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Research Article

Range extension of the Asian green mussel *Perna viridis* (Linnaeus, 1758) into a Marine Extractive Reserve in Brazil

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Abstract

Biological invasions can cause significant changes in the abundance and distribution of species in recipient communities. The effect of invasive species on native biota is now one of the most addressed topics in studies of biological invasions. On March 20, 2022 two juvenile individuals of a never-before-seen mussel were detected on a mussel farm during cleaning activities in the Arraial do Cabo Marine Extractive Reserve (MER), Brazil. The brilliant green color of the margin of the predominantly brown shell was the initial characteristic used to identify the specimens as the Asian green mussel *Perna viridis.* Biometric features of both the farmed mussel *Perna perna* and *P. viridis* were compared to estimate age, and photographs were taken of the anatomical structures. Similar ages of mussel species in farm lanterns suggested that *P. viridis* may have been present, but overlooked, on nearby shores during spat collection, though no mature individuals were seen. Alternatively, marine farms may be more susceptible to invasion by other bivalves, as has been reported at other locations. This third record in the southwest Atlantic (Brazil) extends the range by 110 km eastward from the first report four years previously. With a port and harbor nearby, the most probable vector of introduction to the region was fouling on small boats. We fully expect *P. viridis* to continue its range expansion along the southwest Atlantic coastline. MERs provide a model of ecosystem and resource management which combine sustainable development and the conservation of natural resources to benefit artisanal fishers and other stakeholders. It is therefore important to establish long-term programs for monitoring, prevention and control of biological invasions at MERs, especially at Arraial do Cabo, which seems to be an invasion hotspot and, by its very nature, is particularly sensitive to marine biological invasions.

Key words: bivalvia, geographical distribution, invasive species, marine farm, Mytilidae

Introduction

Biological invasions by non-native species can cause significant changes in the abundance and distribution of species in recipient communities (Carlton and Geller 1993; Lages et al. 2011; Ricciardi and MacIsaac 2011). The effect of invasive species on native species is now one of the most addressed topics in the studies of biological invasions (Lowry et al. 2013). Maritime trade has been linked to increased biological introductions, transposing organisms across biogeographical barriers and expanding the distribution of many species worldwide (Carlton and Ruiz2005; Ignacio et al. 2010).

One important group that contributes to the nonnative fauna in the southwest Atlantic is the Mollusca (Darrigran et al. 2020), specifically the bivalve mollusks (Lopes et al. 2009; Pires-Teixeira and Creed 2020). Worldwide, there have been a large number of successful invasions by bivalve mollusks over the last few decades, both in freshwater and marine environments (Karatayev et al. 1997; Ricciardi 1998; Carlton 1999; Bownes and Mcquaid 2006; Sousa et al. 2009; López et al. 2014). Introduced mollusks are often successful in adapting to new environments, enabling some species to rapidly expand their distribution. The competitive superiority of bivalves stems from some typical characteristics of a successful invader, such as rapid recruitment and growth, gregarious behavior, ability to detach and re-attach with byssus and ability to quickly populate vacant spaces due to natural or human disturbances (Bayne 1976; Morton 1997; Rajagopal et al. 1998, 2006; Gobin et al. 2013), including the use of artificial substrates.

The coast of the southwest Atlantic is increasingly being invaded by nonnative marine species (Munawar et al. 2017; Bailey et al. 2020; Pires-Teixeira and Creed 2020), and the trend is expected to continue (Sardain et al. 2019). The Asian green mussel is native to the tropical waters of the Eastern and Western Indian Ocean and Western Central Pacific Ocean (Siriwardena 2022) (Figure 1A; Table S1). It is nonnative to the Northwest Pacific (China, Hong Kong, Taiwan and Japan), Northeast Pacific (United States), Oceania (Australia, New Zealand, Cook Is., Fiji, French Polynesia, New Caledonia, Western Samoa and Tonga), South Africa, the Northwest Atlantic, Gulf of Mexico (United States) and Caribbean (Jamaica, Trinidad and Tobago, Venezuela and Colombia) (Agard et al. 1992; Kazuhiro and Sekiguchi 2000; Benson et al. 2001; Ingrao et al. 2001; Buddo et al. 2003; Baker et al. 2007; Stafford et al. 2007; Gobin et al. 2013; Spinuzzi et al. 2013; Micklem et al. 2016; Gracia and Rangel-Buitrago 2020; Benson 2022; Siriwardena 2022)(Figure 1A; Table S1). In May 2018 Messano et al. (2019) reported the Asian green mussel, *Perna viridis* (Linnaeus, 1758), in the southwest Atlantic on artificial structures in the Guanabara Bay, Rio de Janeiro, and Soares et al. (2022) also recently reported the species on settlement plates > 2000 km north of Guanabara Bay at Ceará, Brazil (Figure1B).

