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Marine aquaculture as a source of propagules of invasive fouling species

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Exotic species tend to colonize aquaculture installations, especially when they are near international ports. In addition to the local environmental hazard that colonizing exotic species pose, they can also take advantage of local transport opportunities to spread elsewhere. In this study, we examined the risk of spread of eight invasive fouling species that are found in mussel farms in southern Brazil. We used ensemble niche models based on worldwide occurrences of these species, and environmental variables (ocean temperature and salinity) to predict suitable areas for each species with three algorithms (Maxent, Random Forest, Support Vector Machine). As a proxy for propagule pressure, we used the tonnage transported by container-ships from Santa Catarina (the main mariculture region) that travel to other Brazilian ports. We found that ports in the tropical states of Pernambuco, Ceará and Bahia received the largest tonnage, far from Santa Catarina and in a different ecoregion. *Aplidium accarense* and *Didemnum perlucidum* are known from Bahia, with a high risk of invasion in the other states. *Watersipora subtorquata* also has a high risk of establishment in Pernambuco, while *Botrylloides giganteus* has a medium risk in Bahia. Southern states in the ecoregion with Santa Catarina are likely to be invaded by all species (Paraná) and *A. accarense, Megabalanus coccopoma* and *Mytilus galloprovincialis* (Rio Grande do Sul). Global warming is changing species latitudinal distributions and most species will gain rather than lose area in near future (by 2050). As an ideal habitat for fouling organisms and invasive species, aquacultures can increase propagule pressure and the probability that species will expand their distributions. Therefore, to prevent the impact of invasive species from happening far away, marine farms should be far from ports and avenues of travel of cargo ships. The risk maps provided will allow authorities and regional stakeholders to prioritize areas of concern for mitigating future spread of fouling species.

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ABSTRACT

Exotic species tend to colonize aquaculture installations, especially when they are near international ports. In addition to the local environmental hazard that colonizing exotic species pose, they can also take advantage of local transport opportunities to spread elsewhere. In this study, we examined the risk of spread of eight invasive fouling species that are found in mussel farms in southern Brazil. We used ensemble niche models based on worldwide occurrences of these species, and environmental variables (ocean temperature and salinity) to predict suitable areas for each species with three algorithms (Maxent, Random Forest, Support Vector Machine). As a proxy for propagule pressure, we used the tonnage transported by container-ships from Santa Catarina (the main mariculture region) that travel to other Brazilian ports. We found that ports in the tropical states of Pernambuco, Ceará and Bahia received the largest tonnage, far from Santa Catarina and in a different ecoregion. *Aplidium accarense* and *Didemnum perlucidum* are known from Bahia, with a high risk of invasion in the other states. *Watersipora subtorquata* also has a high risk of establishment in Pernambuco, while *Botrylloides giganteus* has a medium risk in Bahia. Southern states in the ecoregion with Santa Catarina are likely to be invaded by all species (Paraná) and *A. accarense, Megabalanus coccopoma* and *Mytilus galloprovincialis* (Rio Grande do Sul). Global warming is changing species latitudinal distributions and most species will gain rather than lose area in near future (by 2050). As an ideal habitat for fouling organisms and invasive species, aquacultures can increase propagule pressure and the probability that species will expand their distributions. Therefore, to prevent the impact of invasive species from happening far away, marine farms should be far from ports and avenues of travel of cargo ships. The risk maps provided will allow authorities and regional stakeholders to prioritize areas of concern for mitigating future spread of fouling species.

INTRODUCTION

Marine aquaculture grows faster than many other agricultural sectors, and can make up for fishery shortfalls and supply protein for many around the world (FAO 2018). While the intentional introduction of non-indigenous marine species (NIMS) for cultivation should be preceded by a comprehensive risk analysis to minimize spillover and spread of NIMS (Code of Conduct on Responsible Fisheries, Article 9 on Aquaculture Development; FAO 1995), indirect negative impacts caused by associated species are poorly understood (Suplicy et al., 2015) and not the object of evaluation in risk analysis.

Biofouling of aquaculture (establishment of other species on the cultivated species and associated structures) increases management costs and decreases profitability (Fitridge et al., 2012), largely as a consequence of the cost of removal of unwanted species and yield reduction of the commercial species (Bannister et al., 2019). Biofouling varies spatially and temporally with community structure among regions (Dürr & Watson, 2010). When fouling species are also exotic invasive species, aquacultures can act as stepping-stones allowing establishment and spread along adjacent coastal areas (Ramsay et al., 2008), and so they share responsibility for the environmental negative impacts caused by those invasive species in natural communities (Simpson et al., 2016; Robinson et al., 2007).

Fouling exotic species are primarily introduced by merchant ships (and other vectors) that travel between oceans (Hewitt & Campbell, 2010). The fundamental propagule pressure in a given region and the likelihood of invasion are determined by the rate at which a species is transported as biofouling (in hydrodynamically protected niche areas on ship hulls, ballast tanks and sea chests; Coutts & Dodgshun 2007; Hulme 2009; Davidson et al., 2010). Transport by such means increases probability of multiple invasions and reduces the effects of demographic and environmental stochasticity at recipient regions (Simberloff, 2009). To become invasive, a species must overcome a number of ecological and environmental barriers, even with many introduction events, and in general only a fraction of the introduced species succeeds in becoming invasive (Blackburn et al., 2011). Continuously available new hard substrates for settlement (ropes, buoys, piers and shells, all found in aquaculture facilities) enhance the probability of invasive species establishment (McKindsey et al., 2007). In addition, shellfish farms are often extensive in high-value, multiple-use, coastal areas (e.g., important and significant habitats for conservation of biodiversity), where ports, marinas, tourism, recreation, and commercial fisheries are all found together, increasing propagule pressure and multiple introduction events (Minchin, 2007; Castro et al., 2017).

