.E., Simone, L., Silveira Júnior, N., Baggio, R., Gernet, M., Birckolz, C., 2020. First record of the Mediterranean mussel *Mytilus galloprovincialis* (Bivalvia, Mytilidae) in Brazil. Pap Zool 60, e20206007. https://doi.org/10.11606/1807-0205/2020.60.07

Benjamini, Y., Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. Ann Stat 29, 1165–1188. https://doi.org/10.1214/aos/1013699998

Benzécri, J.P., 1992. Correspondence analysis handbook. In: Balakrishnan N, Schucany WR, Garvey PR (eds) Statistics: a series of textbooks and monographs, Vol 125. Marcel Dekker, New York, NY.

Bindoff, N.L., Cheung, W.W.L., Kairo, J.G., et al., 2019. Changing Ocean, Marine Ecosystems, and Dependent Communities. In: IPCC Special Report on the Ocean and Cryosphere in a Changing Climate [H.-O. Pörtner, D.C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, A. Alegría, M. Nicolai, A. Okem, J. Petzold, B. Rama, N.M. Weyer (eds.)]. In press

Bownes, S.J., McQuaid, C.D., 2006. Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L. on the south coast of South Africa? J Exp Mar Biol Ecol 338(1), 140–151. https://doi.org/10.1016/j.jembe.2006.07.006

Bownes, S.J., McQuaid, C.D., 2010. Mechanisms of habitat segregation between an invasive (*Mytilus galloprovincialis*) and an indigenous (*Perna perna*) mussel: adult growth and mortality. Mar Biol 157, 1799–1810. https://doi.org/10.1007/s00227-010-1452-2

Branch, G.M., Steffani, C.N., 2004. Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). J Exp Mar Biol Ecol 300, 184–215. https://doi.org/10.1016/j.jembe.2003.12.007

Brenner, M., Buck, B.H., 2010. Attachment properties of blue mussel (*Mytilus edulis* L.) byssus threads on culture-based artificial collector substrates. Aquac Eng 42, 128–139. https://doi.org/10.1016/j.aquaeng.2010.02.001

Castro, M.C.T., Fileman, T.W., Hall-Spencer, J.M., 2017. Invasive species in the Northeastern and Southwestern Atlantic Ocean: A review. Mar Pollut Bull 116, 41–47. https://doi.org/10.1016/j.marpolbul.2016.12.048

Díaz, C., Figueroa, Y., Sobenes, C., 2014. Seasonal effects of the seeding on the growth of Chilean mussel (*Mytilus edulis platensis*, d´Orbigny 1846) cultivated in central Chile. Aquaculture 428-429, 215–222. https://doi.org/10.1016/j.aquaculture.2014.03.013

Efron B, 1983. Estimating the error rate of a prediction rule: improvement on cross-validation. J Am Stat Assoc 78, 316–331.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resourc 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Falush, D., Stephens, M., Pritchard, J.K., 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes 7, 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Ferreira, J.F., Oliveira Neto, F.M., Tureck, C., 2007. Manual do produtor de moluscos 2 - Sementes de Mexilhão. Florianópolis: CNPq/SEAP/FINEP, 12p. http://docweb.epagri.sc.gov.br/website_epagri/Cedap/Publicacao-Seriada/8-Publicacao-seriada-maricultura-mexilhao-semente-engenharia-aplicada-reproducao.pdf

Figueras, A., 2004. Cultured Aquatic Species Information Programme. *Mytilus galloprovincialis*. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 1 January 2004. [assessed June 2020]. http://www.fao.org/fishery/culturedspecies/Mytilus_galloprovincialis

Filipowicz, M., Burzyński, A., Śmietanka, B., Wenne, R., 2008. Recombination in mitochondrial DNA of European mussels *Mytilus*. J Mol Evol 67, 377–388. https://doi.org/10.1007/s00239-008-9157-6

Gabriel, S., Ziaugra, L., Tabbaa, D., 2009. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Curr Prot Hum Gen 60, 2.12.11–12.12.18 https://doi.org/10.1002/0471142905.hg0212s60

Gardner, J.P.A., Zbawicka, M., Westfall, K.M., Wenne, R., 2016. Invasive blue mussels threaten regional scale genetic diversity in mainland and remote offshore locations: the need for baseline data and enhanced protection in the Southern Ocean. Glob Chang Biol 22, 3182–3195. https://doi.org/10.1111/gcb.13332

Giakoumi, S., Katsanevakis, S., Albano, P.G., et al., 2019. Management priorities for marine invasive species. Sci Tot Envir 688, 976–982 https://doi.org/10.1016/j.scitotenv.2019.06.282

Han, Z., Mao, Y., Shui, B., Yanagimoto, T., Gao, T., 2017. Genetic structure and unique origin of the introduced blue mussel *Mytilus galloprovincialis* in the north-western Pacific: clues from mitochondrial cytochrome c oxidase I (COI) sequences. Mar Freshw Res 68, 263–269. https://doi.org/10.1071/mf15186

Han, G.D., Dong, Y.W., 2020. Rapid climate-driven evolution of the invasive species *Mytilus galloprovincialis* over the past century. Anthrop Coasts 3, 14–29. https://doi.org/10.1139/anc-2019-0012

Hanekom, N., 2008. Invasion of an indigenous *Perna perna* mussel bed on the south coast of South Africa by an alien mussel *Mytilus galloprovincialis* and its effect on the associated fauna. Biol Invasions 10, 233–244. https://doi.org/10.1007/s10530-007-9125-x

IdHalla, M., Nhhala, H., Kassila, J., Orbi, A., 2017. Comparative production of two mussel species (*Perna perna* and *Mytilus galloprovincialis*) reared on an offshore submerged longline system in Agadir, Morocco. Int J Sci Eng Res 8, 1203–1213

Klappenbach, M.A., 1965. Lista preliminar de los Mytilidae brasileños con claves para su determinación y notas sobre su distribución. Supl Anais Acad Brasil Cienc 37, 327–352.

Larraín, M.A., Zbawicka, M., Araneda, C., Gardner, J.P.A., Wenne, R., 2018. Native and invasive taxa on the Pacific coast of South America: Impacts on biodiversity and aquaculture of blue mussels (*Mytilus* spp.). Evol Applic 11, 298–311. https://doi.org/10.1111/eva.12553

Lowe, S., Browne, M., Boudjelais, S., De Poorter, M., 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Published by The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN). Aliens Invas Spec Bull 12, 1–12

Mead, A., Carlton, J.T., Griffiths, C.L., Rius, M., 2011. Revealing the scale of marine bioinvasions in developing regions: a South Africa assessment. Biol Invasions 13, 1991–2008. https://doi.org/10. 1007/s10530-011-0016-9

Mizuta, D.D., Silveira Júnior, N., Fischer, C.E., Lemos, D., 2012. Interannual variation in commercial oyster (*Crassostrea gigas*) farming in the sea (Florianópolis, Brazil, 27°44'S; 48°33'W) in relation to temperature, chlorophyll *a* and associated oceanographic conditions. Aquac 366-367, 105–114. https://doi.org/10.1016/j.aquaculture.2012.09.011

Narum, S.R., 2006. Beyond Bonferroni: Less conservative analyses for conservation genetics. Conserv Genet 7, 783–787. https://doi.org/10.1007/s10592-005-9056-y

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C., 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4, 347–354. https://doi.org/10.1111/j.1365-294X.1995.tb00227.x

Pampanin, D.M., Volpato, E., Marangon, I., Nasci, C., 2005. Physiological measurements from native and transplanted mussel (*Mytilus galloprovincialis*) in the canals of Venice. Survival in air and condition index. Comp Bioch Physiol, Part A 140, 41–52. https://doi.org/10.1016/j.cbpb.2004.10.016

Pickett, T., David, A.A., 2018. Global connectivity patterns of the notoriously invasive mussel, *Mytilus galloprovincialis* Lmk using archived CO1 sequence data. BMC Res Notes 11, 231. https://doi.org/10.1186/s13104-018-3328-3

Piry, S., 2004. GENECLASS2: A software for genetic assignment and first-generation migrant detection. J Heredity 95, 536–539. https://doi.org/10.1093/jhered/esh074

Popovic, I., Matias, A.M.A., Bierne, N., Riginos, C., 2020. Twin introductions by independent invader mussel lineages are both associated with recent admixture with a native congener in Australia. Evol Appli 13(3), 515–532 https://doi.org/10.1111/eva.12857

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155(2), 945–959

Rannala, B., Mountain, J.L., 1997. Detecting immigration using multilocus genotypes. PNAS 94, 9197–9201 https://doi.org/10.1073/pnas.94.17.9197

Robinson, T.B., Griffiths, C.L., McQuaid, C.D., Rius, M., 2005. Marine alien species of South Africa - Status and impacts. African J Mar Sci 27, 297–306. https://doi.org/10.2989/18142320509504088

Robinson, T.B., Branch, G.M., Griffiths, C.L., Govender, A., Hockey, P., 2007. Changes in South African rocky intertidal invertebrate community structure associated with the invasion of the mussel *Mytilus galloprovincialis*. Mar Ecol Prog Ser 340, 163–171. https://doi.org/10.3354/meps340163

Rocha, R.M., Kremer, L.P., Baptista, M.S., Metri, R., 2009. Bivalve cultures provide habitat for exotic tunicates in southern Brazil. Aquat Invasions 4, 195–205. https://doi.org/10.3391/ai.2009.4.1.20

Sadchatheeswaran, S., Branch, G.M., Robinson, T.B., 2015. Changes in habitat complexity resulting from sequential invasions of a rocky shore: implications for community structure. Biol Invasions 17, 1799–1816. https://doi.org/10.1007/s10530-014-0837-4

Santos, A.A., Della-Giustina, E.G., 2017. Síntese informativa da maricultura 2017, Florianópolis. Avaiable at: https://cedap.epagri.sc.gov.br/ (accessed 20/11/2020)

SEBRAE, 2012. Estudo setorial da indústria catarinense: Náutico, Santa Catarina, 325p. Avaiable at: https://m.sebrae.com.br/sites/PortalSebrae/ufs/sc/noticias/fique-por-dentro-dos-estudos-setoriais-da-industria-catarinense,49fe2723d790c410VgnVCM1000003b74010aRCRD

Sievers, M., Dempster, T., Fitridge, I., Keough, M.J., 2014. Monitoring biofouling communities could reduce impacts to mussel aquaculture by allowing synchronization of husbandry techniques with peaks in settlement. Biofouling 30, 203–212. https://doi.org/10.1080/08927014.2013.856888

Sievers, M., Fitridge, I., Bui, S., Dempster, T., 2017. To treat or not to treat: a quantitative review of the effect of biofouling and control methods in shellfish aquaculture to evaluate the necessity of removal. Biofouling 33, 755–767. https://doi.org/10.1080/08927014.2017.1361937

Skein, L., Alexander, M., Robinson, T., 2018. Contrasting invasion patterns in intertidal and subtidal mussel communities. African Zool 53, 47–52. https://doi.org/10.1080/15627020.2018.1448720

Smietanka, B., Zbawicka, M., Sanko, T., Wenne, R., Burzynski, A., 2013. Molecular population genetics of male and female mitochondrial genomes in subarctic *Mytilus trossulus*. Mar Biol 160, 17091721. https://doi.org/10.1007/s00227-013-2223-7

Suplicy, F.M., 2017. Cultivo de mexilhões: sistema contínuo e mecanizado. EPAGRI, Florianópolis, 124p. http://docweb.epagri.sc.gov.br/website_epagri/Cedap/Livro/4-%20Livro-maricultura-mexilhao-gestao-sistema-de-cultivo-semente-engorda-risco.pdf

Suplicy, F.M., Vianna, L., Rupp, G., Novaes, A., Garbossa, L., Souza, R., Guzenski, J., Costa, S., Silva, F., Santos, A., 2017. Planning and management for sustainable coastal aquaculture development in Santa Catarina state, south Brazil. Rev Aquac 9, 107–124. https://doi.org/10.1111/raq.12107

Takezaki, N., Nei, M., Tamura, K., 2014. POPTREEW: Web version of POPTREE for constructing population trees from allele frequency data and computing some other quantities. Mol Biol Evol 31, 1622–1624. https://doi.org/10.1093/molbev/msu093

Wenne, R., Bach, L., Zbawicka, M., Strand, J., McDonald, J.H., 2016. A first report on coexistence and hybridization of *Mytilus trossulus* and *M. edulis* mussels in Greenland. Polar Biol 39, 343–355. https://doi.org/10.1007/s00300-015-1785-x

Wenne, R., Zbawicka, M., Bach, L., et al., 2020. Trans-Atlantic distribution and introgression as inferred from single nucleotide polymorphism: mussels *Mytilus* and environmental factors. Gene 11(5), 530. https://doi.org/10.3390/genes11050530

Yildiz, H., Lök, A., Acarli, S., Serdar, S., Köse, A., 2010. A preliminary survey on settlement and recruitment patterns of Mediterranean mussel (*Mytilus galloprovincialis*) in Dardanelles, Turkey. Kafkas Univ Vet Fak Derg 16, S319–S324

Zardi, G.I., Nicastro, K.R., McQuaid, C.D., Rius, M., Porri, F., 2006. Hydrodynamic stress and habitat partitioning between indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels: constraints of an evolutionary strategy. Mar Biol 150, 79–88. https://doi.org/10.1007/s00227-006-0328-y

Zardi, G.I., McQuaid, C.D., Jacinto, R., Lourenco, C.R., Serrao, E.A., Nicastro, K.R., 2018. Re-assessing the origins of the invasive mussel *Mytilus galloprovincialis* in southern Africa. Mar Freshw Res 69, 607–613. https://doi.org/10.1071/MF17132

Zbawicka, M., Skibinski, D.O.F., Wenne, R., 2003. Doubly uniparental transmission of mitochondrial DNA length variants in the mussel *Mytilus trossulus*. Mar Biol 142, 55–460. https://doi.org/10.1007/s00227-002-0969-4

Zbawicka, M., Drywa, A., Śmietanka, B., Wenne, R., 2012. Identification and validation of novel SNP markers in European populations of marine *Mytilus* mussels. Mar Biol 159, 1347–1362 https://doi.org/10.1007/s00227-012-1915-8

Zbawicka, M., Wenne, R., Burzynski, A., 2014. Mitogenomics of recombinant mitochondrial genomes of Baltic Sea *Mytilus* mussels. Mol Genet Genomics 289, 1275–1287 https://doi.org/10.1007/s00438-014-0888-3

Zbawicka, M., Trucco, M.I., Wenne, R., 2018. Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels: Hybridization with invasive European *Mytilus galloprovincialis*. Genet Sel Evol 50, 1–14 https://doi.org/10.1186/s12711-018-0376-z

Zbawicka, M., Gardner, J.P.A., Wenne, R., 2019. Cryptic diversity in smooth-shelled mussels on Southern Ocean islands: connectivity, hybridisation and a marine invasion. Frontiers Zool 16, 32. https://doi.org/10.1186/s12983-019-0332-y

MATERIAL SUPLEMENTAR

CAPÍTULO 3

Table A. 1. Assignment testing to species and to region of origin carried out in GeneClass for mussels from four Brazilian populations and reference samples of different species from Southern and Northern hemisphere.