In the Caribbean and Western Atlantic, *Perna viridis* is considered to be an invasive species as it has substantially expanded beyond its native range through ship ballast and hull fouling (Siriwardena 2022) (Figure 1A). The species can grow on a variety of natural and artificial substrates, including ship hulls and buoys, piers, experimental plates and mariculture gear (Benson et al. 2001; Messano et al. 2019; Siriwardena 2022). It is an edible

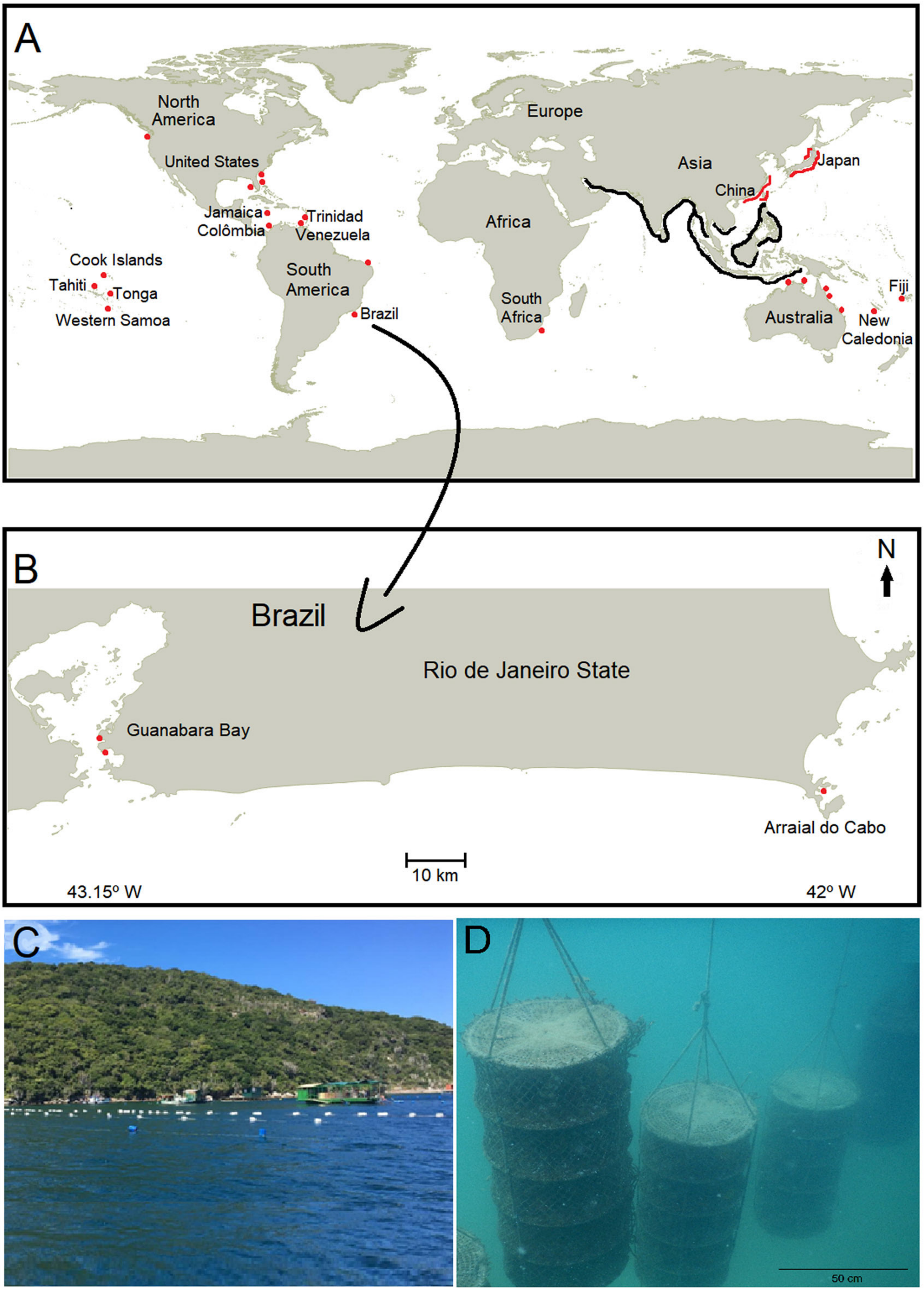

Figure 1. The range expansion of the Asian green mussel *Perna viridis* in Brazil. A) Map showing generalized native distribution (black lines) and generalized introduced range (red lines and points); B) Location of sites where *P. viridis* was discovered in Rio de Janeiro State; C) The marine farm and study area at Arraial do Cabo, Rio de Janeiro; D) Lantern nets where *P. viridis* was recently reported. Photographs by Herick S. dos Santos.

species which is cultivated commercially within its native range (Sivalingam 1977; Rajagopal et al. 1998; Melendres and Largo 2021; Rejeki et al. 2021) and has been introduced into Fiji, French Polynesia, New Caledonia, Samoa and Tonga through mariculture activities (Siddall 1980; Bell and Albert 1983; Coeroli et al. 1984; Vereivalu 1989; Eldredge 1994; Baker et al. 2007; Ng et al. 2013; Dias et al 2018; Siriwardena 2022).

According to Siriwardena (2022), *Perna viridis* is expected to expand throughout Atlantic habitats until it reaches its thermal limits (minimum temperature tolerance: 7 °C, maximum temperature tolerance: 37.7 °C) due to its dispersed spawning nature, lack of local predators, fast growth, and high tolerance of environmental conditions. The present study therefore aims to investigate the range expansion of the Asian green mussel in the southwest Atlantic to better understand its basic biology and how it might be spreading, as well as to identify potential pathways and vectors and future consequences of the invasion for native biota, marine conservation and mariculture in Brazil.

Materials and methods

Study area and site