Mussel farming is very important to the local economy in southern Brazil (Suplicy et al., 2015). The cold and nutrient-rich South Oceanic Central Water is important and has favored aquaculture development in this region (Lopes et al., 2006). Currently, sheltered bays in the state of Santa Catarina are responsible for over 95% of all mussel and oyster production in Brazil, with mussel and oyster farms established along most of the coast (Santos & Della-Giustina, 2017). The region is also notable for having the second largest concentration of shipyards and two of the nation's largest ports, 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. Non-indigenous invasive species are already prevalent fouling species in marine aquaculture facilities (Rocha et al., 2009; Lins & Rocha, 2022). Therefore, risk assessment of the impact of shellfish farming on the regional biodiversity should also consider fouling NIMS, their range expansion, and predictions of future movements of those species that are already known to be problematic in other regions.

Global regulations began to be enforced in 2017 to control marine bioinvasions and require all ships in international traffic to manage their ballast water and sediments (BWM Convention, IMO 2004), yet domestic port to port movement of species is still not the focus of biosecurity management in many countries, including Brazil, with long coastlines. Most of the 17 coastal states of Brazil have at least one major port that receive cargo ships from Santa Catarina. This suggest that the NIMS in aquaculture facilities in Santa Catarina have many opportunities for transport (strong propagule pressure) along the coast. In this study we focused on eight invasive NIMS established in aquaculture facilities at Santa Catarina to understand their potential to be introduced and invasive in other places in the country, based on current domestic shipping trade between ports in Santa Catarina and other states, using species suitability models to address current and future habitat suitability.

MATERIALS & METHODS

Species and occurrences

Eight invasive NIMS recognized to be spread and abundant in aquaculture facilities in Santa Catarina (Lins & Rocha, 2022) 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 (although it is not clear if *A. accarense* is a regional or international invader).

Native and introduced occurrences from these target species were obtained from the global geographic distribution databases (OBIS.org and GBIF.org) and from published taxonomic and ecological studies obtained by searching for the valid names and resolved synonyms of the species in the Web of Science and Google Scholar portals. When studies reported general locations instead of precise geographic coordinates of the register, we acquired those coordinates using Google Maps, considering the existence of ports, marinas and rocky shores as sites with the highest 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 & Mackenzie, 2016) and which are also used to model the future scenarios of gas emissions. To control for strong correlated environmental layers ($r > 0.7$), 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 (2050) projections of the same environmental predictors were obtained under two Representative Concentration Pathways (RCP) for greenhouse gas emissions scenarios: one with low greenhouse gas emission rate (RCP2.6) and one with high greenhouse gas emission rate (RCP6.0) (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 or MaxEnt) (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 & Bell 1997), with which we then evaluated the distributional models using True Skill Statistics (TSS > 0.8). 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 ships, we used the total amount of goods transported during five years' time (2015 to 2019) as a surrogate of the connectivity between states. We ranked states comparatively in three categories: with high (> 1000 thousand tons), intermediate (100-1000 thousand tons) and low connectivity (< 100 thousand tons) with Santa Catarina.

Risk assessment

To assess the risk of species transport and introduction/invasion (connectivity + suitability), we built a matrix that overlaps the information on cargo transport from SC to each

Brazilian coastal state, and environmental suitability for each of the eight focus species (Table 1S). The joint assessment of environmental suitability at possible recipient regions complemented by information on vectors of transport have been previously used to forecast species introduction (Goldsmit et al., 2018; Lins et al., 2018). In our assessment we considered connectivity (propagule pressure) a little more important than environmental adequacy because there have been numerous situations where species can rapidly adapt to new environmental situations and expand their known niche (Broennimann et al., 2007; Early & Sax, 2014).

RESULTS

The bryozoans *S. errata* and *W. subtorquata* are the most widespread species, already in ten and eight states, respectively, between Santa Catarina and Ceará in the northeast. The ascidians *A. accarense*, *D. perlucidum, S. plicata* and the barnacle *M. coccopoma* are in six states, from Santa Catarina to Bahia, with the exception of *A. accarense* (found through Rio Grande do Norte). The ascidian *B. giganteus* occurs in four southern and southeastern states, and the mussel *M. galloprovincialis* occurs in Santa Catarina only (Fig. 1).

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), and is now 97% of all goods shipped from Santa Catarina to elsewhere in Brazil. The northeastern states of Pernambuco, Ceará and Bahia were the main destinations, and so are the states in the highly-connected group and more prone to receive propagules from Santa Catarina (Fig. 1). The intermediate-connected states are São Paulo, Espírito Santo, Rio de Janeiro, in the southeast, and Paraná and Rio Grande do Sul in the south. The least-connected group of states are Paraíba and Rio Grande do Norte in the northeast. Alagoas, Sergipe and Piauí did not receive container ships from Santa Catarina during the time interval under study.