		GeneClass, assigned to species (taxa)									
Name	No ind	*M.trossulus*		*M.edulis*		*M.galloprovincialis*		*M.chilensis*		*M.platensis*	
		No ind	%	No ind	%	No ind	%	No ind	%	No ind	%
PAL	28					28	100				
CGB	27					27	100				
SAF	27					24	100				
RIF	29					28	100				
M.trossulus	30	30	100								
M.edulis	25			25	100						
M.galloprovincialis	21					21	100				
M.chilensis	27							27	100		
M.platensis	30									30	100

		GeneClass, assigned of population to origin region								GeneClass, assigned of individuals to origin region								GeneClass, assigned of individuals to origin region							
										All results								Results with probability above 50%							
		M.galloprovincialis								*M.galloprovincialis*								*M.galloprovincialis*							
Name	No ind	Atlantic		Mediterranean (North-central)		Mediterranean (East)		Black Sea		Atlantic		Mediterranean (North-central)		Mediterranean (East)		Black Sea		Atlantic		Mediterranean (North-central)		Mediterranean (East)		Black Sea	
		No ind	%	No ind	%	No ind	%	No ind	%	No ind	%	No ind	%	No ind	%	No ind	%	No ind	%	No ind	%	No ind	%	No ind	%
PAL	28			28	100					4	14.29	6	21.43	5	17.86	13	46.43	4	14.29	3	10.71	5	17.86	9	32.14
CGB	27							27	100	2	7.41	9	33.33	8	29.63	8	29.63			5	18.52	7	25.93	8	29.63
SAF	27			27	100					3	11.11	6	22.22	10	37.04	8	29.63	2	7.41	5	18.52	10	37.04	7	25.93
RIF	29			29	100					5	17.24	7	24.14	8	27.59	9	31.03	4	13.79	4	13.79	8	27.59	8	27.59

Table A.2. Average and standard deviations of abundance of recruits (log transformed) per settlement structure per 30 days comparing species and substrate types.

Species	Substrate	N	Mean	Std Dev
Mytilus galloprovinciallis	rope	45	0.81	0.49
	plate	60	0.68	0.36
Perna perna	rope	50	0.71	0.39
	plate	70	0.63	0.33

Table A.3. Repeated measures ANOVA comparing monthly recruitment (Time) of *Mytilus galloprovincialis* between sites (Canto Grande and Zimbros, Santa Catarina). N = 160 plates summarized for a total of 22 replicates of site by time.

Variable	df	Sum Sq	*F*	*P*
Site (*Mytilus*)	1	0.03	0.079	0.785
Time	1	4.94	15.04	= 0.001
Residuals [1]	18	5.92		

[1] Normality of residuals: W = 0.970, P = 0.714

Table A.4. Repeated measures ANOVA comparing monthly recruitment (Time) of the invasive mussel (*Mytilus galloprovincialis*) and *Perna perna.* N = 344 plates summarized for a total of 44 replicates of site by time.

Variable	df	Sum Sq	*F*	P
Species	1	0.55	1.66	0.200
Time	1	7.70	23.25	< 0.001
Residuals [1]	40	13.24		

[1] Normality of residuals: W = 0.983, P = 0.797

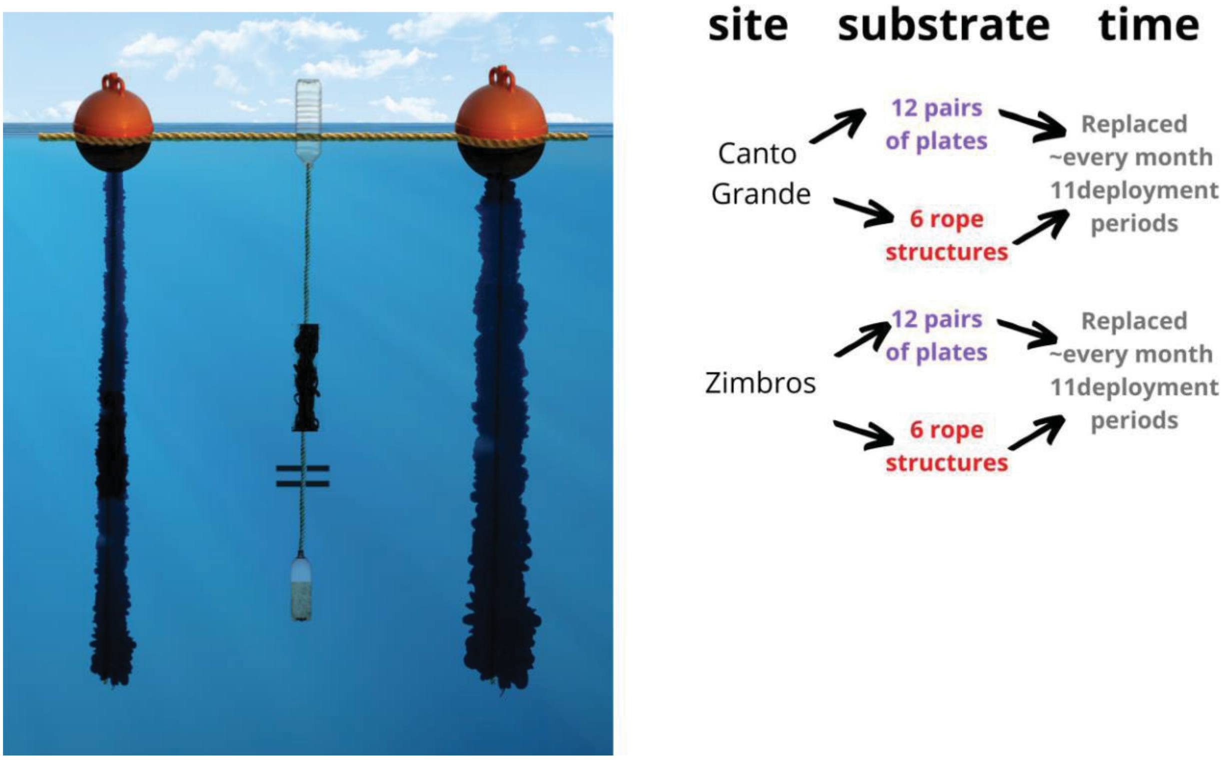


Fig. A. 1. Schematic illustration of each experimental rope hanging from a horizontal long-line with mussel-socks associated to buoys. Each rope had a settlement structure made of a pair of parallel 12 x 12 cm black polyethylene plates. Half of the ropes also had a 30cm-long piece of refurbished rope, used as substrates for mussels settlement. The scheme shows the sampling design.

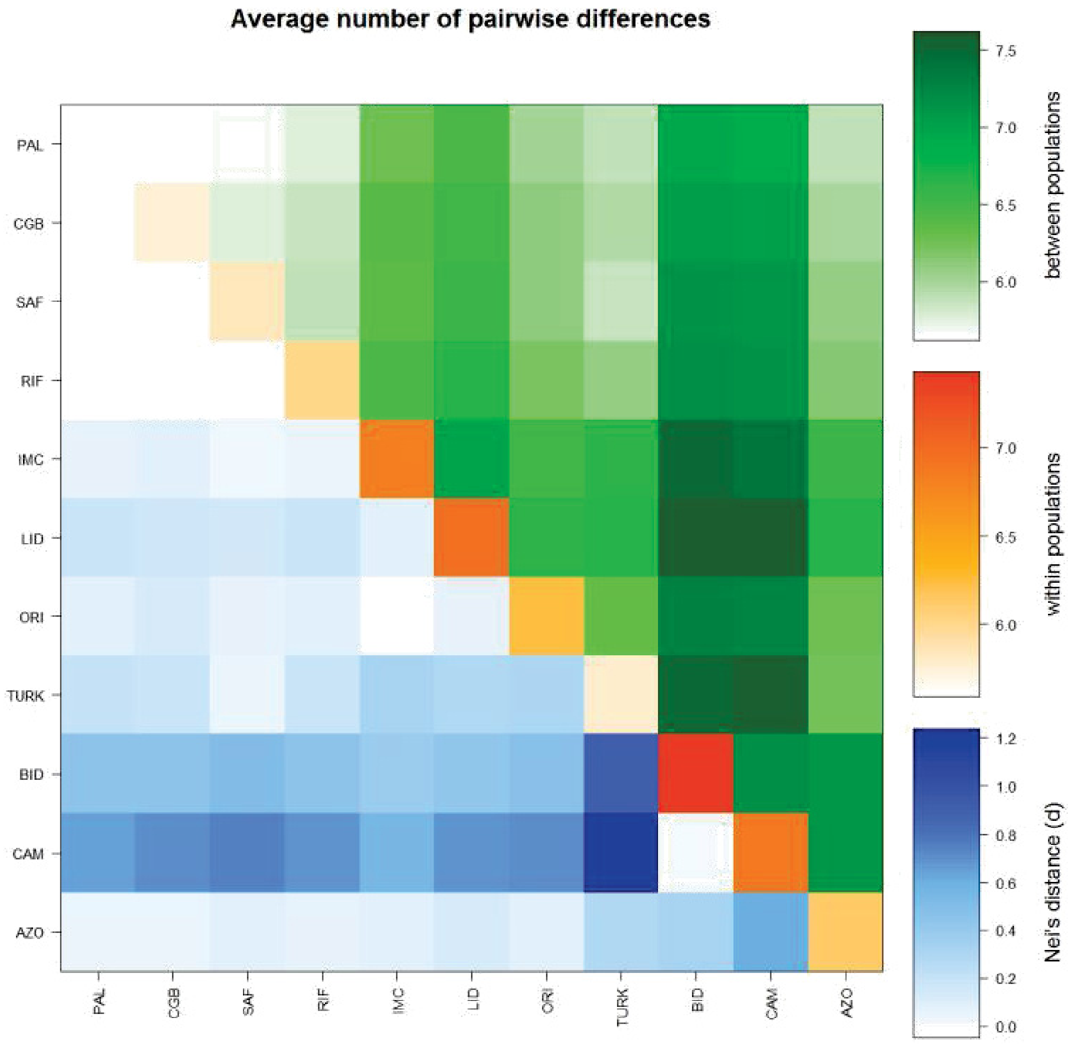
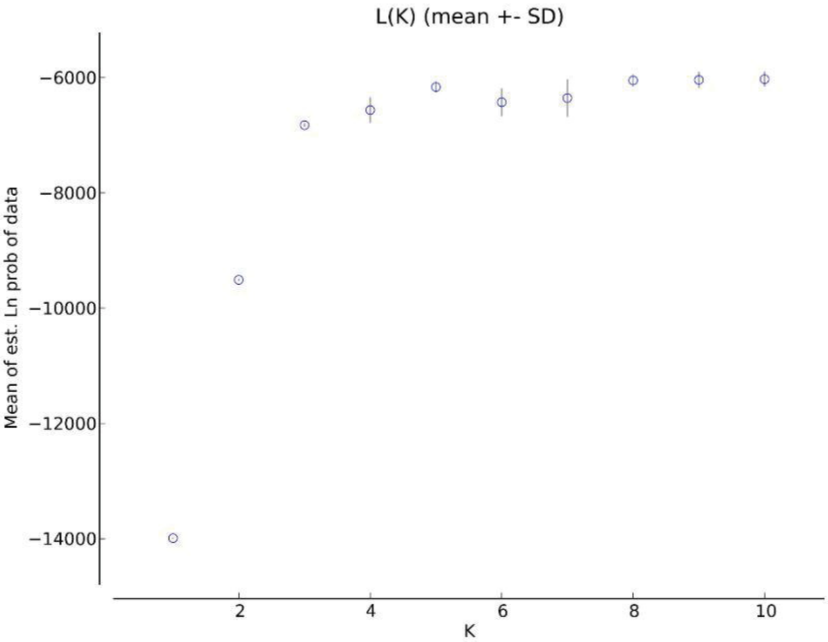


Fig. A. 2. Average number of pairwise differences between and within populations.

a



b

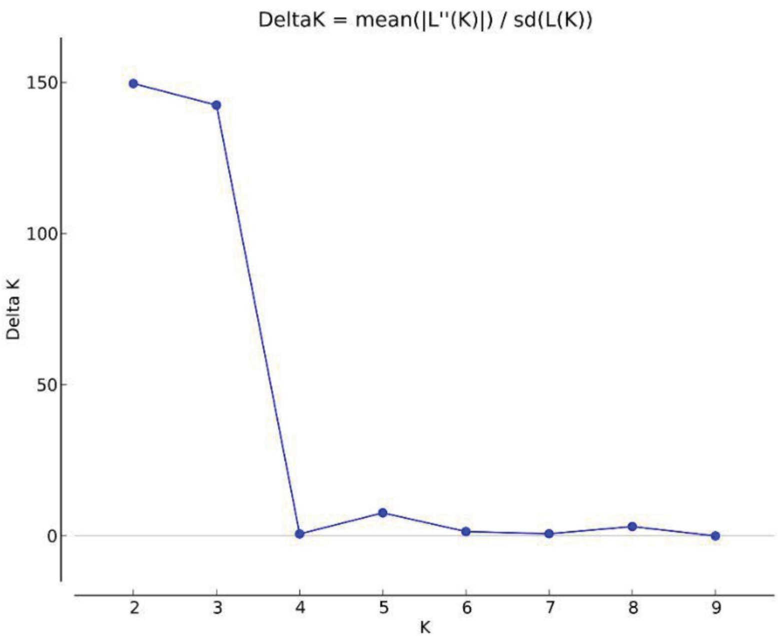


Fig. A. 3. Diagram-based comparison of log-likelihoods for values of *K* and Delta K for some Brazilian samples with the inclusion of reference Northern and Southern hemisphere taxa of *Mytilus* spp.

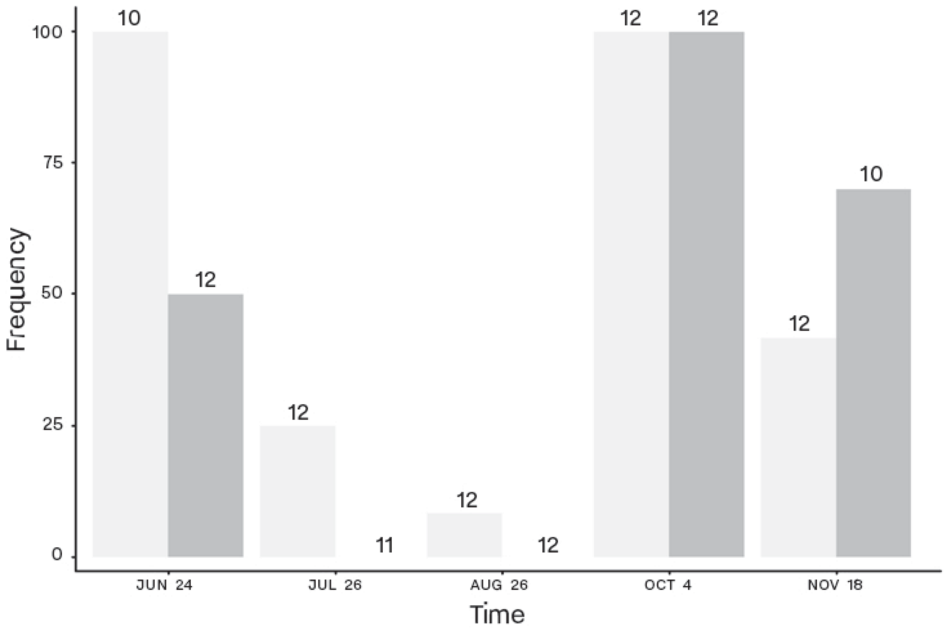


Fig. A. 4. Percentage of black polyethylene plates (12 x 12 cm) with recruits of *Mytilus galloprovincialis* after ~30 days of deployment in Canto Grande (light grey) and Zimbros (dark grey), south Brazil. Numbers over the bars represent sample size recovered at each period.