The mussel farm and study site are contained within the Arraial do Cabo Marine Extractive Reserve at Arraial do Cabo located on the southeastern Brazilian coast (22°49′S; 42°24′W, Figure 1). Extractive Reserves differ from other kinds of protected areas because they originated from the social movement of Amazon rubber tappers during the 1980s (ViVacqua 2018). Marine Extractive Reserves (MERs) therefore provide a model of ecosystem and resource management which combine sustainable development and the conservation of natural resources to benefit artisanal fishers and other stakeholders. The Arraial do Cabo MER, which was created in 1997, is thus a marine protected area under government management in which natural resources are exploited by artisanal fisherfolk. In this area marine farming of mollusks [the brown mussel *Perna perna* (Linnaeus, 1758) and Pacific oyster, *Magallana gigas* (Thunberg, 1793)] is carried out (Figure 1C, D) and is one of the main commercial activities of the local population. Mussel spat (mainly *P. perna*) is collected on nearby shores, and individual juveniles are placed in net lanterns for growth and maturation. The region of Cabo Frio is a tropical marine ecosystem which undergoes periodic upwelling events of colder, nutrient rich water, which contributes to an enhanced coastal productivity and sustains a remarkable marine biodiversity (Ferreira et al. 2001, 2004; Valentin 2001).

Detection, sampling and identification

The mussel farm structure and lantern nets (Figure 1C, D) are constantly monitored and maintained. Never-before-seen mussels were detected on

March 20, 2022 during periodical cleaning activities. Individuals were removed, fixed in 70% alcohol and sent for further analysis (i.e., identification and biometry). The individuals were deposited in the collection of the Laboratory of Benthic Marine Ecology at the State University of Rio de Janeiro – UERJ. To identify the species we used external coloration and morphological characteristics (Siddall 1980; Rajagopal et al. 2006; Leung et al. 2014; Messano et al. 2019). The biometric features of both *P. perna* and *P. viridis* were compared. Photographs of the anatomical structures (shells, hinge and umbo) of both species were taken with a Canon EOS Rebel SL3 camera coupled to an electronic stereoscopic microscope (40×). The individuals were dissected to examine the adductor muscle scar patterns (Siddall 1980). We used the method of McDonald (2012) and Messano et al. (2019) to estimate the "Predicted Age" of individuals using the centered growth rate value of 0.24 $mm.day^{-1}$.