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 2S). The evaluation index indicated that the SVM and RDF models performed similarly and were more accurate than MXL (Table 1S). Except for three species under the RDF model, temperature variables were consistently the main drivers of predictive performances across algorithms (Table 1). 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. 2, 3 and 4).

Among the highly-connected states, Pernambuco is currently environmentally suitable for *A. accarense*, *D. perlucidum* and *W. subtorquata,* while Ceará is suitable for *D. perlucidum* and marginally for *A. accarense*, and Bahia has low suitability for *B. giganteus* (Fig. 1). Among the intermediately-connected states, São Paulo, Rio de Janeiro and Espírito Santo have already been colonized by all species with the exception of *M. galloprovincialis.* Rio de Janeiro has intermediate environmental suitability for this species while the other two states have low suitability. Paraná has high suitability for all species that have not yet colonized, and Rio Grande do Sul has variable suitability for those eight species. Both low-connected states are very suitable for *D. perlucidum* and *W. subtorquata*, and Paraíba is moderately suitable for *A. accarense.*

Considering the connectivity and environmental suitability together (Figs. 2-4), in the northeast, Pernambuco is the state most at risk, and is likely to receive propagules and be invaded by *A. accarense*, *D. perlucidum* and *W. subtorquata* populations coming from Santa Catarina. Ceará is at high risk of invasion by *D. perlucidum* and moderate risk by *A. accarense.* The other states are at a medium risk of invasion by *B. giganteus* (Bahia), *D. perlucidum* (Paraiba, Rio Grande do Norte) and *W. subtroquata* (Paraíba) and low risk by *A. accarense* (Paraíba). In the southeast, only Rio de Janeiro is at moderate risk of being invaded by *M. galloprovincialis,* and São Paulo and Espírito Santo both are at low risk. In the south, Paraná is at high risk of invasion by *A. accarense*, *B. giganteus, S. errata, W. subtorquata* and *M. galloprovincialis.* Rio Grande do Sul is at high risk of invasion by *A. accarense*, *M. coccopoma* and *M. galloprovincialis* and a moderate risk by *B. giganteus, S. errata* and *W. subtorquata,* and low risk by *D. perlucidum* and *S. plicata*.

In the future, all species (with the exception of *A. accarense and D. perlucidum*) are likely to extend their distributions towards lower latitudes and invade more in the northeast, while *B. giganteus* will expand only in the RPC 6.0 scenario, and *M. galloprovincialis* only in the RPC 2.6 scenario (Figs. 2-4).

DISCUSSION

Most invasive species in this study are already found along much of the Brazilian coast, from Santa Catarina to Bahia. *Mytilus galloprovincialis* is an exception and quite recent invasion, so it is still restricted to the shellfish farms of Santa Catarina. Species distribution models indicated that environmentally suitable regions exist but have not yet been invaded. We do not yet understand whether the arrival of these species is simply a matter of time, whether propagule transport is too low or nonexistent, or if there are any other biological or environment constrains on their establishment, and finally, whether they are absent as an artifact of the lack of good monitoring programs. A combination of causes is likely, such as with Alagoas, Sergipe and Piauí that did not receive any propagule from Santa Catarina, but which also do not have good marine biodiversity and monitoring programs.

Landscape features were not accounted for in the environmental suitability models we used, but they may be important to understand community assembly and species presence. One important driver for sessile NIMS is the availability of hard substrates for attachment (Ruiz et al., 2009). In addition to Sergipe and Piauí, hard substrates for attachment are absent in the southern state of Rio Grande do Sul, and the long sandy beaches may have prevented the introduction of the focal species, despite the environmental suitability for most species and intermediate connectivity with Santa Catarina.

Predation may also limit species distributions and is not included in climate suitability models even though predation is known to alter benthic communities along latitudinal gradients increasingly towards the tropics (Freestone et al., 2021). Predation and competition for space have a strong influence on the composition of benthic communities in different latitudes in Brazil (Kremer & 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). In Santa Catarina, 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 & Rocha 2016), which could favor the barnacle spread to lower latitudes predicted under climate change.

Temperature was the most important driver for suitable areas and it is known to interact with or influence other drivers. When seawater temperature increases, other correlated factors can become important, such as primary productivity, which can lead to context-dependent shifts in competition (Ruiz et al., 2009). Competition, in polluted waters, between the bryozoans *S. errata* and *W. subtorquata* was strengthened at higher temperatures (McKenzie et al., 2011). Santa Catarina is at a higher latitude, in a different marine realm and province (*sensu* Spalding (

al., 2007) than the tropical states in the country, yet habitat is still suitable for four species (*Aplidium accarense, Didemnum perlucidum, Schizoporella errata* and *Watersipora subtorquata*). Global warming is expected to generate range shifts of NIMS towards higher latitudes (Sorte et al., 2010, Canning-Clode & Carlton, 2017), however our predictions indicate that in global warming scenarios most species will also gain suitable areas towards the equator, which suggests that for invasive species range shift predictions could be more complex, given that widespread invasive species have high phenotype plasticity, allowing them to occupy climatic niches distinct from those they occupy in the regions of origin (Broennimann et al., 2007; Rocha et al., 2017). We used registers of occurrences of native and introduced ranges without distinction to calibrate and test the models, and which usually perform better than when using occurrences from the native range only because they better reflect phenotype variation of the species of interest (Broennimann & Guisan, 2008).