CAPÍTULO 4

MARICULTURE AS A PROPAGULE SOURCE FOR THE SPREAD OF INVASIVE FOULING SPECIES

Abstract

Eight fouling species are invasive in mussel farms of Santa Catarina state, southern Brazil. Given the extended distribution of inshore aquaculture installations and the presence of two ports, the region is considered important source of propagules. Our assessment aim to forecast the spread of these fouling species to other connected regions along the Brazilian coast. We used ensemble niche models with three algorithms (Maxent, Random Forrest and Support Vector Machine), parameterized with worldwide occurrences of these species, and environmental variables related to ocean temperature and salinity to predict suitable areas to each species. As a proxy for propagule pressure, we considered the amount of goods transported by container-ships from Santa Catarina to other states in the country. States in the northeastern region were the most connected and, although in a different province and ecoregion, the states of Pernambuco and Ceará could be invaded by, respectively, three and one of the focal species. States within the same ecoregion of Santa Catarina could be invaded by *Mytilus galloprovincialis* and by the other species. In the south, Rio Grande do Sul belongs to another ecoregion but it is still highly suitable for *Styela plicata*, *Schizoporella errata*, *Megabalanus coccopoma* and *Mytilus galloprovincialis*. Global warming is changing species latitudinal boundaries but most of the coast will remain suitable in near future (year, 2050). As an ideal habitat for fouling organisms and invasive species, mariculture facilities can interfere with the probability of species establishment and spread, and thus should be leased away from transport vectors. The risk maps provided will allow state authorities and regional stakeholders to prioritize areas of concern of future fouling species invasion.

Keywords: invasion debt; exotic species; connectivity index; aquaculture; risk assessment

Introduction

Marine aquaculture grows faster than other food production sectors to make up for the shortfall of capture fisheries and to supply protein needs of the world´s population (FAO 2018). Much of the production in tropical and sub-tropical, mostly underdeveloped countries, is dedicated to non-indigenous species due to preexisting cultivation protocols and markets (Hewitt at el., 2006). This is a serious threat to native biodiversity given the risk of subsequent escapes of these species and their associated pathogens and parasites that may become invasive when introduced outside their native range (Cook et al., 2008). The evidence of human-induced species introduction leading to invasions is overwhelming and widely seen as a major biological component of global change

(Olden et al., 2004; Molnar et al., 2008; Simberloff et al., 2013), with estimated monetary costs around US$26.8 billion worldwide per year (1970-2017) (Diagne et al., 2021).

Although the intentional introduction of non-indigenous marine species (NIMS) for cultivation has an important effect to the economic development and growth of marine aquaculture, the unintended introduction of epibionts/biofouling, have a negative impact to the industry profitability (Fitridge et al., 2012). The settlement of fouling species increases management costs, which varies considerably with community structure spatially and temporally between regions (Dürr and Watson 2010). The direct negative economic impact of fouling is largely related to the cost of removal of unwanted species and reduction of the commercial species yield (Bannister et al., 2019). Furthermore, indirect negative impacts such as the decrease in native biodiversity is poorly understood and not accounted for, given an inflated value for profit (Suplicy et al., 2015).

Because the ocean is an open environment, very rarely an invasion is associated with a single particular anthropogenic event of NIMS introduction. Fouling species are frequently introduced by merchant-ships that travel between oceans (Hewitt and Campbell 2010) and are considered models for Invasion Science. The rate in which a species gets unintentionally transported as biofouling in hydrodynamically protected niche areas on ship hulls, ballast tanks and sea chests (Coutts and Dodgshun 2007; Hulme 2009; Davidson et al., 2010) determines the fundamental propagule pressure in a given region and eventually the likelihood of invasion, by reducing the effects of demographic and environmental stochasticity at recipient regions (Simberloff 2009). Even with many transport and introduction events, the species must overcome a number of ecological and environmental barriers that in general limit population growth and only a fraction of the introduced species succeeds in becoming invasive (Blackburn et al., 2011). Each successful introduction event is a result of the match of biotic traits with environmental factors that allow species to overcome ecological barriers (Sakai et al., 2001). Broad physiological plasticity is part of the suit of traits owned by widespread invasive species (Broennimann et al., 2007), a characteristic that enable NIMS persistence found at some recipient environments of ports, marinas and mooring bays (Piola and Johnston 2008). The likelihood of invasions depends, in large part, of the abundance of individuals available in the donor region for translocation and the realized propagule pressure that gets introduced, because of persistent genetic or Allee effects involved in the establishment phase (Blackburn et al., 2015).

Once a new region is reached, the availability of hard substrate for settlement is imperative for a translocated fouling NIMS. Thus, extensive availability of artificial structures is expected in recipient regions to support the new population of introduced organism. In fact, globally fouling

NIMS are abundant and diverse on artificial structures in coastal areas (Ruiz et al., 2009; Mineur et al., 2012) that may be used as stepping stones by introduced species to regionally expand their ranges (Adams et al., 2014; Mesel et al., 2015). Marine aquaculture facilitates fouling species accumulation and spread due to the wide variety of artificial structures such as ropes, buoys and livestock shells that provide unlimited space for attachment (McKindsey et al., 2007). In addition, farming sites often extends throughout many high-value coastal areas (e.g., important and significant habitats for conservation of biodiversity) with multiple-uses, in which shipping, ports, aquaculture, recreational SCUBA diving, boating and fishing may occur simultaneously, playing a key role as a pathway of species transportation and introduction (Minchin 2007; Castro et al., 2017).

The introduction of *Mytilus galloprovincialis* in South Africa is a good example. A single farm introduced the mussel in the southern region of South Africa from where has spread to adjacent natural environments (McQuaid and Phillips 2000) with negative effects to the biodiversity (Sadchatheeswaran et al., 2015). The importance of the population cultivated in this farm as a source of propagules was evident after the abandonment of the activity at the site, which led to the subsequent drastic reduction of the density of the invasive species at the adjacent natural environments (Rius et al., 2011). Thus, mariculture can create problems for biodiversity by mediating the spread of propagules of fouling NIMS and also of commercial species.

In southern Brazil mussel farming is very important to the local economy (Suplicy et al., 2015), but the installation of farming structures has created a new impact to the seascape, which have favored the prevalence of fouling NIMS (Rocha et al., 2009). Therefore, risk assessments on the impacts of farming installations to regional biodiversity should consider the spread of fouling NIMS and include the prediction of future translocations of those species that are already known to be problematic in other regions.

The cold and rich South Oceanic Central Water is an important force that have favored aquaculture development in the southern Brazilian coast (Lopes et al., 2006). Nowadays, Santa Catarina nutrient-rich waters and sheltered bays are responsible for over 95% of all the mussels and oysters produced in the country with mussel and oyster farms spread along the 560 km of the state, mostly inshore; (Santos and Della-Giustina 2017). The region is notable for having the second largest concentration of shipyards and two of the largest ports in the country located to the north (Port of Itajaí) and to the south (Port of Imbituba) of the farming areas (SEBRAE 2012;

ANTAQ 2021), which increases the risks of unintentional regional and international transport of fouling species.

While global regulations to control marine bioinvasions entered into force in 2017, which require all ships in international traffic to manage their ballast water and sediments (BWM Convention, IMO 2004), domestic transfers from port to port has yet to become the focus of biosecurity management in countries with long coastlines such as Brazil. There are 17 coastal states, most of them with at least one major port receiving container vessels originated in ports of Santa Catarina. This suggest that the NIMS present in aquaculture facilities in this region have numerous opportunities to spread along the coast and contribute to large propagule pressure for all those ports. In this study we focused on six invasive NIMS established in aquaculture facilities at Santa Catarina to understand their potential to be introduced and invade other regions in the country, considering the current domestic shipping trade between ports in Santa Catarina and in other states as the main pathway and species suitability models to address current and future habitat.

Methods

Species and occurrences

Eight invasive NIMS recognized to be spread and abundant in aquaculture facilities in Santa Catarina (1st |chapter) were target in the present study: the ascidians *Aplidium accarense* (Millar, 1953), *Botrylloides giganteus* (Pérès, 1949), *Didemnum perlucidum* Monniot F., 1983 and *Styela plicata* (Lesueur, 1823), the bryozoans *Schizoporella errata* (Waters, 1878) and *Watersipora subtorquata* (d'Orbigny, 1852), the barnacle *Megabalanus coccopoma* (Darwin, 1854) and the bivalve *Mytilus galloprovincialis* Lamarck, 1819. These species are worldwide hitchhikers that have been introduced in Brazil unintended by domestic and/or international shipping.

Native and introduced occurrences from these target species were obtained from the global geographic distribution databases (OBIS and GBIF) and from published taxonomic, ecological and oceanographic studies searching for accepted names and resolved synonyms of the species in Web of Science and Google Scholar portals. When studies reported general locations instead of precise geographic coordinates at sea of species occurrence, we acquired those coordinates using Google Maps, considering the existence of ports, marinas and rocky shores to guess the locality with higher probability of presence in the location mentioned in the study. Finally, we plotted species occurrences on maps and excluded outliers (points far outside the species known distribution).

Current and future environment predictors

We modeled environmental suitability using variables from the Bio-Oracle v2.0 dataset (Assis et al., 2018), which has global marine layers with spatial resolution associated with a grid of cells of approximately 9 km² (5 arc-minutes) of average values for the period 2000 – 2014. We selected variables associated with seawater temperature and salinity which are considered main drivers of the distribution of marine invertebrates (Hauton 2016; Whiteley and Mackenzie 2016). To control for strong correlated environmental layers (r >.7), we systematically selected one and dropped the other in each pair of correlated variables (Dormann et al., 2013), resulting in a final set of four predictors that comprised maximum sea surface temperature, minimum sea surface salinity and ranges of both variables. Future (2040-50) projections of the same environmental predictors were obtained under two Representative Concentration Pathways (RCP) for greenhouse gas emissions scenarios; one very stringent (RCP26) and one more conservative with high greenhouse gas emission rate (RCP60) (see Assis et al., 2018 for details).

Ecological niche modelling

After gathering occurrence locations (presence) and environmental parameters in those locations we used the ENMTML package (Andrade et al., 2020) to fit species current environmental suitability and projected models of future environmental conditions for the Brazilian coast. In the models, we employed the ensemble of the following algorithms: Maximum Entropy with default tuning (MXS) (Phillips et al., 2006), Random Forest (RDF) (Prasad et al., 2006), and Support Vector Machine (SVM) (Guo et al. 2005). To reduce the effects of sampling bias, we randomly filtered species occurrences considering one presence only within each grid cell of a grid with a grain 2x the resolution of the environmental variables. It is a simple procedure with good performance (Fourcade et al., 2014). We used an absence ratio of 1 to 10 presences, which were randomly allocated within the lowest suitability areas predicted by a Bioclim model (Engler at al., 2004), inside the area accessible to each species delimited by the Exclusive Economic Zone (i.e., within 370 km of the coast). Models were validated by random bootstrap partition between 70% of the occurrence records for model training and 30% for testing the results (Fielding and Bell 1997), with which we then evaluated the distributional models using True Skill Statistics (TSS). We repeated this procedure 10 times for each algorithm and used the suitability value that maximizes the TSS to transform each map in binary (Allouche et al., 2006). Final models were constructed by an ensemble of all the algorithms using the average of suitability values weighted by the algorithms performance (TSS) (Thuiller et al., 2009). Environmentally suitable cells were categorized using a gradient from deep blue to red to indicate low to high suitability for each species. All the procedures were performed in R 4.0.0 (R Core Team 2020).

Connectivity between ports

Merchant shipping is the main vector of transport and introductions of juvenile or adult marine organisms either by ballast water, hull fouling or sea chests (Coutts and Dodgshun, 2007; Hewitt et al., 2009). Container ships moved 97% of the cargo from Santa Catarina to other states in Brazil according to the data acquired online at the Brazilian national aquatic transport agency (ANTAQ). In the absence of data on the number of voyages and containers ships, we used the total amount of goods transported during five years' time (2015 to 2019) as surrogate of the connectivity between states. We ranked ports comparatively in three categories, high, intermediate and low connectivity and also, excluded from the connectivity analysis the states of Amazonas and Pará with ports outside the species environmental suitability (salinity < 0,5%).

Risk assessment

To assess the risk of species transport and introduction/invasion (suitability + connectivity), we built a matrix that overlaps the cargo transported from SC to each state and environmental suitability. The joint assessment of donor region with environmental suitability at possible recipient regions complemented by information on vectors of transport constitute an index to propagule pressure (sensu Lockwood et al., 2009) that have been used to forecast species introduction and invasion (Goldsmit et al, 2017; Lins et al., 2018).

Results

Currently, the bryozoans *S. errata* and *W. subtorquata* are present in ten and eight states in the Brazilian coast, between Santa Catarina and Ceará in the northeastern region. The ascidians *D. perlucidum* and *S. plicata* and the barnacle *M. coccopoma* are present in six states, the ascidians *B. giganteus* is present in four and *A. accarense* in two mainly within the southern and southeastern regions. The mussel *M. galloprovincialis* occurs only in Santa Catarina (Table 1).

Table 1. Amount of goods (thousand tons) transported by container ships from Santa Catarina ports to other Brazilian coastal states in five years (2015 – 2019) with predicted environmental suitability. Red cells correspond to high environmental suitability, yellow medium suitability and blue low suitability. Asterisks indicates where the species already occurs and empty cells, where there is no environment suitability.

Federation states	Total	*Aplidium*	*Botrylloides*	*Didemnum*	*Styela*	*Schizoporella*	*Watersipora*	*Megabalanus*	*Mytilus*
Pernambuco (PE)	3083			(red)		*	(red)	(yellow)	
Ceará (CE)	2220			(red)		*	*		
Bahia (BA)	1336		(yellow)	*	*	*	*	*	
São Paulo (SP)	752	*	*	*	*	*	*	*	(yellow)
Rio Grande do Sul (RS)	597	(red)	(yellow)	(blue)	(blue)	(yellow)	(yellow)	(red)	(red)
Rio de Janeiro (RJ)	360	(red)	*	*	*	*	*	*	(yellow)
Espirito Santo (ES)	327	(yellow)	*	*	*	*	*	*	(blue)
Paraná (PR)	236	(red)	(red)	*	*	(yellow)	(blue)	*	(red)
Paraíba (PB)	13			(red)		*	(red)	(blue)	
Rio Grande do Norte (RN)	4			(red)		*	*	(blue)	
Alagoas (AL)	0			(red)		*	*	(blue)	
Sergipe (SE)	0			(red)		(blue)	(red)	(blue)	
Piauí (PI)	0			(red)				(blue)	

The number of unique occurrences per species used for modeling ranged from 20 to 678, with accurate predictions (TSS > 0.8) and little variation (Table 2). The evaluation index indicated that the SVM and RDF models performed similarly and more accurate than MaxEnt (Table 2). Except for three species under the RDF model, temperature variables were consistently the main drivers of predictive performances across algorithms (Table 3). The ensemble of the models showed that there are suitable areas not yet occupied to which species can expand their distribution, both currently and under future global warming scenarios (Figs. 1 to 8).

Table 2. Summary of predictive performance validated by random bootstrap partition of test occurrences (30% of N) of ten replications for the models: MaxEnt (MXS); Random Forrest (RDF); Support Vector Machine (SVM) and Ensemble (WMEA) evaluated using True Skill Statistics (TSS average and standard deviation in parenthesis).

Species	N	MXS	RDF	SVM	WMEA
A. accarense	32	0.894 (0.045)	0.952 (0.062)	0.942 (0.070)	0.922 (0.071)
B. giganteus	20	0.693 (0.066)	0.896 (0.032)	0.883 (0.020)	0.853 (0.032)
D. perlucidum	48	0.772 (0.045)	0.887 (0.088)	0.867 (0.096)	0.877 (0.083)
S. plicata	121	0.843 (0.037)	0.944 (0.041)	0.894 (0.054)	0.932 (0.059)
S. errata	55	0.764 (0.082)	0.947 (0.019)	0.918 (0.037)	0.923 (0.018)
W. subtorquata	48	0.821 (0.109)	0.965 (0.034)	0.972 (0.022)	0.954 (0.038)
M. coccopoma	80	0.814 (0.005)	0.954 (0.042)	0.914 (0.043)	0.906 (0.059)
M. galloprovincialis	678	0.932 (0.015)	0.981 (0.005)	0.972 (0.005)	0.977 (0.009)

Santa Catarina delivered a total of 10.758 thousand tons in goods from 2015 to 2019, with a remarkable increase in containerized cargo from 1113 to 2351 thousand tons in five years' time, which is now 97% of the total goods transported from Santa Catarina to other ports in the Brazilian coast. The states of Pernambuco, Ceará and Bahia in the northeastern region have been the main destination of goods delivered, in other words, these are the states more prone to receive the propagule supply originated in Santa Catarina (Table 1). A second group of intermediate connected states is formed by Espírito Santo, Rio de Janeiro, São Paulo in the southeastern and Paraná and Rio Grande do Sul in the southern. The least connected states are Rio Grande do Norte, and Paraíba all in the northeastern region. Piauí, Sergipe and Alagoas did not receive container ships from Santa Catarina.