Results

On March 20, 2022 two juvenile individuals of the Asian green mussel *Perna viridis* were detected on a marine farm located in the Arraial do Cabo MER during the maintenance and management activities of mussels (*Perna perna*) cultivation (Figure 1C, D). No individuals were observed on the natural substrate on the shores adjacent to the marine farm area, where *P. perna* spat are usually collected. This population represents the third invaded location in Brazil and is 112 km east of the first reported location for *P. viridis* in Brazil, Guanabara Bay, reported four years previously (Figure 1B).

The brilliant green color of the margin of the predominantly brown shell (Figure 2A) were the initial characteristics used to identify specimens as belonging to *P. viridis* (Buddo et al. 2003). Specimens presented other morphological features characteristic of *P. viridis*, such as green coloration of the edges of the shell present in growth rings, a uniformly bright green coloration of the periostracum (Figure 2A), posterior adductor scars extending beyond the pallial line (Figure 2B) and smooth elongated shells with a curved shape (Figure 2C).

The greenish color of *P. viridis* shells contrasted with the dark brown tone of the mussel *P. perna* (Figure 2B, C), quickly drawing attention because it was the first time that individuals with these characteristics have been spotted after > 10 years performing the same type of maintenance at the site. Taxonomic structures such as the umbo and hinge of both specimens were compared (Figure 3A–F, Table 1) (Siddall 1980; Rajagopal et al. 2006; Messano et al. 2019). The predicted age analysis of *P. viridis* showed that individuals were approximately 110 (*P. viridis* 1) and 90 days (*P. viridis* 2) old, similar to *P. perna* (Table 1).