Propagule pressure and environmental suitability make *Aplidium accarense*, *Didemnum perlucidum* and *Watersipora subtorquata* the species with greatest risk of invasion of tropical regions. The first is already widespread in different continents (Monniot, F., 1969, Rocha et al., 2010; Lopez-Legentil et al., 2015) but as of yet, it has no known impact. *Didemnum perlucidum* is known to spread from artificial substrates to seagrass beds (*Halophila ovalis*) in Western Australia (Simpson et al., 2016) with possible impact on plant photosynthesis, and the abundance of seagrass-associated mud snails. In Santa Catarina mussel farms, this species reduces mussel yield (Lins & Rocha 2020). *Watersipora subtorquata* is a bioengineer species that can have variable impacts on the sessile and mobile species on hard substrates communities (Scott & terHorst, 2020).

The mussel *Mytilus galloprovincialis* merits concern and ranks among the 100 most invasive species worldwide (Lowe et al., 2000) with the important environmental impact of reducing biodiversity in natural communities. This mussel was introduced for cultivation in South Africa from where it has spread to adjacent natural environments (McQuaid & Phillips 2000) with negative impacts on biodiversity (Sadchatheeswaran et al., 2015). Rio Grande do Sul and Paraná are environmentally suitable for the species, and, as neighbor states of Santa Catarina, are at high risk of invasion. Rio de Janeiro is the next state in risk, followed by São Paulo and Espírito Santo. Given the already observed rapid adaptive genetic variation associated with temperature enabling this species to invade a wide range of thermal habitats successfully

(Han & Dong 2020) it is not surprising that our model of future warming scenario (RCP 2.6) predicts the establishment of the species up to Bahia.

Managers should be aware that a continuous propagule supply from Santa Catarina could further intensify invasions by introducing adaptive genetic variation, even in states with these NIMS (Ghabooli et al., 2013). The main source of information of these NIMS are from Rapid Assessment Surveys or experimental studies carried out in marinas and ports, and so do not indicate that they are already established on natural substrates elsewhere.

The species distribution model also indicates that offshore habitats inside the exclusive economic zone are suitable for these species. Although farther from the coast, these regions have several human activities, including domestic shipping routes, industrial fishing, oil and natural gas exploratory blocks, that are associated with hard surfaces susceptible to fouling by NIMS acting as stepping stones for their dispersal and subsequent expansion to natural communities (De Mesel et al., 2015).

Risk maps, such as those in this study, are valuable tools for decision-making to determine where to allocate resources where NIMS introductions are most likely. Predictive models can help to determine the most probable pathways of population movement and help to choose sampling sites and reduce costs of molecular studies to detect origins of introduced species (Pritchard et al., 2000; Falush et al., 2007). Predictive models also have the advantage of using public information and have already successfully predicted marine species introduction elsewhere (e.g., *C. lepadiformis* to Australia and *S. clava* to Argentina, Lins et al., 2018).

Our results highlight how NIMS associated with aquaculture can increase the probability of invasion in distant regions, and that this tendency should be considered in any decision-making processes for the expansion or establishment of aquaculture farms. Stakeholder perceptions may vary about the importance of an ecosystem approach when locating aquaculture parks (Vianna & Filho, 2018), and so these results should be made clear to them. Ideally, the location of aquacultures should avoid multiple use areas (where recreational, fishing, and international shipping vessels increase the chance of dispersal). Few studies estimate natural dispersal of NIMS from aquacultures, and that is required to determine the extent of buffer zones around them. Coastal shipping should also become the focus of biosecurity management and risk analysis, both national and international, in addition to concern with the international transport of NIMS.

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Figures Caption

Figure 1. **Tonnage (thousand tons) transported by container ships from Santa Catarina to other states in Brazil over five years (2015 – 2019) and current environmental suitability (colored cells)**. Red cells indicate greatest environmental suitability, yellow are medium suitability, blue are low suitability, and uncolored are not suitable. Asterisks indicates current occurrences.

Figure 2. **Maps of coastal Brazil and two oceanic island regions in which current and future species distributions are projected for *Aplidium accarense, Botrylloides giganteus* and *Didemnum perlucidum***. (A) Current, with species occurrences (circles) and the states at most risk of introduction and invasion, based on both connectivity and environment suitability (black = high risk, grey = medium risk and light grey = low risk). (B) Future climate scenario, RCP 2.6 in year 2050. (C) Future climate scenario, RCP 6.0 in year 2050. Environmental suitability maps were generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest).

Figure 3. **Maps of coastal Brazil and two oceanic island regions in which current and future species distributions are projected for *Styela plicata, Schizoporella errata* and *Watersipora subtorquata***. (A) Current, with species occurrences (circles) and the states at most risk of introduction and invasion, based on both connectivity and environment suitability (black = high risk, grey = medium risk and light grey = low risk). (B) Future climate scenario, RCP 2.6 in year 2050. (C) Future climate scenario, RCP 6.0 in year 2050. Environmental suitability maps were generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest).

