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Article

One more place to call home: the invasive bivalve *Mytilopsis leucophaeata* reaches the Maricá-Guarapina lagoon system (Rio de Janeiro, Brazil)

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Abstract: *Mytilopsis leucophaeata* is an estuarine bivalve native from the Gulf of Mexico and Southeast USA, and it was introduced in Europe, Asia, Caribbean, South America and Northeast USA, showing massive colonization skills. In Brazil, the single invasion records of *M. leucophaeata* occur in the city of Rio de Janeiro, i.e., in the Rodrigo de Freitas Lagoon and in the Marapendi Lagoon. We conducted a new series of fieldworks in estuaries from the Rio de Janeiro state in order to evaluate the propagation of this invasive bivalve, aiming sites with proper salinities for the establishment of *M. leucophaeata*. A new record is given for the Maricá-Guarapina lagoon system, where *M. leucophaeata* mainly colonizes hard substrata (such as piers and rocks), reaching a mean density up to 43,375 specimens/m^2; however, aggregates of *M. leucophaeata* were also observed in the soft substratum. Based on mitochondrial sequences, the taxonomic identification of the invasive bivalve was confirmed. The associated fauna to the agglomerates of *M. leucophaeata* in the lagoon system comprises amphipods, barnacles, tanaidaceans, isopods, crabs, polychaetes and snails. The expansion of *M. leucophaeata* requires a continuous investigation due to the great circulation of boats in the littoral of the Rio de Janeiro state and the increased chance of new introductions.
Keywords: *Biological invasion; secondary dispersion; ecosystem engineer; associated fauna; estuary; Dreissenidae.*

Mais um lugar para chamar de casa: o bivalve invasor *Mytilopsis leucophaeata* alcança o sistema lagunar Maricá-Guarapina (Rio de Janeiro, Brasil)

Resumo: *Mytilopsis leucophaeata* é um bivalve estuarino originário do Golfo do México e Sudeste dos EUA, tendo sido introduzido na Europa, Ásia, Caribe, América do Sul e Nordeste dos EUA, e apresentando massiva capacidade de colonização. No Brasil, os únicos registros de invasão de *M. leucophaeata* ocorrem no município do Rio de Janeiro, i.e., na Lagoa Rodrigo de Freitas e na Lagoa de Marapendi. Este estudo propõe um novo levantamento de campo em estuários fluminenses para avaliar a propagação desse bivalve invasor, visando locais com salinidade propícia para o estabelecimento de *M. leucophaeata*. Um novo registro é feito para o complexo lagunar Maricá-Guarapina, onde *M. leucophaeata* coloniza principalmente substratos duros (como píers e rochas), chegando a uma densidade média de 43.375 indíviduos/m^2; porém, agregados de *M. leucophaeata* também foram observados em substrato inconsolidado. Com base em sequências mitocondriais, a identificação taxonômica do bivalve invasor foi confirmada. A fauna associada aos aglomerados de *M. leucophaeata* no complexo lagunar compreende anfípodes, cracas, tanaidáceos, isópodes, caranguejos, poliquetas e gastrópodes. A expansão de *M. leucophaeata* demanda uma investigação contínua devido ao alto fluxo de embarcações no litoral fluminense e elevada probabilidade de novas introduções.
Palavras-chave: *Invasão biológica; dispersão secundária; espécie engenheira; fauna associada; estuário; Dreissenidae.*

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Introduction

Mytilopsis leucophaeata (Conrad, 1831) is an estuarine bivalve with planktotrophic development and massive colonization in several types of hard substrata (Verween et al. 2010, Kennedy 2011), but occasionally also forming agglomerates in soft substrata (Fernandes et al. 2020, Rodrigues et al. 2022). The required salinity for the survivorship and reproduction of *M. leucophaeata* are below 18–21 ppt (Kennedy 2011, Van der Gaag et al. 2016, Maia-Neto et al. 2020). This species is native from the Gulf of Mexico and Southeast USA, where it has low densities, and it was introduced in Europe, Asia, Caribbean, South America and Northeast USA (Fernandes et al. 2018, 2021, Lodeiros et al. 2019, Zhulidov et al. 2021, Rodrigues et al. 2022). In Brazil, the confirmed records of *M. leucophaeata* are in the city of Rio de Janeiro, i.e., in the Rodrigo de Freitas Lagoon and in the Marapendi Lagoon (Rizzo et al. 2014, Fernandes et al. 2020). This bivalve may form huge aggregates in invaded sites; for example, at the Rodrigo de Freitas Lagoon, a mean density of up to 84,560 specimens/m^2 was recorded (Maia-Neto et al. 2020), enabling the filtration of a large amount of water (Neves et al. 2020). The arrival of *M. leucophaeata* in a new site may alter the nutrient cycling and the structure of the benthic, planktonic and nektonic communities, acting as an ecosystem engineer (Kennedy 2011, Neves et al. 2020, Rodrigues et al. 2021), and may promote economic losses (Rajagopal et al. 1997, Verween et al. 2010, Florin et al. 2013, Lodeiros et al. 2019).

The primary dispersal (long distance) of *M. leucophaeata* into new areas may occur through the transference of larval forms by the inadequate discharge of ballast water, whereas the incrustation of specimens in the hull of a ship or boat may also promote secondary dispersion (medium or short distance) (Kennedy 2011, Fernandes et al. 2018, 2020, 2021). Even with the existence of Brazilian laws that regulate the discharge of ballast water (Normam-20/DPC), the surveillance is inefficient. Because of that, Brazil is a great receptor of marine and estuarine non-native species, comprising diverse taxonomic groups and with the constant record of new species (Teixeira & Creed 2020), which brings a series of damages (sometimes irreversible) to the native biodiversity (Pysek et al. 2020).