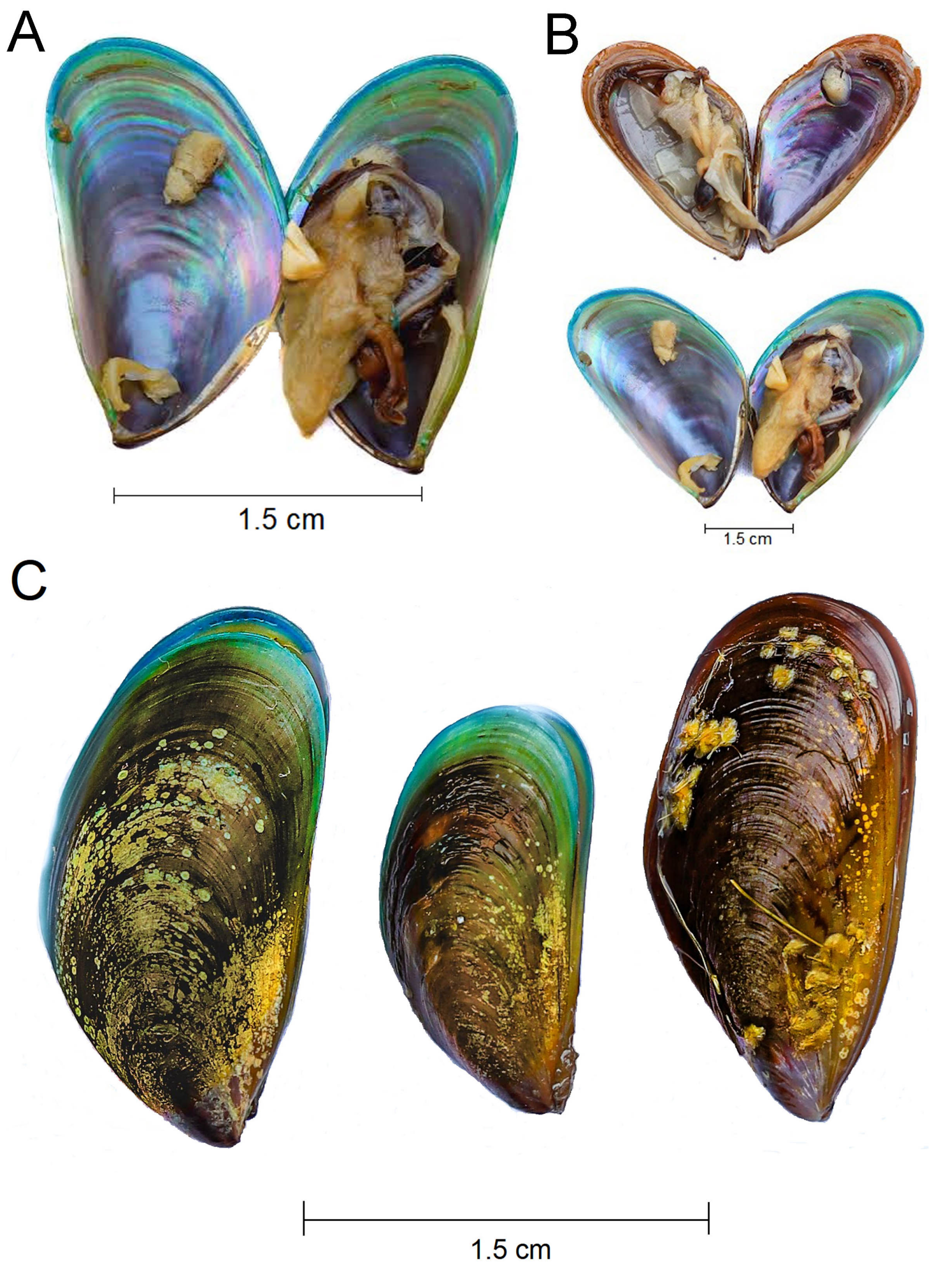

Figure 2. A) Internal view of *Perna viridis* (Ind. 1) with muscle tissue; B) Comparison between brown mussel *Perna perna* (above) and the green mussel *P. viridis* (Ind.1, below); C) Shells of *P. viridis* 1 (left), *P. viridis* 2 (centre) and *P. perna* (right). Photographs by Júlia C. Bertollo.

Discussion

This study represents the third record of *P. viridis* in Brazil and a range expansion of over 110 km eastwards from the first detection in Guanabara Bay four years previously. This confirms the prediction of Siriwardena (2022) who expected that the species would expand in Atlantic habitats. This study allows us some new insights into the following aspects of the *P. viridis* invasion in Brazil: 1) probable mechanisms for dispersal; 2) susceptibility of shellfish farms to invasion by mussels; 3) management and the future.

The study site is within a relatively pristine and protected conservation area (i.e., the Arraial do Cabo MER). In contrast, Guanabara Bay is a highly