Figure 4. **Maps of coastal Brazil and two oceanic island regions in which current and future species distributions are projected for *Megabalanus coccopoma* and *Mytilus galloprovincialis***. (A) Current, with species occurrences (circles) and the states at most risk of introduction and invasion, based on both connectivity and environment suitability (black = high risk, grey = medium risk and light grey = low risk). (B) Future climate scenario, RCP 2.6 in year

2050. (C) Future climate scenario, RCP 6.0 in year 2050. Environmental suitability maps were generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest).

Table 1 (on next page)

Summary of contributions (as %) of environmental variables used for predicting performance of species suitability models.

Sea surface salinity (SSS) minimum and range, sea surface temperature (SST) maximum and range obtained from Bio-Oracle v2.0 database. Maximum values for each species and model in bold.

Table 1. **Summary of contributions (as %) of environmental variables used for predicting performance of species suitability models**. Sea surface salinity (SSS) minimum and range, sea surface temperature (SST) maximum and range obtained from Bio-Oracle v2.0 database. Maximum values for each species and model in bold.

Species	MXS[1]				RDF				SVM			
	SSS		SST		SSS		SST		SSS		SST	
	Min	Range	Max	Range	Min	Range	Max	Range	Min	Range	Max	Range
Aplidium accarense	25	2	29	**42**	**32**	18	26	22	24	4	22	**50**
Botrylloides giganteus	25	11	**33**	31	26	26	17	**28**	19	7	26	**47**
Didemnum perlucidum	23	5	**67**	5	22	18	**31**	29	28	3	**64**	5
Styela plicata	25	8	27	**50**	28	14	**37**	21	22	11	31	**36**
Schizoporella errata	25	3	27	**43**	**38**	16	21	23	23	2	35	**38**
Watersipora subtorquata	28	11	**42**	18	**38**	7	29	24	26	11	**39**	22
Megabalanus coccopoma	8	7	42	**43**	25	7	31	**37**	15	5	**55**	25
Mytilus galloprovincialis	20	13	5	**62**	19	10	**51**	21	16	12	3	**69**

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

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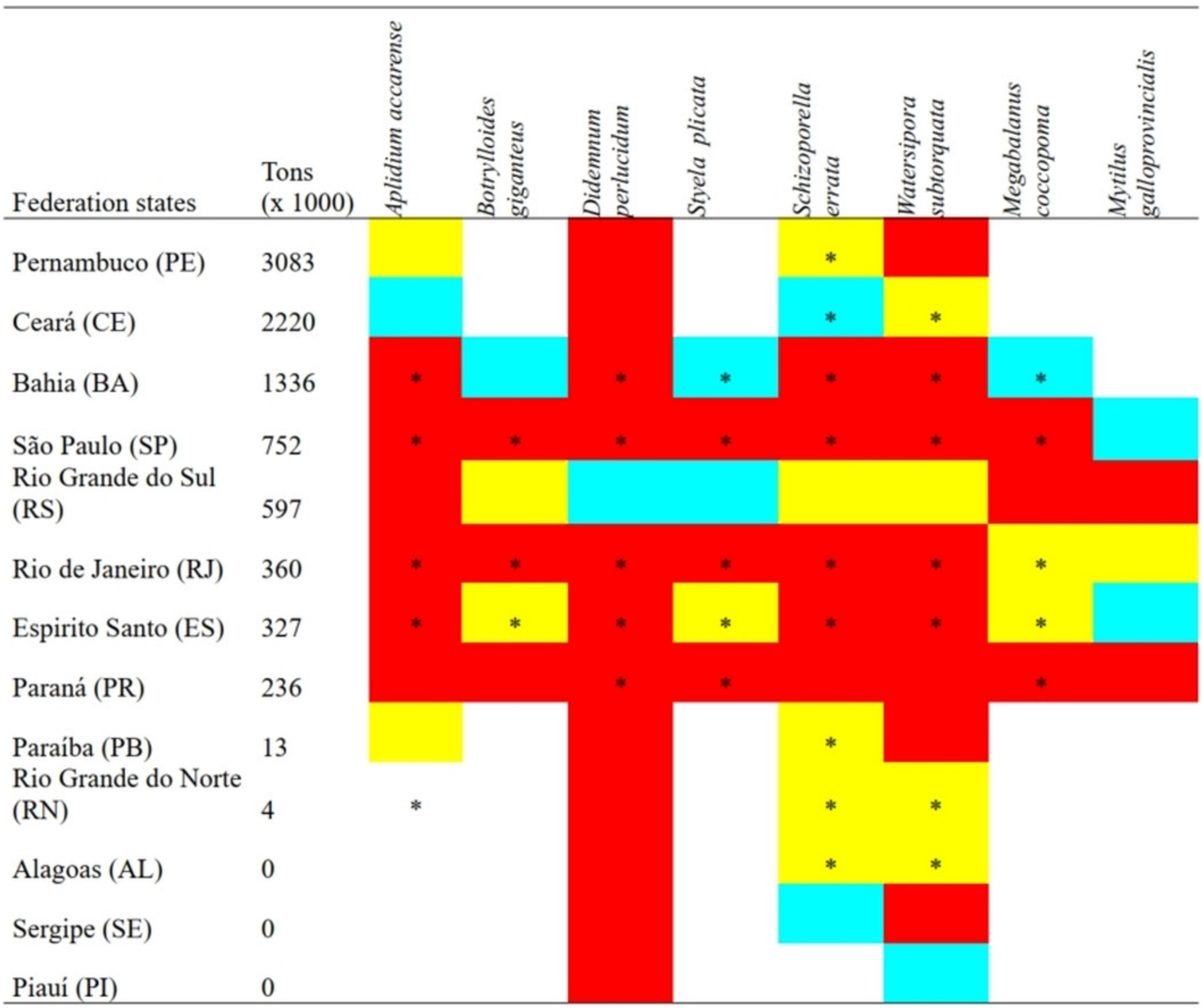
Figure 1

Tonnage (thousand tons) transported by container ships from Santa Catarina to other states in Brazil over five years (2015 – 2019) and current environmental suitability (colored cells).