Table 3. Summary of the percentage contribution of environmental variables used for the predictive performance of species suitability models. Sea surface salinity (SSS) minimum, sea surface temperature (SST) maximum and ranges obtained from Bio-Oracle v.2.0 database.

Species	MXS[1]				RDF				SVM			
	SSS Min	SSS Range	SST Max	SST Range	SSS Min	SSS Range	SST Max	SST Range	SSS Min	SSS Range	SST Max	SST Range
A. accarense	24	3	20	53	34	15	24	26	25	2	17	56
B. giganteus	25	11	33	31	26	26	17	28	19	7	26	47
D. perlucidum	23	5	67	5	22	18	31	29	28	3	64	5
S. plicata	25	8	27	50	28	14	37	21	22	11	31	36
S. errata	25	3	27	43	38	16	21	23	23	2	35	38
W. subtorquata	28	11	42	18	38	7	29	24	26	11	39	22
M. coccopoma	8	7	42	43	25	7	31	37	15	5	55	25
M. galloprovincialis	20	13	5	62	19	10	51	21	16	12	3	69

[1]MXS = MaxEnt; RDF = Random Forrest and SVM = Support Vector Machine

Pernambuco has high environment suitability for the species *D. perlucidum* and *W. subtorquata* and in the future, for *M. coccopoma* too, which together with the species *S. errata* that already occurs in the region present high risks of invasion given the high propagule pressure from Santa Catarina in the state. The bryozoans *S. errata* and *W. subtorquata* can also invade Ceará where they already occur, while the species *D. perlucidum* can be introduced. The whole northeastern region is suitable for the species *D. perlucidum*, however, due to the low propagule pressure, the states of Paraíba and Rio Grande do Norte have low risk of introduction, while Bahia can be invaded. The southern and southeastern regions present intermediate connectivity and several species, which already occur in the southeastern can invade. Rio Grande do Sul, the only state to the south of Santa Catarina has high suitability and high risk of the introduction of *M. coccopoma* and *M. galloprovincialis*, followed by *S. errata*, *W. subtorquata*, *A. accarense* and *B. giganteus* with intermediate risk and *D. perlucidum* and *S. plicata* with low risk. In the state of Paraná, also with intermediate connectivity, only *M. galloprovincialis* presents a high risk of introduction while *A. accarense*, *B. giganteus* and *S. errata* is of intermediate risk and *W. subtorquata* low risk. The invasive mussel *M. galloprovincialis* can also reach the state of São Paulo and Rio de Janeiro, but given the intermediate suitability of these regions the risk is low, while for *M. coccopoma* and *B. giganteus* the suitability in the northeastern region increases in the future, increasing also the risk of invasion (Table 1; Figs. 1 to 68).

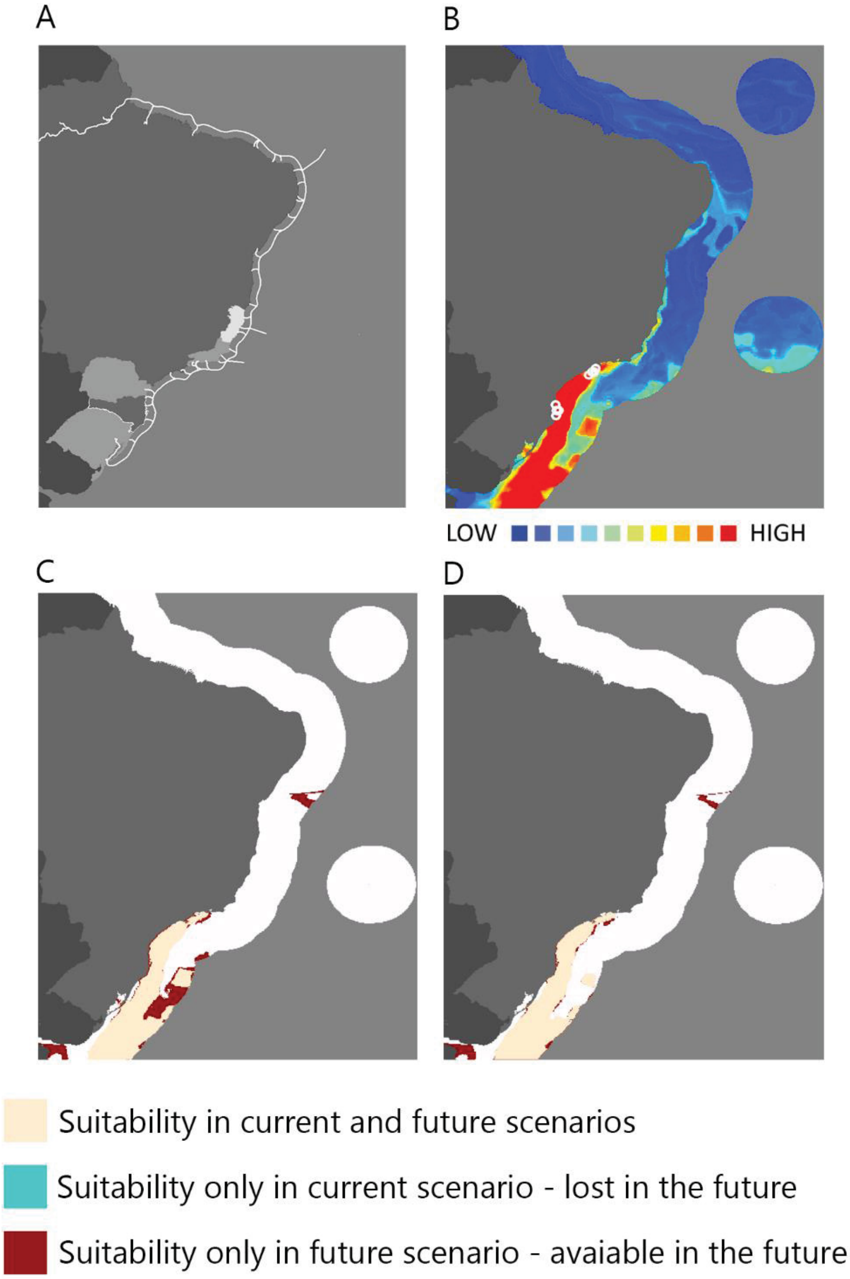


Figure 1. *Aplidium accarense*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

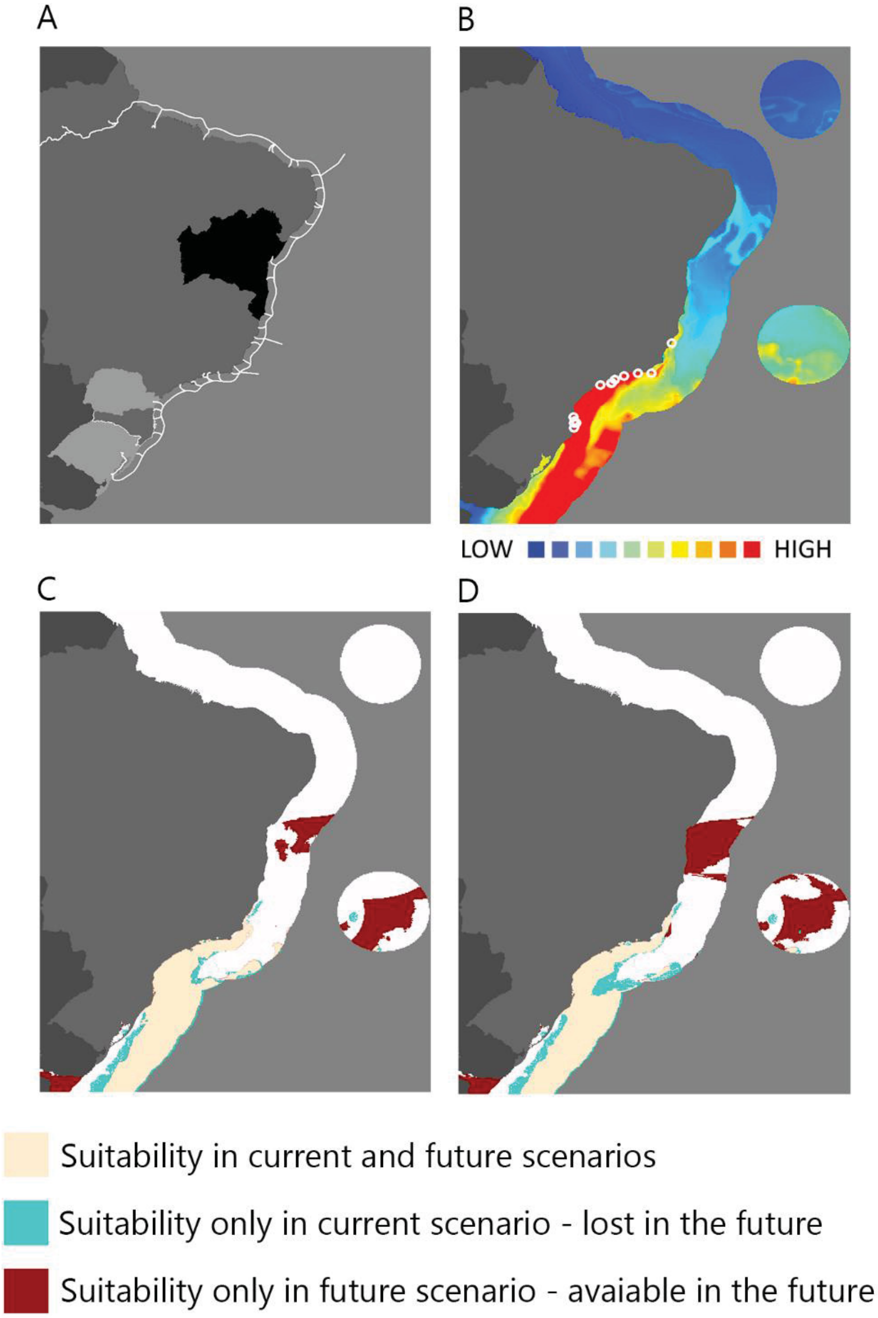


Figure 2. *Botrylloides giganteus*. A. Domestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

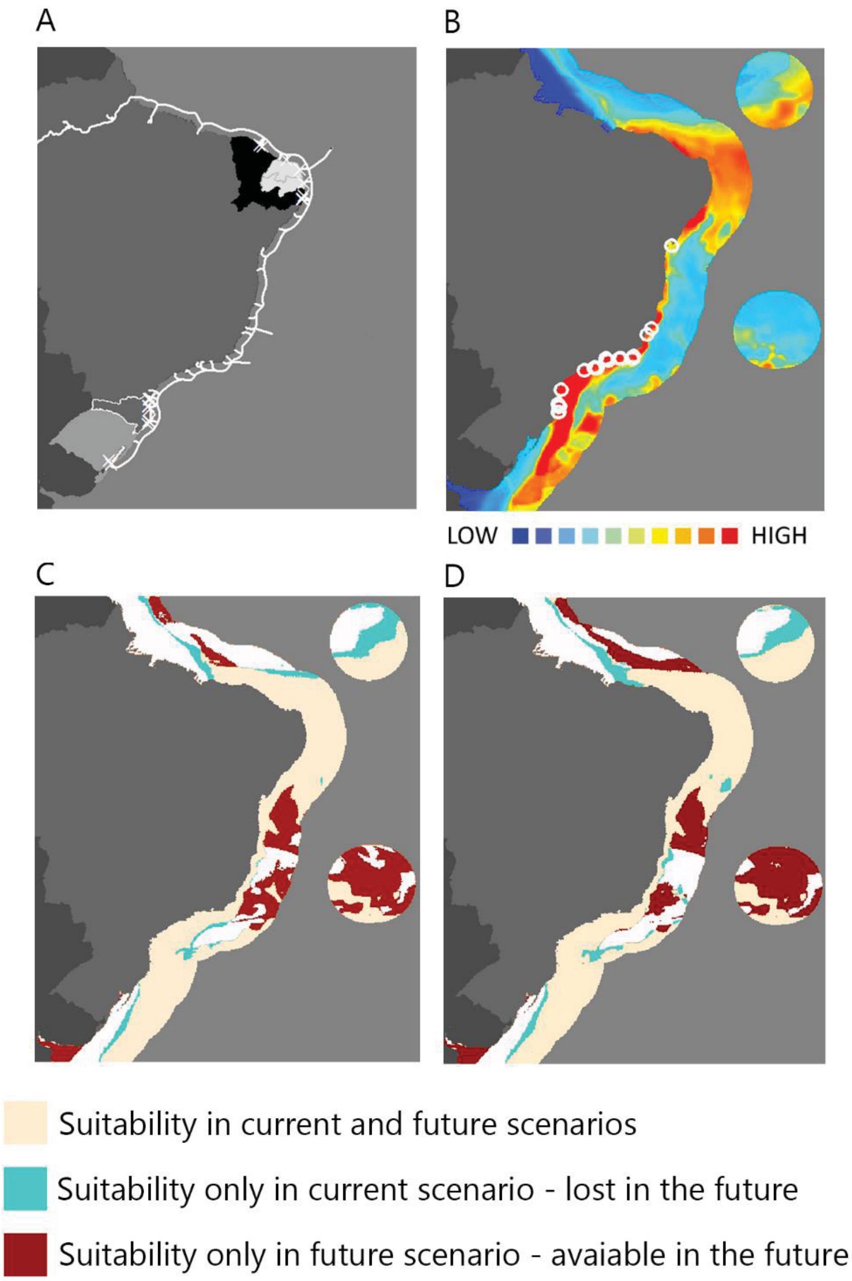


Figure 13. *Didemnum perlucidum*. A. cabotage container route (white line) that originate in the state of Santa Catarina and indication ofDomestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