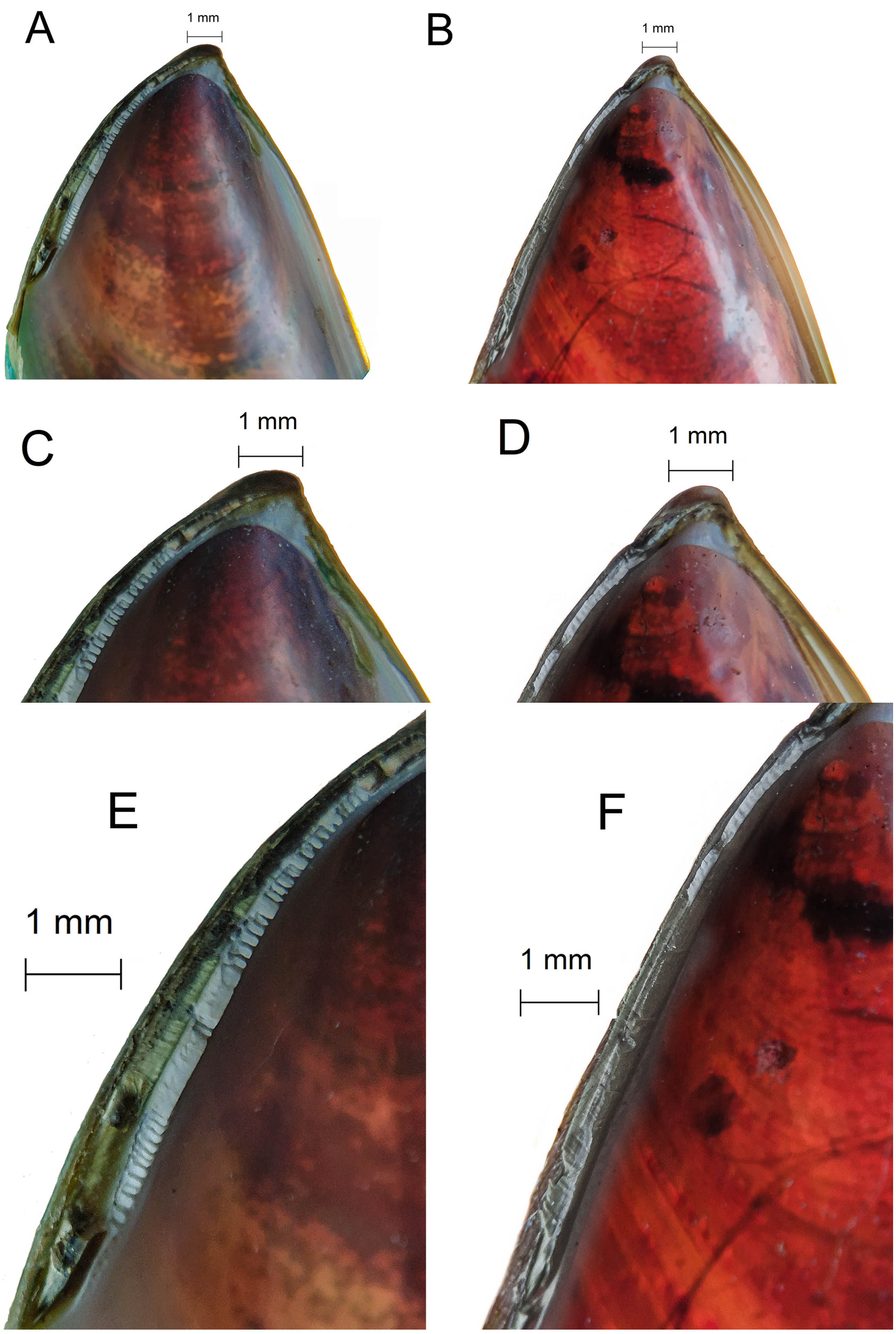

Figure 3. Morphological details of umbo (A, C) and hinge (E) of the Asian green mussel *Perna viridis* and *P. perna* (B, D and F) respectively (40×) collected from artificial structures in the Arraial do Cabo, Rio de Janeiro, Brazil in March 2022.

Table 1. Biometric measures and predicted age of two individuals of *Perna viridis* and one *Perna perna* collected from artificial structures in the Arraial do Cabo, Rio de Janeiro in March 2022.

	Lenght (cm)	Width (cm)	Height (cm)	Hinge (cm)	Pedicted Age (days)
P. viridis 1	2.6	1.4	1	1.1	110
P. viridis 2	2.1	1.1	0.7	0.8	90
P. perna	2.7	1.4	0.7	1.5	111

polluted and anthropogenic estuarine system with a number of ports, located near the city of Rio de Janeiro. Despite these quite different habitats, *P. viridis* has been successful in establishing at both sites, so what do the two locations have in common?