Red cells indicate greatest environmental suitability, yellow are medium suitability, blue are low suitability, and uncolored are not suitable. Asterisks indicates current occurrences.



Federation states	Tons (x 1000)	*Aplidium accarense*	*Botrylloides giganteus*	*Didemnum perlucidum*	*Styela plicata*	*Schizoporella errata*	*Watersipora subtorquata*	*Megabalanus coccopoma*	*Mytilus galloprovincialis*
Pernambuco (PE)	3083					*			
Ceará (CE)	2220					*	*		
Bahia (BA)	1336	*		*	*	*	*	*	
São Paulo (SP)	752	*	*	*	*	*	*	*	
Rio Grande do Sul (RS)	597								
Rio de Janeiro (RJ)	360	*	*	*	*	*	*	*	
Espirito Santo (ES)	327	*	*	*	*	*	*	*	
Paraná (PR)	236			*	*			*	
Paraíba (PB)	13					*			
Rio Grande do Norte (RN)	4	*				*	*		
Alagoas (AL)	0					*	*		
Sergipe (SE)	0								
Piauí (PI)	0								

Figure 2

Maps of coastal Brazil and two oceanic island regions in which current and future species distributions are projected for *Aplidium accarense, Botrylloides giganteus* and *Didemnum perlucidum*.

(A) Current, with species occurrences (circles) and the states at most risk of introduction and invasion, based on both connectivity and environment suitability (black = high risk, grey = medium risk and light grey = low risk). (B) Future climate scenario, RCP 2.6 in year 2050. (C) Future climate scenario, RCP 6.0 in year 2050. Environmental suitability maps were generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest).