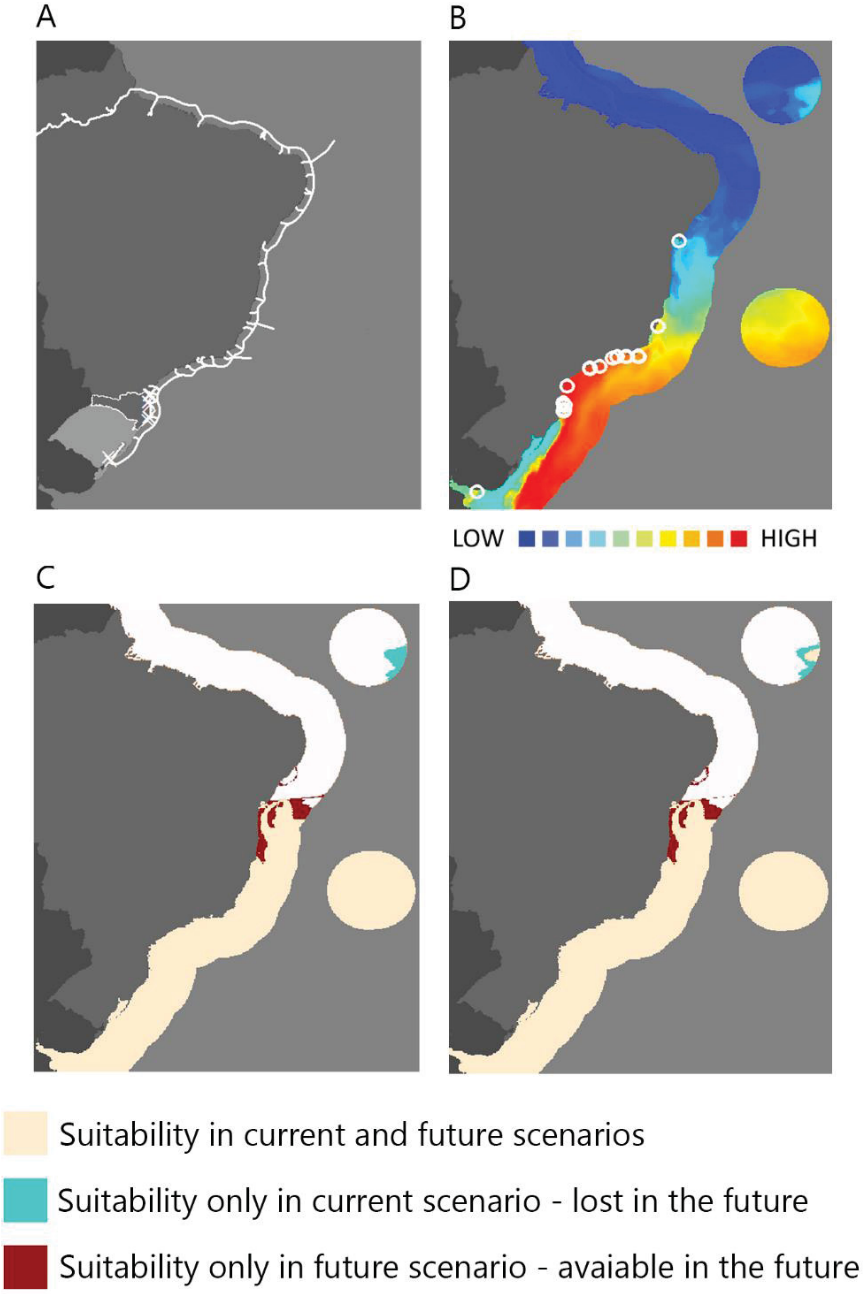


Figure 24. *Styela plicata*. A. cabotage container route (white line) that originate in the state of Santa Catarina and indication ofDomestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

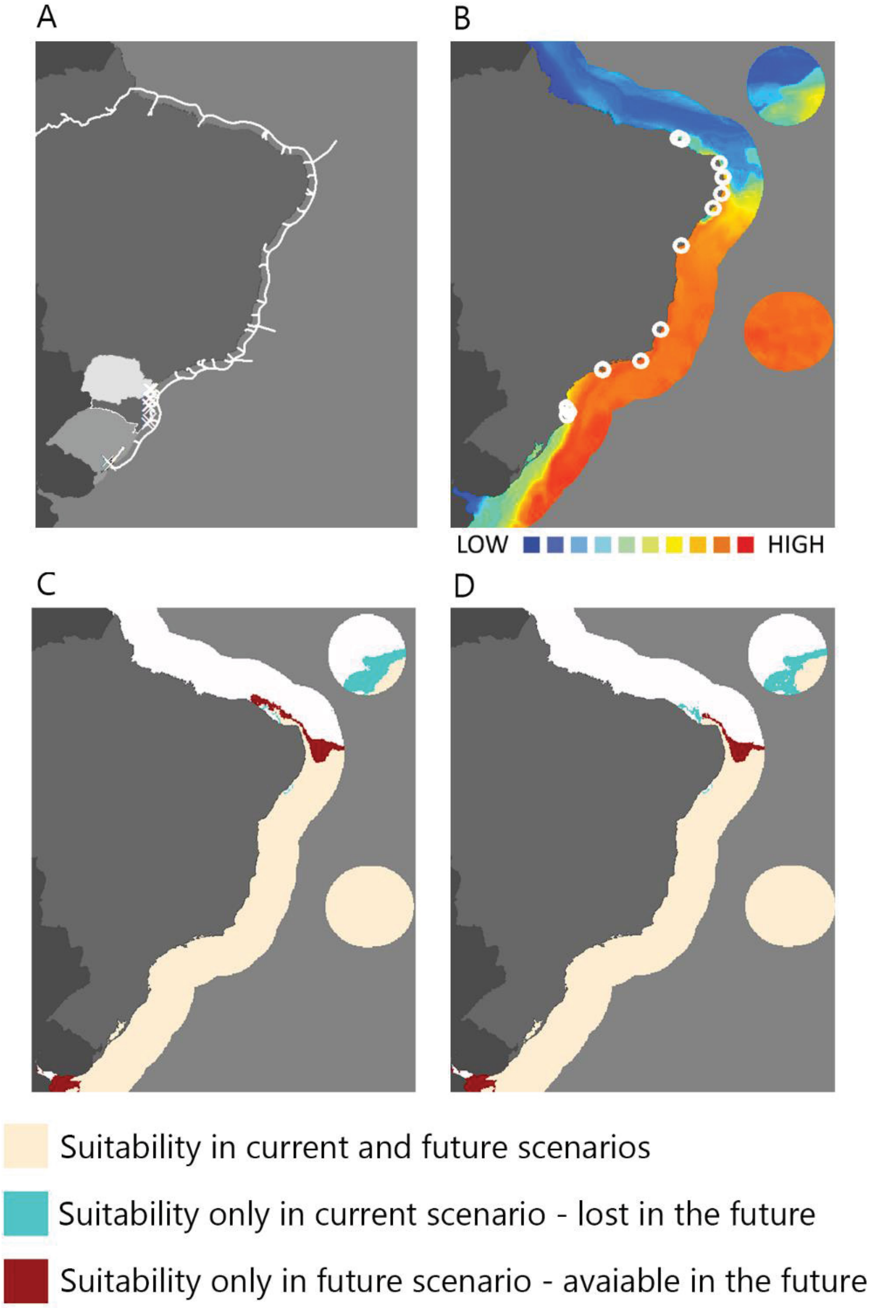


Figure 35. *Schizoporella errata*. A. cabotage container route (white line) that originate in the state of Santa Catarina and indication ofDomestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

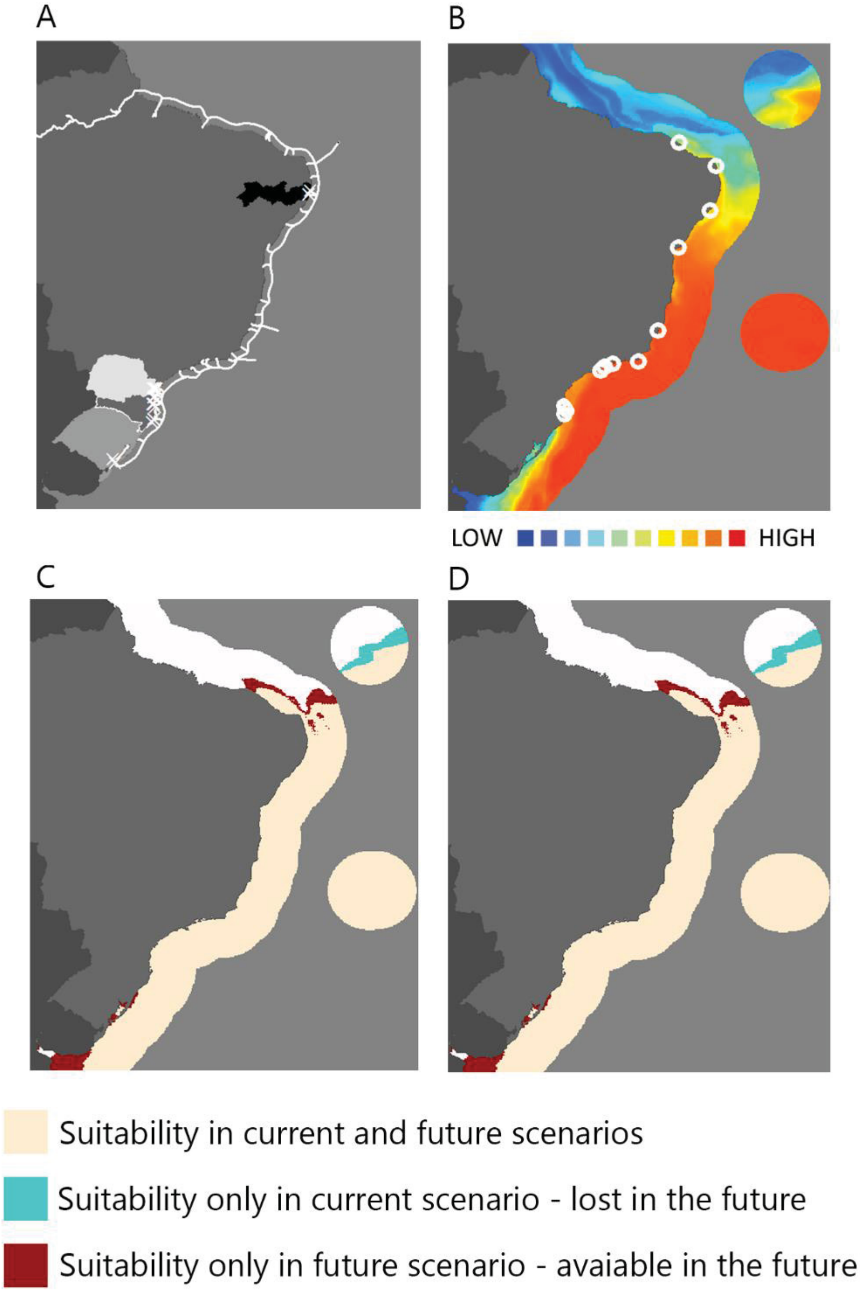


Figure 46. *Watersipora subtorquata*. A. cabotage container route (white line) that originate in the state of Santa Catarina and indication ofDomestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

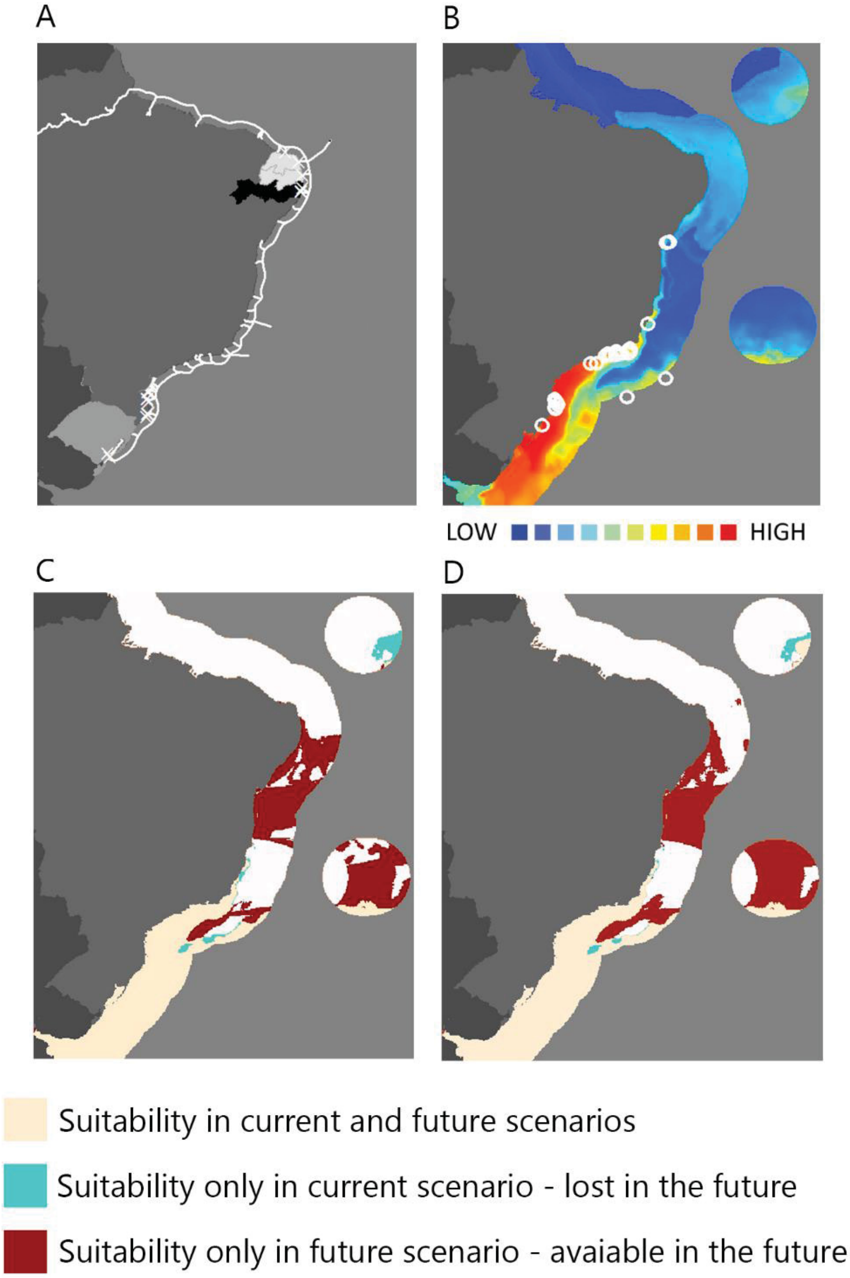


Figure 57. *Megabalanus coccopoma*. A. cabotage container route (white line) that originate in the state of Santa Catarina and indication ofDomestic shipping container route (white line) and the states at most risk of this species introduction (black = high risk, grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP26 in year 2050. D. future climate scenario, RCP60 in year 2050.

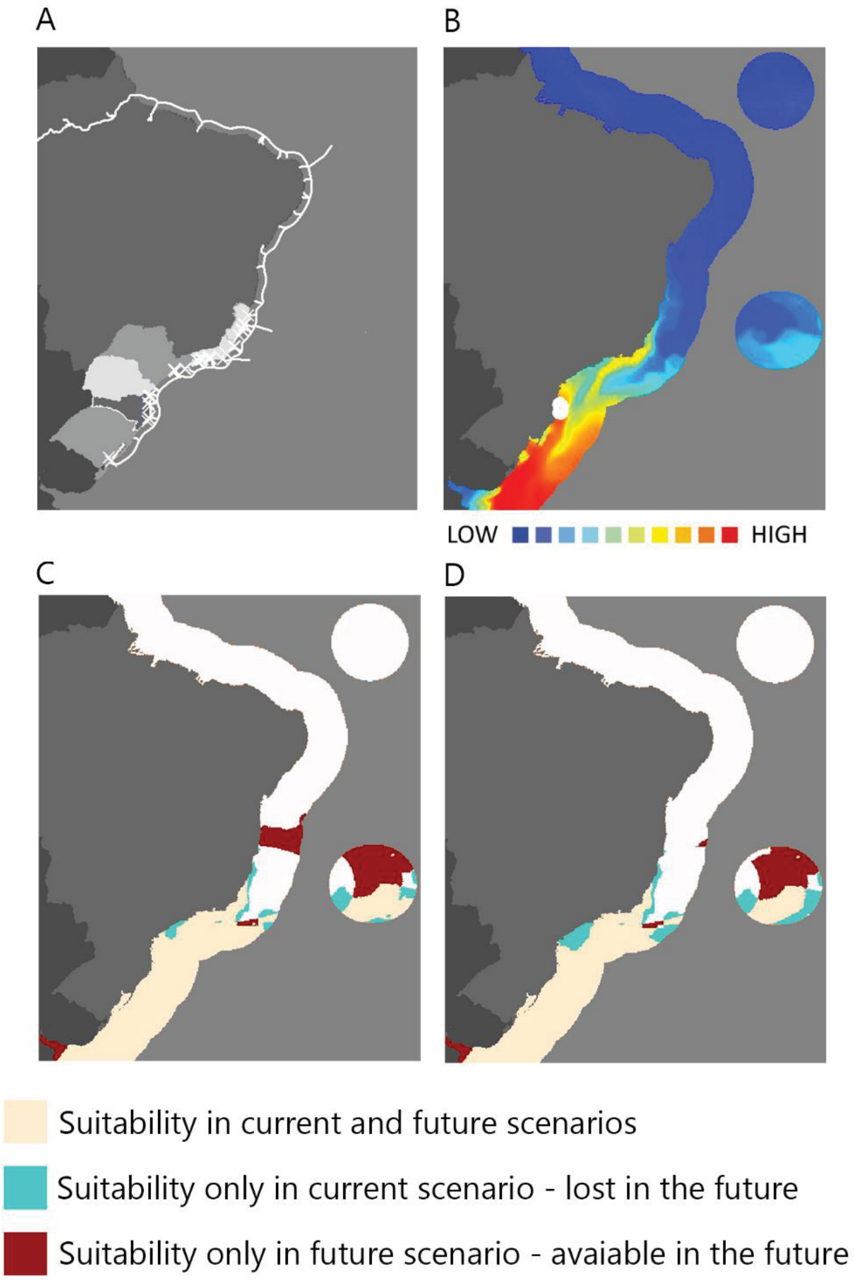


Figure 8. *Mytilus galloprovincialis*. A. cabotage container route (white line) that originate in the state of Santa Catarina and indication ofDomestic shipping container route (white line) and the states at most risk of this species introduction (grey = medium risk and light grey = low risk). B – D. Environmental suitability maps generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest). B. Current scenario with species occurrences. C. Future climate scenario, RCP2,6 in year 2050. D. future climate scenario, RCP6,0 in year 2050.