Examples around the world suggest that the introduction of *P. viridis* may be through mariculture activity or shipping (ballast water or fouling) (Siddall 1980; Bell and Albert 1983; Coeroli et al. 1984; Vereivalu 1989; Eldredge 1994; Baker et al. 2007; Ng et al. 2013; Dias et al. 2018; Siriwardena 2022). Several cases of biological introductions of other nonnative marine species have been reported over the 20 years at Arraial de Cabo, and all of them have been attributed to the local port activities (Silva et al. 2004; López et al. 2005; Ferreira 2003; Santos et al. 2019). Port environments are considered major entry points for invasive species (Seebens et al. 2013). Although ballast water has also been suggested as a potential invasion vector for *P. viridis* (Baker et al. 2007), it is unlikely that the introduction of these organisms into the MER region was via ballast water. The presence of individuals on ships hulls as fouling and on other artificial structures is more widely reported (Coutts and Taylor 2001; Peebles 2004; Minchin et al. 2016; Dias et al. 2018), including in the Atlantic Ocean (Penchaszadeh and Valez 1996). The Arraial do Cabo region has been reported as a donor of ballast water to other ports (Silveira et al. 2006) due to the import of coarse salt to supply a local factory. No deballasting activity has been reported for this location in the literature or in local port authority records. It is also very unlikely that these organisms reached this new region via natural dispersal at either the adult or larval stage. Floating marine litter has frequently been reported as an important vector for the dispersal of several nonnative adult marine benthic species (Carlton et al. 2017; Mantelatto et al. 2020) including *P. viridis* in the Caribbean (Gracia and Rangel-Buitrago 2020). However rafting or larval dispersal from Guanabara Bay to Arraial de Cabo is unlikely given the local oceanographic conditions and currents. In addition, the individuals found were juveniles, suggest recent settlement and recruitment processes at this location.

The most likely vector of introduction is, therefore, fouling on hulls of vessels that have traveled between Arraial do Cabo and Guanabara Bay. In spite of the Arraial do Cabo MER being a marine protected area, the cleaning and repair activities of vessels and oil platforms have historically been carried out by the Port of Forno, which may have been the entry point for most of these organisms as it is only 1 km away from the study site. *Perna viridis* has also been reported as hull fouling on an oil platform supply vessel (PSV) docked in Guanabara Bay, Rio de Janeiro, Brazil in 2019 (JCC *unpublished data*). Other potential secondary dispersal vectors of these organisms may be fishing and tourism boats, which have been suggested as the vehicle of local spread of many species (Ramalho and

Muricy 2004; Ferreira et al. 2006; Mizrahi 2008; Santos 2018; Santos et al. 2019). This is reinforced by the fact that the vast majority of other introduced species in this region are also reported to occur near the Porto do Forno area and adjacent rocky shores, having subsequently expanded their distributional ranges (Lacombe and Rangel 1978; Ferreira 2003; Lages 2003; Fernandes et al. 2004; Ferreira et al. 2004, 2006; Breves-Ramos et al. 2010).

The fact that the predicted ages of *P. viridis* and *P. perna* were very similar suggests that they may have been collected together, as spat, for mariculture from the nearby coastline. It is quite possible that the spat of *P. viridis* passed undetected amongst that of *P. perna*. However, as mature *P. viridis* were not detected on neighboring shores, another possible explanation is that the mariculture systems are more susceptible to invasion by other bivalves. Urbano et al. (2005) described experimental tests in mariculture systems in the Gulf of Cariaco, Venezuela, in which *P. viridis* and *P. perna* were also found together under conditions similar to which we describe here. This is not surprising as the cultivation systems presumably provide optimal conditions for growth, for both target species and invasives.

Perna viridis is not the only nonnative mussel to threaten mariculture in the southwest Atlantic, as the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819) was detected in marine farms in September 2016 in southern Brazil, also growing with *Perna perna* (Belz et al. 2020). There, *M. galloprovincialis* outcompeted *P. perna* spat for space, damaged cultivation socks and was not accepted by consumers as a dietary alternative to *P. perna* (Lins et al. 2021). Bivalve cultures also provide habitat for a range of other nonnative species in southern Brazil (Rocha et al. 2009). According to Tyrrell and Byers (2007), artificial surfaces such as those found in marine farms may provide a novel context for competitive interactions, giving nonnative species a more "level playing field" in an evolutionary setting where they are not so well adapted. This is called selection regime modification, where additions of artificial substrates to the nearshore environment may disproportionately favor nonnative species.

Whichever the explanation, *P. viridis* can out-compete many other benthic species, causing changes in community structure and food web relationships. For example, since the appearance of *P. viridis* in the Paria Gulf, Venezuela in 1993, the habitat of *P. perna*, a species that has demonstrated a similar physiological response to *P. viridis* (Urbano et al. 2005) has been altered (Rajagopal et al. 1998; Bravo et al. 1998). In fact *P. perna* itself is actually considered by some as nonnative to Brazil, as there is some evidence that it may be a "deep invader", having been introduced as fouling on boats during the slave trade from Africa (Wood et al. 2007; Silva et al. 2018).