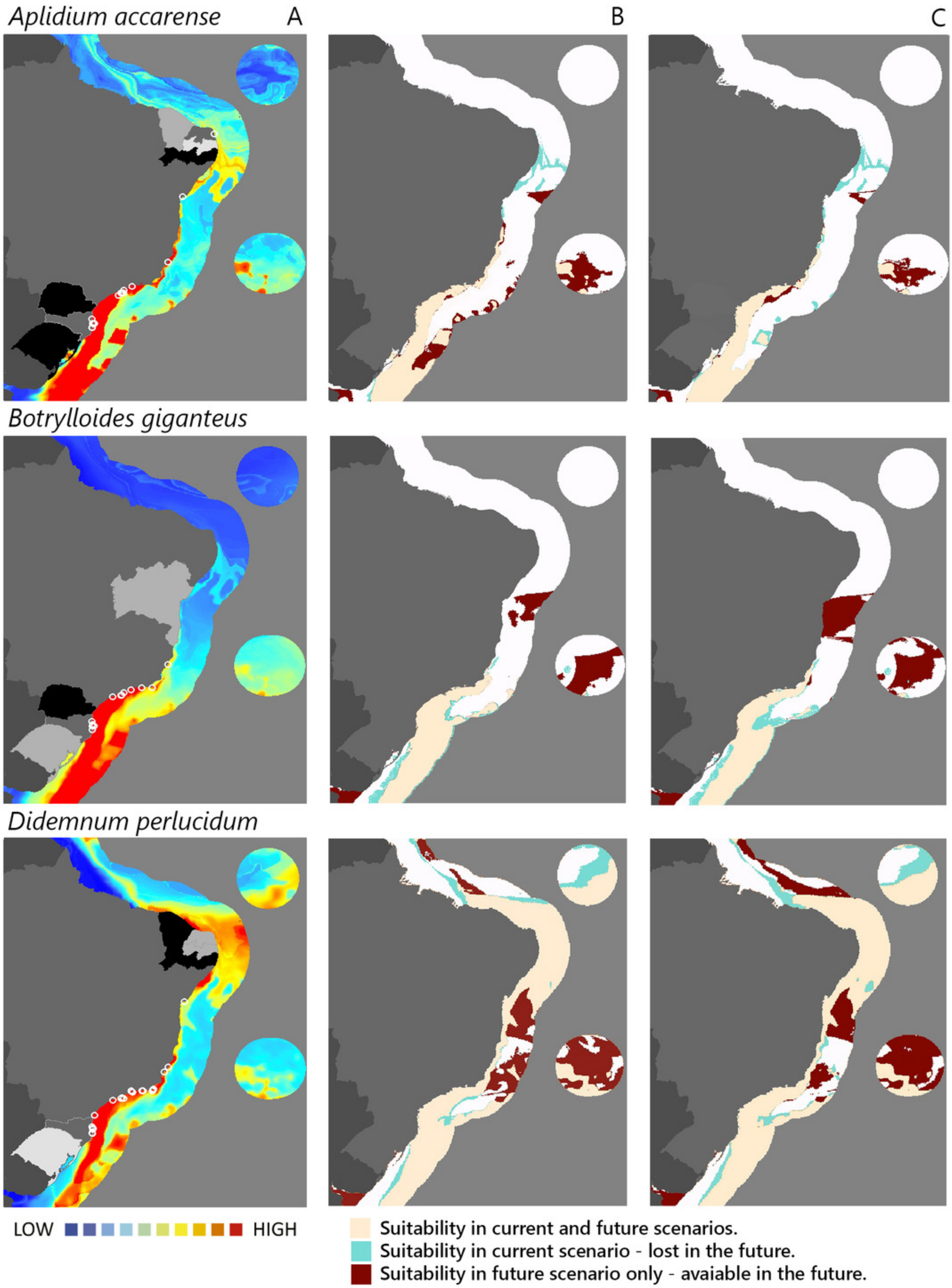

Aplidium accarense
A
B
C
Botrylloides giganteus
Didemnum perlucidum
LOW
HIGH
Suitability in current and future scenarios.
Suitability in current scenario - lost in the future.
Suitability in future scenario only - avaiable in the future.

Figure 3

Maps of coastal Brazil and two oceanic island regions in which current and future species distributions are projected for *Styela plicata, Schizoporella errata* and *Watersipora subtorquata*.

(A) Current, with species occurrences (circles) and the states at most risk of introduction and invasion, based on both connectivity and environment suitability (black = high risk, grey = medium risk and light grey = low risk). (B) Future climate scenario, RCP 2.6 in year 2050. (C) Future climate scenario, RCP 6.0 in year 2050. Environmental suitability maps were generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest).