Discussion

Models successfully recognized regions of environmental suitability to each hazardous species, that when overlapped with the connectivity between Santa Catarina and each recipient state resulted in a risk matrix designed to alert state authorities and stakeholders along the Brazilian coast about target species of concern. The detection of NIMS sometimes occurs too late in the invasion process and only after the negative effects of the species worsen (Coutts and Forrest 2007). Unfortunately, most regions in Brazil do not sustain long-term biodiversity assessments and research programs to cover the entire extension of the coast (UNESCO, 2015), one possible explanation for why we have observed such extensions of suitable areas and so few correspondent registers of species occurrence outside the southeastern region (mostly, Rio de Janeiro and São Paulo). An alternative to explain the absence of occurrences in some states is the alleged lower Gross Domestic Product of Sergipe, Piauí, Alagoas, Paraíba and Rio Grande do Norte (IBGE), which is an indicator of the flow of goods and services and is correlated to disturbance and propagule pressure (Clark and Johnston 2009; Hulme 2009). The International Marine Organization (IMO) by means of the Ballast Water Management Convention (BWMC), which came into force by September 2017 will likely reduce the number of NIMS transported. Thus, cabotage domestic shipping will become the focus of biosecurity management and in possession of these maps, federal authorities can allocate resources more securely to the neediest states and coast stretches most at risk of NIMS introductions.

The joint use of target species, environmental suitability models and connectivity, allowed the identification of Pernambuco and Ceará with high risk of *D. perlucidum* introduction and establishment. *Didemnum perlucidum* is known to spread from artificial substrates to seagrass beds (*Halophila ovalis*) in Western Australia (Simpson et al., 2016) and also to reduce mussel yield in Santa Catarina mussel farms (Lins and Rocha 2020). It is the only species with primary high introduction risk in Ceará. The prevalence of unconsolidated sediments in Ceará coast possibly prevented the natural dispersal of *D. perlucidum* but the presence of artificial structures could assist the species introduction in the future.

São Paulo is the largest international hub in Brazil for maritime trade and is at constant risk of NIMS introduction. São Paulo and Rio de Janeiro had medium environmental suitability to the species *M. galloprovincialis* but Rio de Janeiro connectivity with Santa Catarina is half the value of São Paulo. This mussel ranks among the 100 most invasive species worldwide (Lowe et al., 2000) and the states of Rio Grande do Sul and Paraná had high environmental suitability for the species. Beyond *M. galloprovincialis, S. errata* can also be introduced to Paraná, although the presence of ports inside estuaries such as Paranaguá, could work as a natural barrier to the species due to water turbidity and strong spatial and temporal salinity gradients (Angulo et al., 2006; Hauton 2016). Nevertheless, unexpected marine species have already been registered in this estuary such as the ascidians *D. perlucidum* and *S. plicata* (Bumbeer and Rocha 2016), and hydroids common to both Paraná and Santa Catarina (Ajala-Batista et al., 2020).

There are other biotic and landscape factors unaccounted in environmental suitability models, that are important to understand community assemble and presence or absences of species. One important driver for sessile NIMS occurrence is the availability of consolidated substrates for attachment. The southern and southeastern Brazilian region concentrates most of the rock shores that are scattered from the Espírito Santo state to Santa Catarina and where native biodiversity is most at risk of species invasion. These regions are also highly anthropized, with many changes to coastal environments and the installation of artificial structures, which has also been demonstrated to be important corridors for NIMS (Airoldi et al., 2015) increasing the risk of multiple events of introduction of new propagules.

In the opposite direction, the state of Rio Grande do Sul lack natural consolidated substrates, and the presence of wide sandy plains could have prevented *M. galloprovincialis* and *M. coccopoma* and the bryozoans *S. errata* and *W. subtorquata* introduction despite its environmental suitability and intermediate connectivity.

Predation is another example of limiting interaction of species distribution that is not considered in climate suitability models but is recognized for altering benthic communities along latitudinal gradients with increased pressure in the tropics (Freestone et al., 2021). Predation and competition for space have a strong influence on the composition of benthic communities in the Brazilian coast (Kremer and Rocha 2016; Hiebert et al., 2019), but varies greatly depending on the stages of development of the species and size of the predators (Oricchio et al., 2016). For example, in Ribeirão da Ilha-SC, ascidians are dominant over barnacles at artificial structures when not controlled by predation, but when *S. plicata* is strongly preyed upon it liberates space for *M. coccopoma* (Kremer and Rocha 2016), which could favor the barnacle spread to lower latitudes predicted under climate change. Despite the increased predation, ascidians acquire large biomass during the summer, which may buffer negative effects of predation, while the bryozoan mineralized colony is not affected by biotic interactions or predation (Hiebert et al., 2019; Oricchio and Dias 2020). *Didemnum perlucidum* and *Schizoporella errata* show seasonality in growth rate and favor warmer waters (Oricchio and Dias 2020) which explains the increase in habitat suitability with global warming and the risk of introduction to lower latitudes.

Widespread invasive species have high phenotypic plasticity, allowing them to occupy climatic niches distinct from those they occupy in the regions of origin (Broennimann et al., 2007).

We used registers of occurrences of native and introduced ranges without distinction to calibrate and test the suitability models that usually perform better than when using occurrences from the native range only (Broennimann and Guisan 2008). Temperature was the most important driver of suitable areas. When seawater temperature increases, other correlated factors may emerge, as for example primary productivity, that may lead to context-dependent shifts in competition (Ruiz et al., 2009). For example, temperature strengthens competition between the bryozoans *S. errata* and *W. subtorquata*, and favors the latter but only in polluted habitats (McKenzie et al., 2011). Global warming is expected to generate poleward range shifts of NIMS (Sorte et al., 2010). However, our predictions indicate that with the exception of the mussel *M. galloprovincialis*, target species will gain areas of suitability in lower latitudes of Brazilian coast. Nevertheless, NIMS range shifts are likely to happen more slowly than the rate in which species are transported and introduced so, other non-climatic limiting factors, such as propagule pressure and the availability of artificial substrates for species introductions could be more limiting (Early and Sax 2014)

Additionally, to the great risk of Santa Catarina invasive populations reaching new areas, these regions can also receive propagules from other regions where the species are established. For example, *D. perlucidum*, *S plicata*, *S. errata*, *W. subtorquata* and *M. coccopoma* occur in other states of Brazil, thus authorities and stakeholders should also identify other alternative pathways for species introduction and rank their risk based both on degree of connectivity and propagule abundance (considering the species abundance in donor regions is a good proxy). Even in states with established populations of the NIMS here studied, managers should study different pathways and donors' regions because propagule pressure further intensify invasions by introducing adaptive genetic variation (Ghabooli et al., 2013). Molecular studies are able to illustrate the genetic relationships among disjunct populations and detect the possible origin of introduced species (Pritchard et al., 2000; Falush et al., 2007). However, this type of analysis can be very expensive when species are distributed globally at multiple regions. Connectivity indexes can reduce these costs by narrowing the most probable pathways of gene flow to be taken as sampling references. Predictive models such as we used here have the advantage of using public information and have successfully predicted species introduction (e.g., *C. lepadiformis* to Australia and *S. clava* to Argentina, Lins et al., 2018).

Invasive species can become a problem with enormous negative effects to human well-being and to biodiversity. Predictive studies of invasion risk can guide authorities and stakeholders by pointing which species are more likely to invade and where to focus monitoring efforts that aim to prevent invasions in the early stages, when actions are more successful. For example, marine protected areas (MPA´s) are likely to be broadly expanded to meet Sustainable Development Goal (SDG) 14 to protect aquatic life and SDG 2 to guarantee food security, by 2030. The placement of MPA´s have been planned based on socioeconomic features that favors anthropogenic activities to the detriment of conservation objectives (Ojeda-Martínez et al., 2009) which have led to very few suitable areas for threatened marine invertebrate species in Brazilian no-take MPAs (Magris and Déstro 2010). In face of increasing human pressure, the identification of invasive species suitable areas and connectivity networks among of the target species presented here, should be incorporated in the planning of MPA´s to avoid further threatening of biodiversity.

Our maps also show offshore habitats inside the exclusive economic zone suitable for the six target species. These regions encompass several human activities such as cabotage domestic shippingroutes, industrial fishing, oil and natural gas exploratory blocks that are susceptible to fouling NIMS impacts. Offshore facilities reinforce the strategic position of fouling species as a stepping stone for dispersion (De Mesel et al., 2015).

The results here highlight how local NIMS can interfere with the probability of marine invasions in quite distant regions of the country and that this should be considered in the decision-making process of establishing new aquaculture plants and their expansion. Currently in Santa Catarina stakeholders diverge on the perception about the importance of an ecosystem approach when locating aquaculture parks (Vianna and Filho 2018). The location and size of mariculture installations may alter the risk of NIMS spreading to adjacent regions. Ideally the location of aquaculture parks should avoid areas of multiple uses that could increase the chance of dispersal of propagules by vessels and smaller recreational and fishing boats to other areas. Further studies should estimate the natural dispersion of propagules from farm areas (of known densities of fouling NIMS) -by recruitment experiments at various distances, to a radius from the farms. This could help to determine safe buffer zones around farm installations and reduce the spread. There is an urgency to integrate the best available scientific, traditional and stakeholders' knowledge to ensure the best practices and management strategies to reduce NIMS in mariculture, given not only local as well as regional benefits.

References

Airoldi L, Turon X, Perkol-Finkel S, Rius M (2015) Corridors for aliens but not for natives: Effects of marine urban sprawl at a regional scale. Divers. Distrib. 21, 755–768. doi:10.1111/ddi.12301

Allouche O, Tsoar A, Kadmon R (2006) Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). J. Appl. Ecol. 43, 1223–1232. doi: 10.1111/j.1365-2664.2006.01214.x

Ajala-Batista L, Lins DM, Haddad MA (2020) Diversity of estuarine and marine hydroids (Cnidaria, Hydrozoa) from subtropical ecosystems of Brazil. Mar. Biodivers. 50. doi:10.1007/s12526-020-01133-0

Anderson MJ, Crist TO, Chase JM, et al., (2011) Navigating the multiple meanings of β diversity: A roadmap for the practicing ecologist. Ecol. Lett. 14: 19–28. doi:10.1111/j.1461-0248.2010.01552.x

Angulo RJ, Souza MC, Lamour MR (2006) Coastal erosion problems induced by dredging activities in the navigation channel of Paranaguá and São Francisco do Sul harbor, southern Brazil. Journal of Coastal Research, SI 39 (Proccendigs of the 8th International Coastal Symposium), 1801 - 1803. Itajaí, SC, Brazil, ISSN 0749-0208.

ANTAQ (2021) Agência Nacional de Transportes Aquaviários. Statistical Panel. http://web.antaq.gov.br/anuario/

Assis, J, Tyberghein, L, Bosh, S, Verbruggen, H, Serrão, E A, & De Clerck, O (2018) Bio-ORACLE v2.0: Extending marine data layers for bioclimatic modelling. Global Ecology and Biogeography, 27: 277–284. doi: 10.1111/geb.12693

Bannister J, Sievers M, Bush F, Bloecher N (2019): Biofouling in marine aquaculture: a review of recent research and developments, Biofouling 35(6): 631–648. doi: 10.1080/08927014.2019.1640214

Blackburn TM, Pys P, Bacher S, et al (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26: 333–339. doi: 10.1016/j.tree.2011.03.023

Broennimann O, Treier UA, Muller-Scharer H, et al (2007) Evidence of climatic niche shift during biological invasion. Ecol Lett 10: 701–709. doi: 10.1111/j.1461-0248.2007.01060.x

Broennimann O, Guisan A (2008) Predicting current and future biological invasions: both native and invaded ranges matter. Biol. Lett. 4: 585–589. doi:10.1098/rsbl.2008.0254

Brugère C, Aguilar-Manjarrez J, Beveridge MCM, Soto D (2019) The ecosystem approach to aquaculture 10 years on – a critical review and consideration of its future role in blue growth. Rev Aquacult, 11: 493-514. doi:10.1111/raq.12242

Bueno GW, Ostrensky A, Canzi C, de Matos FT, Roubach R (2015) Implementation of aquaculture parks in Federal Government waters in Brazil. Rev. Aquac. 7: 1–12. doi: 10.1111/raq.12045

Castro MC, Fileman TW, Hall-Spencer J (2017) Invasive species in the Northeastern and Southwestern Atlantic Ocean: A review, Mar Pol Bul 116 (1-2): 41–47. doi: 10.1016/j.marpolbul.2016.12.048.

Clark GF, Johnston EL (2009) Propagule pressure and disturbance interact to overcome biotic resistance of marine invertebrate communities. Oikos 118: 1679–1686. doi: 10.1111/j.1600-0706.2009.17564.x

Cole E, Keller RP, Garbach K (2019). Risk of invasive species spread by recreational boaters remains high despite widespread adoption of conservation behaviors. J. Environ. Manage. 229: 112–119. doi: 10.1016/j.jenvman.2018.06.078

Cook EJ, Ashton G, Campbell M, et al., (2008) Non-Native Aquaculture Species Releases: Implications for Aquatic Ecosystems. In: Holmer M., Black K., Duarte C.M., Marbà N., Karakassis I. (eds)

Aquaculture in the Ecosystem. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6810-2_5

Coutts ADM and Dodgshun TJ (2007) The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. Mar Pollut Bull 54:875–886. doi: 10.1016/j.marpolbul.2007.03.011

Coutts ADM, Forrest BM (2007) Development and application of tools for incursion response: Lessons learned from the management of the fouling pest Didemnum vexillum. J. Exp. Mar. Bio. Ecol. 342: 154–162. doi: 10.1016/j.jembe.2006.10.042

Cole E, Keller RP, Garbach K (2018) Risk of invasive species spread by recreational boaters remains high despite widespread adoption of conservation behaviors. Journal of Environmental Management 229: 112– 119. doi: 10.1016/j.jenvman.2018.06.078

Davidson IC, Zabin CJ, Chang AL, et al (2010) Recreational boats as potential vectors of marine organisms at an invasion hotspot. Aquat Biol 11: 179–191. doi: 10.3354/ab00302

De Mesel I, Kerckhof F, Norro A, Rumes B, Degraer S (2015) Succession and seasonal dynamics of the epifauna community on offshore wind farm foundations and their role as stepping stones for non-indigenous species. Hydrobiologia 756, 37–50. Doi: 10.1007/s10750-014-2157-1

Diagne C, Leroy B, Vaissière AC, et al., (2021) High and rising economic costs of biological invasions worldwide. Nature 592: 571–576 (2021). https://doi.org/10.1038/s41586-021-03405-6

Dias PJ, Rocha R, Godwin S, et al (2016) Investigating the cryptogenic status of the sea squirt Didemnum perlucidum (Tunicata, ascidiacea) in Australia based on a molecular study of its global distribution. Aquat Invasions 11: 239–245. doi: 10.3391/ai.2016.11.3.02

Dürr S, Watson DI, (2010) Biofouling and antifouling in aquaculture. In: Dürr S, Thomason JC, editors. Biofouling. West Sussex (UK): Wiley-Blackwell; p. 267–287.