This *P. viridis* range expansion is worrying, as the introduction of new species to environments in which they did not evolve has been widely recognized as one of the top five threats to marine ecosystem function and biodiversity (Carlton 2001). Over the past decades the worldwide dispersal of species has increased by orders of magnitude, and this has contributed to some regions now being invaded by several new species per year (Seebens et al. 2013). The main vectors of these introductions are related to navigation, either by water used as ballast in ships (Carlton and Geller 1993; Miller et al. 2011) or by fouling of ships hulls, anchors and support structures (Carlton 1999; De Paula and Creed 2004; Ferreira et al. 2006; Page et al. 2006; Sammarco et al. 2012; Creed et al. 2017). As the Arraial do Cabo MER is near a port and it is still unclear whether *P. viridis* settled and recruited onto the marine farm or was present but undetected amongst the spat collected on nearby shores, the presence of this species on natural substrates will need to be monitored. If populations are detected in native communities, ecological interactions with native species should be investigated.

We fully expect *P. viridis* to continue its range expansion along the southwest Atlantic coastline. Early detection is one of the premises of bioinvasion management and prevention, in addition to pre- and post-border cleaning practices and efficient anti-fouling systems (Ferreira et al. 2009; Olenin et al. 2011; Seebens et al. 2013). Currently, predictive approaches based on risk analysis and other pre-border control actions have also been developed to manage biological invasions. Risk assessment describes an array of methods and techniques that estimate the likelihood and consequences of undesired events, either using qualitative or quantitative methods, providing a valuable decision aid if completed in a systematic and rigorous manner (Hewitt and Campbell 2007). These models integrate variables such as propagule pressure, invasibility levels (expressed by the environmental matching between origin and destination sites) and community mismatch (expressed by biogeographic dissimilarity) (Seebens et al. 2013; Santos et al. 2019). These approaches may be used to take a new look at the problem, helping to recognize priority locations for management actions.

Conclusions

The present study provides the third record of *P. viridis* for the Brazilian coast, the second for the Rio de Janeiro State and the first within a marine protected area (MER) and on a marine farm. Although the occurrence of these organisms in the South Atlantic is currently restricted to artificial structures, the range expansion and increase in the number of records is an alert to the probable further expansion of this species onto natural substrates and into native communities. Our results suggest the existence of different vectors acting in the secondary spread of this species, possibly with the occurrence of multiple introduction events in a relatively short period of time.

Research on marine biological invasions in Brazil has increased over the last three decades, and some important goals have been achieved (Ferreira et al. 2009; Oigman-Pszczol et al. 2017; Creed et al. 2017). In this study we have reported on yet another invasive species in expansion along the coast of the southwest Atlantic and yet another species with potential to impact the livelihoods and local ecosystems in Brazil. It will be important to establish long-term programs for monitoring, preventing and controlling biological invasions, especially in the Arraial do Cabo MER, which seems to be an invasion hotspot and, by its very nature, is particularly sensitive to marine biological invasions.

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Authors' contribution

HSS, JCB and JCC were responsible for research conceptualization. HSS and JCB were responsible for sample design and methodology, investigation and data collection, data analysis and interpretation and ethics approval; HSS carried out the morphological analyses and identification of the specimens. JCB was responsible for high resolution photomicrographs and identification of specimens. JCC obtained some of the funding. HSS wrote the first draft of the manuscript and all authors participated in the writing of the manuscript. All authors read and approved the final version of the manuscript.

Ethics and permits

Mussels of the *Perna* genus are not listed as species at risk of extinction. The authors have complied with all policies relative to the collection and handling of marine species, and no ethics approval was required. This research was carried out under the Brazilian Federal Government Sistema de Autorização e Informação em Biodiversidade – SISBIO license no. 71757-1 to HSS.

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Supplementary material

The following supplementary material is available for this article:

Table S1. List of geo-referenced occurrence records of *Perna viridis* throughout the world used to construct the maps in Figure 1 and categorized by range (native or nonnative) and country or territory.

Table S2. List of sources used for compiling occurrence records for *Perna viridis* in Table S1.

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