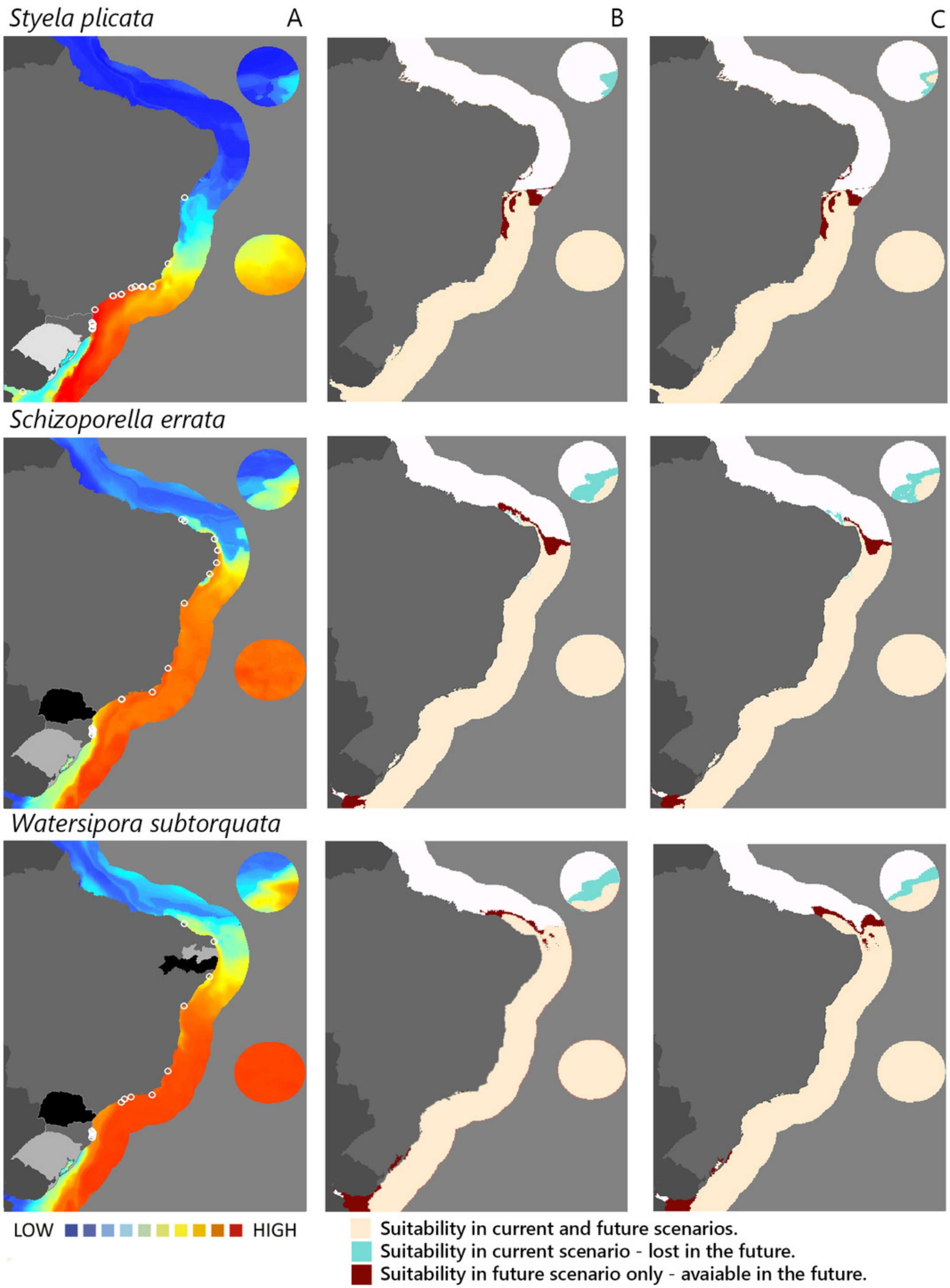
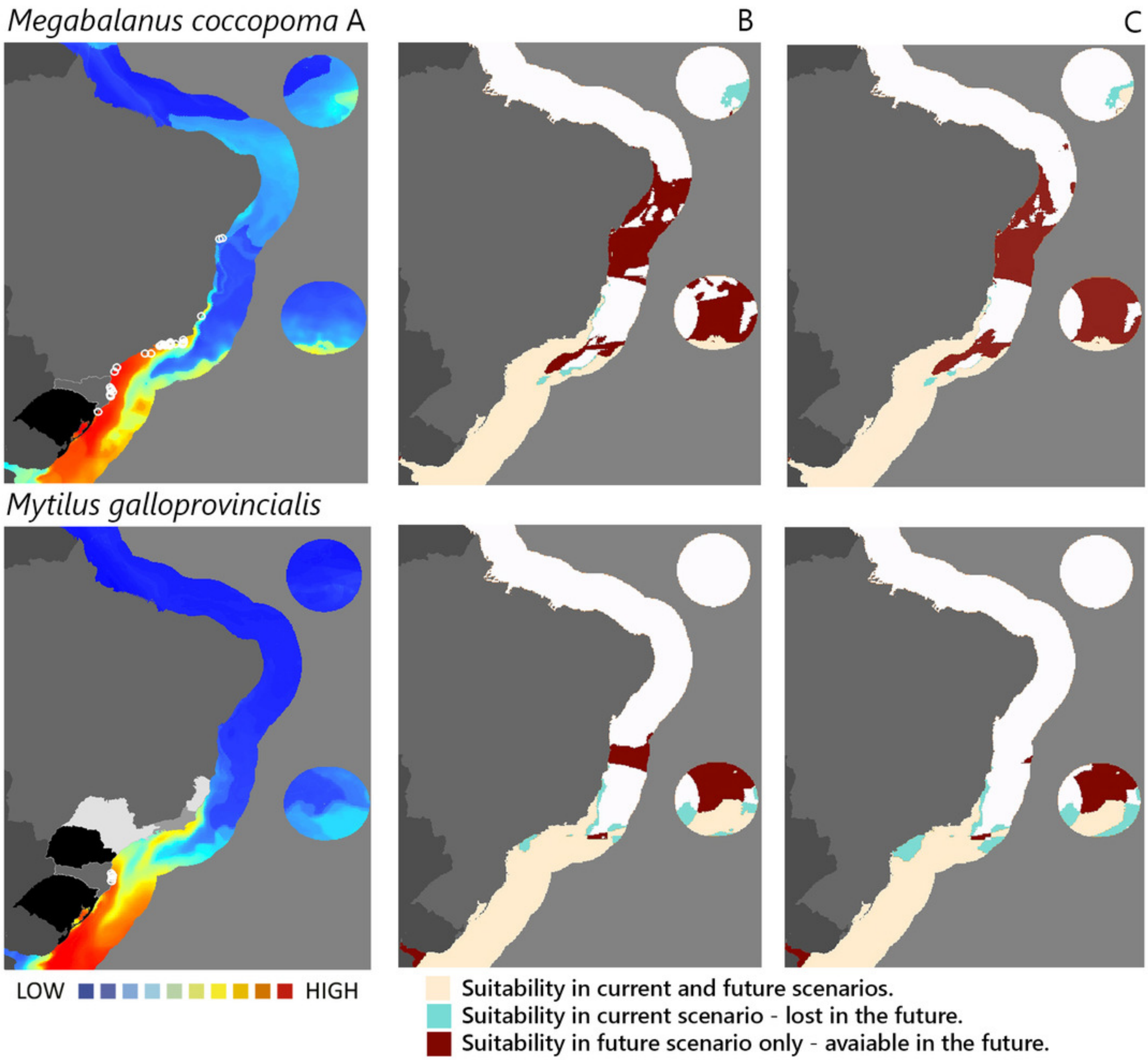

Styela plicata
A
B
C
Schizoporella errata
Watersipora subtorquata
LOW
HIGH
Suitability in current and future scenarios.
Suitability in current scenario - lost in the future.
Suitability in future scenario only - avaiable in the future.

Figure 4

Maps of coastal Brazil and two oceanic island regions in which current and future species distributions are projected for *Megabalanus coccopoma* and *Mytilus galloprovincialis*.

(A) Current, with species occurrences (circles) and the states at most risk of introduction and invasion, based on both connectivity and environment suitability (black = high risk, grey = medium risk and light grey = low risk). (B) Future climate scenario, RCP 2.6 in year 2050. (C) Future climate scenario, RCP 6.0 in year 2050. Environmental suitability maps were generated by the ensemble procedure of three types of Ecological Niche Models (MaxEnt, Support Vector Machine and Random Forest).


Megabalanus coccopoma A
B
C
Mytilus galloprovincialis
LOW
HIGH
Suitability in current and future scenarios.
Suitability in current scenario - lost in the future.
Suitability in future scenario only - avaiable in the future.