Early R and Sax DF (2014) Niche shift during naturalization. Global Ecology and Biogeography, 23: 1356-1365. doi: 10.1111/geb.12208

Engler R, Guisan A, Rechsteiner L (2004) An improved approach for predicting the distribution of rare and endangered species from occurrence and pseudo-absence data. J. Appl. Ecol. 41: 263–274.

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes 7: 574–578. doi: 10.1111/j.1471-8286.2007.01758.x

FAO (2018) The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals. Rome. http://www.fao.org/3/i9540en/I9540EN.pdf

Ferrario J, Caronni S, Occhipinti-Ambrogi A, Marchini A (2017) Role of commercial harbours and recreational marinas in the spread of non-indigenous fouling species. Biofouling, 33(8): 651-660. doi: 10.1080/08927014.2017.1351958

Freestone AL, Torchin ME, Jurgens LJ, Bonfim M, et al., (2021) Stronger predation intensity and impact on prey communities in the tropics. Ecology e03428. doi:10.1002/ecy.3428

Fielding AH, and Bell JF (1997) A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental Conservation 24: 38 – 49, doi: 10.1017/S0376892997000088

Fitridge I, Dempster T, Guenther J, de Nys R (2012) The impact and control of biofouling in marine aquaculture: a review, Biofouling, 28(7): 649-669, doi: 10.1080/08927014.2012.700478

Fofonoff PW, Ruiz GM, Steves B, Simkanin C, Carlton JT (2021) National Exotic Marine and Estuarine Species Information System. http://invasions.si.edu/nemesis. Access Date: 2021-06-25

Fourcade Y, Engler JO, Rödder D, Secondi J (2014) Mapping species distributions with MAXENT using a geographically biased sample of presence data: A performance assessment of methods for correcting sampling bias. PLoS ONE, 9: e97122. doi: 10.1371/journal.pone.0097122

Ghabooli S, Zhan A, Sardiña P, … MacIsaac HJ (2013) Genetic diversity in introduced golden mussel populations corresponds to vector activity. PLoS ONE, 8, e59328. doi: 10.1371/journal.pone.0059328

Goldsmit J, Archambault P, Chust G, et al. (2018) Projecting present and future habitat suitability of ship-mediated aquatic invasive species in the Canadian Arctic. Biol Invasions 20:501–517. doi: 10.1007/s10530-017-1553-7

Guo Q, Kelly M, Graham CH (2005) Support vector machines for predicting distribution of Sudden Oak Death in California, Ecological Modelling 182:75-90, doi: 10.1016/j.ecolmodel.2004.07.012.

Halpern BS, Frazier M, Potapenko J, et al (2015) Spatial and temporal changes in cumulative human impacts on the world's ocean. Nat Commun 6:1–7. doi: 10.1038/ncomms8615

Hauton, C (2016) Effects of salinity as a stressor to aquatic invertebrates. In: (M Solan & N Whiteley eds.), Stressors in the Marine Environment: Physiological and ecological responses; societal implications. Oxford. doi: 10.1093/acprof:oso/9780198718826.003.0001

Hayes K, Sliwa C, Migus S, McEnnulty F, Dunstan P (2005) National priority pests: Part II Ranking of Australian marine pests. An independent report undertaken for the Department of Environment and Heritage by CSIRO Marine Research. http://www.marine.csiro.au/crimp/reports/PriorityPestsFinalreport.pdf

Hewitt CL, Campbell ML, Gollasch S (2006) Alien Species in Aquaculture. Considerations for responsible use. IUCN, Gland, Switzerland and Cambridge, UK. viii + 32 pp.

Hewitt CL, Gollasch S, Minchin D (2009) Ballast water, sediments and hull fouling in: (G Rilov & J Crooks eds.), Biological Invasions in Marine Ecosystems: Ecology, Conservation and Management Perspectives, Springer, Heidelberg, pp. 117–129.

Hewitt C, and Campbell ML (2010) The relative contribution of vectors to the introduction and translocation of invasive marine species. Australian government department of agriculture, fisheries and forestry report. https://www.marinepests.gov.au/sites/default/files/Documents/relative-contribution-vectors-introduction-translocation-invasive-marine-species.pdf

Hiebert LS, Vieira EA, Dias GM, Tiozzo S, Brown FD (2019) Colonial ascidians strongly preyed upon, yet dominate the substrate in a subtropical fouling community. Proceedings. Biol. Sci. 286: 20190396. doi: 10.1098/rspb.2019.0396

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. J Appl Ecol 46:10–18. doi: 10.1111/j.1365-2664.2008.01600.x

Kremer LP, Rocha RM (2016) The biotic resistance role of fish predation in fouling communities. Biol Invasions 18, 3223–3237 doi: 10.1007/s10530-016-1210-6

Lins DM, de Marco P, Andrade AFA, Rocha RM (2018) Predicting global ascidian invasions. Divers. Distrib. 24: 692–704. https://doi.org/10.1111/ddi.12711

Lins and Rocha (2020) Cultivated brown mussel (*Perna perna*) size is reduced through the impact of three invasive fouling species in southern Brazil. Aquatic Invasions 15(1):114-126. doi: 10.3391/ai.2020.15.1.08

Lopes, R.M., Katsuragawa, M., Dias, J.F., et al., (2006) Zooplankton and ichthyoplankton distribution on the southern Brazilian shelf: An overview. Sci. Mar. 70: 189–202. https://doi.org/10.3989/scimar.2006.70n2189

Lowe S, Browne M, Boudjelais S, De Poorter M (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. Published by The Invasive Species Specialist

Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN). Aliens. Invas. Spec. Bull. 12: 1–12.

Magris RA, Déstro GFG (2010) Predictive modeling of suitable habitats for threatened marine invertebrate and implications for conservation assessment in Brazil. Brazilian Journal of Oceanography 58:57–68

McQuaid CD, Phillips TE (2000) Limited wind-driven dispersal of intertidal mussel larvae: in situ evidence from the plankton and the spread of the invasive species Mytilus galloprovincialis in South Africa. Marine Ecology Progress Series 201: 211–220

McKenzie L., Brooks R, Johnston EL (2011) Heritable pollution tolerance in a marine invader. Environ. Res. 111: 926–932. Doi: 10.1016/j.envres.2010.12.007

McKindsey CW, Landry T, O'beirn FX, Davies IM (2007) Bivalve Aquaculture and Exotic Species: a Review of Ecological Considerations and Management Issues. J. Shellfish Res. 26: 281–294. doi: 10.2983/0730-8000

Mesel I De, Kerckhof F, Norro A, et al (2015) Succession and seasonal dynamics of the epifauna community on offshore wind farm foundations and their role as stepping stones for non-indigenous species. Hydrobiologia 756: 37–50. doi: 10.1007/s10750-014-2157-1

Minchin D (2007) Aquaculture and transport in a changing environment: Overlap and links in the spread of alien biota. Mar Pol Bul, 55(7–9): 302-313. doi: 10.1016/j.marpolbul.2006.11.017

Mineur F, Cottier-cook EJ, Minchin D, et al (2012) Changing coasts: Marine aliens and artificial structures. Oceanogr Mar Biol An Annu Rev 50:189–234. doi: 10.1201/b12157-5

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Front Ecol Environ 6:485–492. doi: 10.1890/070064

Ojeda-Martínez C, Casalduero FG, Baylesempere JT, et al., (2009) A conceptual framework for the integral management of marine protected areas. Ocean coast. Mgmt, 52 (2):89–101

Olden JD, Poff NL, Douglas MR, et al (2004) Ecological and evolutionary consequences of biotic homogenization. Trends Ecol Evol 19:18–24. doi: 10.1016/j.tree.2003.09.010

Oricchio FT Flores AAV, Dias GM (2016) The importance of predation and predator size on the development and structure of a subtropical fouling community. Hydrobiologia 776: 209–219. doi: 10.1007/s10750-016-2752-4

Oricchio FT, Marques AC, Hajdu E, et al., (2019) Exotic species dominate marinas between the two most populated regions in the southwestern Atlantic Ocean. Mar. Pollut. Bull. 146: 884–892. doi: 10.1016/j.marpolbul.2019.07.013

Oricchio FT, Dias GM (2020) Predation and competition interact to determine space monopolization by non-indigenous species in a sessile community from the Southwestern Atlantic Ocean. Aquat. Invasions 15: 127–139. doi: 10.3391/AI.2020.15.1.09

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecological Modelling 190(3–4):231-259, doi: 10.1016/j.ecolmodel.2005.03.026

Piola RF, Johnston EL (2008) Pollution reduces native diversity and increases invader dominance in marine hard-substrate communities. Divers Distrib 14:329–342. doi: 10.1111/j.1472-4642.2007.00430.x

Prasad AM, Iverson LR, Liaw A (2006) Newer Classification and Regression Tree Techniques: Bagging and Random Forests for Ecological Prediction. Ecosystems 9, 181–199, doi: 10.1007/s10021-005-0054-1

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data, Genetics, 155(2): 945–959. doi: 10.1093/genetics/155.2.945

Rius M, Heasman KG, McQuaid CD (2011) Long-term coexistence of non-indigenous species in aquaculture facilities. Mar. Pollut. Bull. 62: 2395–2403. doi:10.1016/j.marpolbul.2011.08.030

Robinson TB, Griffiths CL, McQuaid CD, Rius M (2005) Marine alien species of South Africa — status and impacts, African Journal of Marine Science, 27(1): 297-306. doi: 10.2989/18142320509504088

Rocha RM, Kremer LP, Baptista MS, Metri R (2009) Bivalve cultures provide habitat for exotic tunicates in Southern Brazil. Aquat Invasions 4: 195–205. doi: 10.3391/ai.2009.4.1.20

Ruiz GM, Freestone AL, Fofonoff PW, Simkanin C (2009) Habitat distribution and heterogeneity in marine invasion dynamics: the importance of hard substrate and artificial structure. Wahl M Mar Hard Bottom Communities Patterns, Dyn Divers Chang Berlin, Springer 321–332. doi: 10.1007/978-3-540-92704-4

Sadchatheeswaran S, Branch GM, Robinson TB (2015) Changes in habitat complexity resulting from sequential invasions of a rocky shore: implications for community structure. Biol. Invasions 17: 1799–1816. doi:10.1007/s10530-014-0837-4

Sakai AK, Allendorf FW, Holt JS, et al (2001) The population biology of invasive species. Annu Rev Ecol Syst 32: 305–332

Santos AA and Della-Giustina EG (2017) Síntese informativa da maricultura 2017. https://cedap.epagri.sc.gov.br/ (accessed 10/7/2018)

Santos AA, Mondardo M, Marchiori NC (2019) Presença de Mytilus cf. edulis platensis em fazendas marinhas de Bombinhas, Santa Catarina, Brasil. Agropecuária Catarinense 32: 53–55.

SEBRAE (2012) Estudo setorial da indústria catarinense: Náutico, Santa Catarina, 325p. Avaiable at: https://m.sebrae.com.br/sites/PortalSebrae/ufs/sc/noticias/fique-por-dentro-dos-estudos-setoriais-da-industria-catarinense,49fe2723d790c410VgnVCM1000003b74010aRCRD

Simberloff D (2009) The Role of Propagule Pressure in Biological Invasions. Annu Rev Ecol Evol Syst 40: 81–102. doi: 10.1146/annurev.ecolsys.110308.120304

Simberloff D, Martin J, Genovesi P, et al (2013) Impacts of biological invasions: what' s what and the way forward. Trends Ecol Evol 28: 58–66. doi: 10.1016/j.tree.2012.07.013

Simpson TS, Wernberg T, Mcdonald JI (2016) Distribution and Localised Effects of the Invasive Ascidian Didemnum perlucidum (Monniot 1983) in an Urban Estuary. 1–17. doi: 10.1371/journal.pone.0154201

Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, et al., (2007) Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. Bioscience 57, 573–583. https://doi.org/10.1641/B570707

Sorte CJB, Williams SL Carlton JT (2010) Marine range shifts and species introductions: comparative spread rates and community impacts. Global Ecology and Biogeography, 19: 303-316. https://doi.org/10.1111/j.1466-8238.2009.00519.x

Sorte CJB, Williams SL, Zerebecki RA (2010) Ocean warming increases the threat of invasive species in a marine fouling community. Ecology, 91: 2198–2204.

Sorte CJB, Jones SJ, Mille, LP (2011) Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. J. Exp. Mar. Bio. Ecol. 400: 209–217. Doi: 10.1016/j.jembe.2011.02.009

Suplicy FM, Vianna LF, Rupp GS, Novaes ALT et al., (2015) Planning and management for sustainable coastal aquaculture development in Santa Catarina State, south Brazil. Rev. Aquac. 1–18. doi:10.1111/raq.12107

Thuiller W, Lafourcade B, Engler R and Araújo MB (2009) BIOMOD – a platform for ensemble forecasting of species distributions. Ecography, 32:369-373, doi: 10.1111/j.1600-0587.2008.05742.x

UNESCO (2015) Science Report: towards 2030 – Executive Summary - Visão Geral e Cenário Brasileiro. Paris: UNESCO. Available at: http://www.peaunesco-sp.com.br/encontros/nacional_2016/1%20Objetivos%20de%20Desenvolvimento%20Sustentavel%20-%20ODS%20-%20Rumo%20a%202030/6_Arquivo.pdf

Vianna LF, and Filho JB (2018) Spatial analysis for site selection in marine aquaculture: An ecosystem approach applied to Baía Sul, Santa Catarina, Brazil. Aquaculture 489: 162–174. doi: 10.1016/j.aquaculture.2017.12.039.

Walther GR, Roques A, Hulme PE et al. (2009) Alien species in a warmer world: risks and opportunities. Trends in Ecology and Evolution, 24: 686–93.

Whiteley NM, Mackenzie CL (2016) Physiological responses of marine invertebrates to thermal stress. In: (M Solan & N Whiteley eds.), Stressors in the Marine Environment: Physiological and ecological responses; societal implications. Oxford. doi: 10.1093/acprof:oso/9780198718826.003.0004

CONCLUSÕES

A globalização promove a introdução e o estabelecimento de espécies fora de suas regiões nativas. Fenômeno que aumenta fortemente sem sinal de estabilização o risco de espécies se tornarem invasoras e causarem impactos (Simberloff et al., 2013; Seebens et al., 2017), principalmente em ambientes costeiros devido à suscetibilidade dessas regiões à inoculação de propágulos de espécies transportadas acidentalmente através do transporte transoceânico (Coutts & Dodgshun 2007; Kaluza et al. 2010). Além do impacto causado por espécies invasoras, ocorre sinergia com o impacto causado por outras atividades humanas como sobrepesca, poluição (incluindo plástico) e mudanças climáticas (Diaz et al. 2019). A maricultura é responsável tanto pela introdução intencional de espécies não nativas para fins comerciais, como pelo transporte de forma acidental de diversas espécies epibiontes associadas às espécies cultivadas e às estruturas dos cultivos, quando estoques da espécie-comercial são transportados entre regiões de cultivo (Mckindsey et al. 2007). Ironicamente, além de facilitar o transporte e fornecer substrato para as espécies introduzidas, a maricultura também sofre com os impactos das invasoras (Fitridge et al. 2012) que reduzem a qualidade dos bivalves e causam a depreciação de equipamentos (bóias, cordas, embarcações, etc.).

Há mais de vinte anos a produção nacional de mexilhões *Perna perna* no Brasil é liderada pelo estado de Santa Catarina. O mercado de mais de 20 mil toneladas de moluscos é a principal fonte de renda para grande parte dos cerca de 550 produtores do estado (Santos & Della Giustina 2017). A maricultura proporciona emprego e renda para muitas famílias, gerando autossutentabilidade e atendendo às demandas globais de produção de proteína (FAO 2014). No Brasil, a grande maioria dos empreendimentos são negócios artesanais de propriedade familiar, nos quais o impacto socioeconômico das espécies invasoras é difícil de gerenciar. Diversas espécies não nativas da classe Ascidiacea são consideradas modelos para o estudo de bioinvasão e ocorrem na maricultura de Santa Catarina, sugerindo-se a necessidade de estudos de risco (Rocha et al. 2009). Entretanto, avaliações anteriores não foram padronizadas e estavam limitadas a duas regiões. Nesse sentido, no primeiro capítulo da tese avaliamos a distribuição de espécies introduzidas em oito fazendas marinhas ao longo das áreas mais produtivas do estado e, através de busca ativa e senso visual, avaliamos a susceptibilidade de estruturas de cultivo (boias, cordas e pencas de mexilhões) e regiões às incrustações. Os resultados indicam que tanto regiões quanto substratos são diferentes na proporção relativa em que as espécies estão distribuídas. A ascídia *Styela plicata* foi a espécie mais amplamente distribuída e ocorreu em mais de 70% de todos os substratos avaliados em cada local. De norte a sul, outras espécies muito frequentes (> 50% dos substratos) foram a craca *Megabalanus coccopoma* e as ascídias *Aplidium accarense* e *Didemnum perlucidum* em Penha, o briozoário *Schizoporella errata* em Gov. Celso Ramos (GCR), o poliqueta *Branchiomma luctuosum* em Palhoça, e *M. coccopoma* em Florianópolis. As espécies *S. errata* em Penha, *M. coccopoma* em GCR, *Botrylloides giganteus* e *M. galloprovincialis* em Palhoça, foram encontradas com frequências intermediárias (>30%). Posteriormente as espécies foram ranqueadas considerando a proposta de Bacher et al. (2018), que classifica as espécies com base em impactos socioeconômicos (SEICAT). Com base nesta avaliação o mexilhão invasor *M. galloprovincialis* é a espécies com maior impacto negativo, enquanto as outras espécies apresentam menor impacto aos cultivos.

Há um interesse constante em experimentar formas inovadoras que auxiliem a mitigar o impacto de espécies invasoras nos cultivos (LeBlanc et al. 2003; Ross et al. 2004; Bullard et al. 2010). A competição entre espécies comerciais e invasoras se dá por espaço e alimento o que resulta em impactos econômicos na produção dos mexilhões (Sievers et al. 2013). No entanto, as técnicas de manejo de incrustação não são espécie específicas e por isso se tornam controversas, pois podem afetar também o desenvolvimento e produtividade dos mexilhões cultivados (Sievers et al. 2017) e ainda, se realizadas no oceano, podem promover a disseminação das espécies e a reinfecção de equipamentos (Paetzold & Davidson 2010). Assim, no Brasil sugeriu-se evitar a remoção das incrustações durante a fase de crescimento de mexilhões (Metri et al. 2002; Lodeiros et al. 2007) e limitar intervenções à seleção e limpeza das conchas e disposição adequada dos rejeitos orgânicos após a colheita, por exemplo, para mercados onde a aparência do mexilhão é importante, em oposição àqueles que comercializam o produto descascado e cozido.

No entanto, o efeito negativo das espécies invasoras em Santa Catarina na produtividade dos mexilhões ainda havia sido pouco explorado. Neste capítulo, reportamos o estudo do impacto das incrustações no crescimento do mexilhão (*Perna perna*) cultivado em Santa Catarina e descobrimos que os mexilhões são 19-36% menores em tamanho e pesam 60% menos quando recobertos pelas espécies invasoras *Didemnum perlucidum*, *Megabalanus coccopoma* e/ou *Schizoporella errata*. Com base nas espécies com maior efeito negativo no tamanho e peso de *P. perna*, sugerimos que a maricultura deve focar ações de manejo na contenção destas espécies para reduzir a impacto das incrustações.

Em algumas regiões do globo a densidade das invasões tornou o manejo inevitável. Espécies invasoras incrustantes podem alcançar grandes abundâncias rapidamente e com extrema biomassa resultar em impactos devastadores. Por exemplo, no Canadá, onde a ascídia *Ciona intestinalis* atinge grande biomassa e a invasão foi associada à mortalidade dos mexilhões *Mytilus edulis* cultivados (Daigle & Herbinger 2009). Outras invasoras notórias incluem a ascídia *Ciona robusta* e o mexilhão *Mytilus galloprovincialis*, invasores dominantes na África do Sul (Robinson et al. 2005; Rius et al. 2011) e também na Nova Zelândia, onde o impacto econômico associado à invasão gira em torno de US$16.4 milhões por ano (Forrest & Atalah 2017) e podem aumentar o peso dos cultivos em mais de 50% (Woods et al. 2012). Na África do Sul *M. galloprovincialis* também invadiu costões rochosos e deslocou o mexilhão marrom (*P. perna*) que hoje ocorre em fragmentos diminutos do seu ambiente natural (Robinson et al. 2007).

O mexilhão *Mytilus galloprovincialis* foi introduzido recentemente em Santa Catarina e tem causado prejuízos sem precedentes à produção na região de Bombinhas, principalmente por substituir *P. perna* nas cordas de recrutamento de sementes (Santos et al., 2019). A alta abundância da espécie invasora nos cultivos de Bombinhas é ainda mais preocupante devido à proximidade da região com bancos naturais de *P. perna* e também da Reserva Biológica Marinha do Arvoredo que inclui as ilhas de Galés, Arvoredo, Deserta e Calhau de São Pedro, importantes para a manutenção da biodiversidade e de estoques pesqueiros. Realizamos o monitoramento do recrutamento do mexilhão invasor e de *P. perna* em Bombinhas, ao longo de um ano, com o objetivo de identificar se através da sincronização de técnicas de recrutamento com picos de assentamento de sementes distinto entre as espécies seria possível evitar a espécie invasora nos coletores. Os resultados revelaram que ambas as espécies têm tendências de recrutamento sazonais semelhantes ao longo do ano, o que torna impossível controlar a espécie invasora pelo manejo de recrutamento de *Perna perna*. Também utilizamos análises moleculares com base em indivíduos (SNPs) para confirmar a identificação da espécie e a origem dos propágulos. Resultados apontam para a possibilidade de um único evento de introdução e o Mar Mediterrâneo como a região de origem das populações invasoras em Santa Catarina (Capítulo 3).

O mexilhão *Mytilus galloprovincialis* foi introduzido na África do Sul por uma única fazenda marinha na região sul do país que acidentalmente em menos de cinco anos mediou o espalhamento da espécie para ambientes naturais adjacentes (McQuaid & Phillips 2000). A importância da população cultivada nessa fazenda como fonte de propágulos ficou evidente após o abandono da atividade no local, que levou à subsequente redução drástica da densidade da espécie invasora nos ambientes da região (Rius et al., 2011). Desta forma, além do impacto econômico direto das espécies invasoras nos cultivos, a maricultura pode criar problemas à biodiversidade ao mediar o estabelecimento e espalhamento das espécies. Devido aos riscos para os ecossistemas e para a subsistência da atividade econômica, cultivos de bivalves devem ser prioritários nos estudos e ações para prever e controlar espécies introduzidas e potencialmente invasoras com risco de espalhamento para outras regiões.

No capítulo final desta tese, apresentamos um estudo do potencial da maricultura de Santa Catarina em tornar-se fonte de propágulos de espécies introduzidas para outras regiões do país. Utilizamos os registros de ocorrências mundiais das espécies invasoras aqui estudadas e a quantidade de mercadorias transportadas por navios-contêineres entre os portos da região e outros portos ao longo da costa para prever a potencial introdução de espécies a outros estados brasileiros considerando o efeito de mudanças climáticas. Considerando dois cenários representativos de emissões de gases do efeito estufa, um otimista (RCP2,6) e um mais conservador (RCP6,0), descobrimos que áreas de habitat com alta adequabilidade permanecem no futuro (2040-50) para todas as espécies. Os estados de Pernambuco e Ceará, no nordeste, estão em maior risco devido à conectividade relativamente maior e alta adequabilidade climática às espécies *D. perlucidum* e *M. coccopoma*. No sul, Rio Grande do Sul e Paraná são regiões de grande preocupação devido à proximidade da região doadora e áreas de alta adequabilidade para *Styela plicata*, *Watersipora subtorquata* no RS, e *S. errata*, *M. coccopoma* e *M. galloprovincialis* no Paraná. Atualmente, as espécies estudadas ocorrem principalmente no estado de São Paulo, seguido pelo Rio de Janeiro, Espírito Santo na região sudeste e Bahia no Nordeste, mas ainda assim *M. galloprovincialis* poderiam ser introduzidos nos estados do Sudeste e a Bahia devido à conectividade relativamente alta com Santa Catarina. Todos os estados brasileiros estudados possuem áreas de adequabilidade climática e são conectados através de rotas de navegação com os portos de Santa Catarina. Como resultado importante, mapas de risco permitirão que as autoridades priorizem espécies em cada estado e rota de transporte, tanto para monitorar futuras introduções quanto para impor ações para diminuir o risco de invasões.

REFERÊNCIAS

Carlton JT (1996) Pattern, process, and prediction in marine invasion ecology. Biological Conservation 3207(78): 97–106

Coutts ADM, Dodgshun TJ (2007) The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. Marine Pollution Bulletin 54: 875–886, doi: 10.1016/j.marpolbul.2007.03.011

Daigle RM, Herbinger CM (2009) Ecological interactions between the vase tunicate (Ciona intestinalis) and the farmed blue mussel (Mytilus edulis) in Nova Scotia, Canada. Aquatic Invasions 4(1): 177–187, doi: 10.3391/ai.2009.4.1.18

Díaz S, Settele J, Brondízio ES, Ngo HT, Agard J, Arneth A, Balvanera P, Brauman KA, Butchart SHM, Chan KMA, Garibaldi LA, Ichii K, Liu J, Subramanian SM, Midgley GF, Miloslavich P, Molnár Z, Obura D, Pfaff A, Polasky S, Purvis A, Razzaque J, Reyers B, Chowdhury RR, Shin Y-J, Visseren-Hamakers I, Willis KJ, Zayas CN (2019) Pervasive human-driven decline of life on Earth points to the need for transformative change. Science 366: eaax3100, doi: 10.1126/science.aax3100

Fitridge I, Dempster T, Guenther J, Nys R (2012) The impact and control of biofouling in marine aquaculture: a review. Biofouling 28(7): 649–669, doi: 10.1080/08927014.2012.700478

Forrest BM, Atalah J (2017) Significant impact from blue mussel Mytilus galloprovincialis biofouling on aquaculture production of green-lipped mussels in New Zealand. Aquaculture Environment Interactions 9: 115–126

Kaluza P, Ko A, Gastner MT (2010) The complex network of global cargo ship movements. Journal of The Royal Society Interface 7: 1093–1103

LeBlanc AR, Landry T, Miron G (2003) Identification of fouling oganisms covering mussel lines and impact of a common defouling method on the abundance of foulers in Tracadie bay, Prince Edward Island. Canadian Technical Report Fisheries and Aquaculture Science 2477(VII): 18

Lodeiros C, Galindo L, Buitrago E, Himmelman JH (2007) Effects of mass and position of artificial fouling added to the upper valve of the mangrove oyster Crassostrea rhizophorae on its growth and survival. Aquaculture 262: 168–171, doi: 10.1016/j.aquaculture.2006.10.002

Mckindsey CW, Landry T, Beirn FXO, Davies IM (2007) Bivalve Aquaculture and Exotic Species: a Review of Ecological Considerations and Management Issues Bivalve Aquaculture and Exotic Specie: a Review of Ecological Considerations and Management Issues. Journal of Shellfish Research 26(2): 281–294

Mcquaid CD, Phillips TE (2000) Limited wind-driven dispersal of intertidal mussel larvae: in situ evidence from the plankton and the spread of the invasive species Mytilus galloprovincialis in South Africa. Marine Ecology Progress Series 201: 211–220

Metri R, Rocha RM, Marenzi A (2002) Epibiosis reduction on productivity in a mussel culture of Perna perna (Linné, 1758). Brazilian Archives of Biology and Technology 45(3): 325–331

Paetzold SC, Davidson J (2010) Viability of golden star tunicate fragments after high-pressure water treatment. Aquaculture 303:105–107, doi: 10.1016/j.aquaculture.2010.03.004

Rius M, Heasman KG, McQuaid CD (2011) Long-term coexistence of non-indigenous species in aquaculture facilities. Mar. Pollut. Bull. 62: 2395–2403. doi:10.1016/j.marpolbul.2011.08.030

Robinson TB, Griffiths CL, McQuaid CD, Rius M (2005) Marine alien species of South Africa — status and impacts, African Journal of Marine Science, 27(1): 297-306, DOI: 10.2989/18142320509504088

Robinson TB, Branch GM, Griffiths CL, Govender A, Hockey PAR (2007) Changes in South African rocky intertidal invertebrate community structure associated with the invasion of the mussel Mytilus galloprovincialis. Marine Ecology Progress Series 340: 163–171, doi: 10.3354/meps340163

Rocha RM, Kremer LP, Baptista MS, Metri R (2009) Bivalve cultures provide habitat for exotic tunicates in Southern Brazil. Aquatic Invasions 4(1): 195–205, doi: 10.3391/ai.2009.4.1.20

Ross KA, Thorpe JP, Brand AR (2004) Biological control of fouling in suspended scallop cultivation. Aquaculture 229: 99–116, doi: 10.1016/S0044-8486(03)00328-4

Santos AA, Giustina EG Della (2017) Síntese informativa da maricultura 2017

Santos AA, Mondardo M, Marchiori NC (2019) Presença de *Mytilus cf. edulis platensis* em fazendas marinhas de Bombinhas, Santa Catarina, Brasil. Agropecuária Catarinense 32, 53–55, doi: 10.22491/rac.2019.v32n3.6

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S (2017) No saturation in the accumulation of alien species worldwide. Nature Communications 8: 1–9, doi: 10.1038/ncomms14435

Sievers M, Fitridge I, Dempster T, Keough MJ (2013) Biofouling leads to reduced shell growth and flesh weight in the cultured mussel *Mytilus galloprovincialis*. Biofouling 29(1): 97–107, doi: 10.1080/08927014.2012.749869

Sievers M, Dempster T, Fitridge I, Keough MJ, (2014) Monitoring biofouling communities could reduce impacts to mussel aquaculture by allowing synchronization of husbandry techniques with peaks in settlement. Biofouling 30: 203–212, doi: 10.1080/08927014.2013.856888

Sievers M, Fitridge I, Bui S, Dempster T (2017) To treat or not to treat: a quantitative review of the effect of biofouling and control methods in shellfish aquaculture to evaluate the necessity of removal. Biofouling 7014: 1–13, doi: 10.1080/08927014.2017.1361937

Woods C, Floerl O, Hayden BJ (2012) Biofouling on Greenshell TM mussel (Perna canaliculus) farms: A preliminary assessment and potential implications for sustainable aquaculture practices. Aquaculture International 20(3): 537–557, doi: 10.1007/s10499-011-9484-2