This study aims to improve the knowledge about the distribution of *M. leucophaeata* in the Rio de Janeiro state, complementing the previous data of Fernandes et al. (2020). After a new series of fieldworks, guided to sites with proper salinities for the establishment of the bivalve, a new record is provided, leading us to study the composition of the fauna associated to agglomerates of *M. leucophaeata* and how some environmental and anthropic aspects may influence the distribution of this invader.

Material and Methods

1. Study area

The study area comprises part of the littoral of Rio de Janeiro state (Figure 1), additional to the data of Fernandes et al. (2020). Samplings were mainly performed at 'Região dos Lagos' (Figure 1A; including the Maricá-Guarapina lagoon system, the Saquarema lagoon system and the lagoons of Jaconé and Jacarepiá) and at Sepetiba Bay (Figure 1B; including the vicinities of the Port of Itaguaí, the mouth of Guandu River and a mangrove area at Guaratiba). In addition, the Jacuecanga River (city of Angra dos Reis) was also visited (Figure 1B).

Sepetiba Bay is a semi-closed waterbody with an area of 305 km^2, receiving the discharge of 12.6 to 14.5 million m^3 of freshwater per day, mainly through the canal of São Francisco and the rivers Guarda, Guandu and Mazomba-Cação (Carreira et al. 2009). This bay receives impacts through the polluted rivers, the local presence of industries and the dense urbanization, including domestic/industrial sewage and leakage of heavy metals (Ribeiro et al. 2015, PACS 2015, Rodrigues et al. 2017). The Port of Itaguaí was inaugurated in 1982 under the

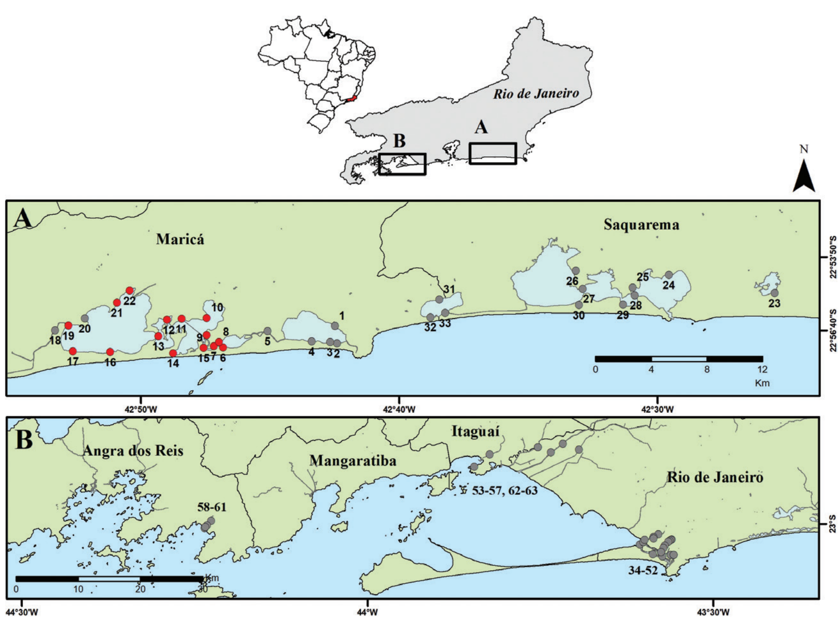

Figure 1. Map of Rio de Janeiro state with detailed location of the sampling stations: A- Municipalities of Maricá and Saquarema; B- Municipalities of Angra dos Reis, Mangaratiba, Itaguaí and Rio de Janeiro. Circles indicate absence (grey) or presence (red) of *Mytilopsis leucophaeata*, whether by live specimens or empty shells/valves. Coordinates available in Appendix 2.

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old name Port of Sepetiba, having an important role in the transport of iron ore; it was the sixth Brazilian port in terms of cargo transport in 2021 (ANTAQ 2022). However, the growing expansion of the port has substantially increased the socio-environmental impacts in the area (PACS 2015, Cordeiro & Amaral 2017).

The conservation unit 'Reserva Biológica Estadual de Guaratiba' (RBG) was created in 1974 and aims to preserve a mangrove area of 360,000 m² in the city of Rio de Janeiro, having a strong influence of the Sepetiba Bay (INEA 2021a). Such ecosystem has an evident environmental value (high biodiversity; sites of resting and feeding for migratory birds; prevention of flooding; source of organic matter for adjacent waters), in addition to economic (artisanal fishing; crab and mussel collectors) and historical (containing 18 'sambaquis', i.e., pre-Columbian shell mounds) values (INEA 2021a). Among the anthropic impacts in the area, there are illegal hunting and fishing, construction of irregular houses, domestic sewage without any prior treatment, and tourism.

At the Ilha Grande Bay, the Jacuecanga River discharges at ~5.5 km away from the fourth Brazilian port (Terminal Aquaviário de Angra dos Reis) in terms of cargo transport at 2021 (ANTAQ 2022). About 0.5 km away from the mouth of the Jacuecanga River, there are several shipyards and a large marina (Verolme), which may influence the arrival of exotic species.

The Maricá-Guarapina lagoon system covers a total area of ~35 km² and it is composed of four interconnected waterbodies: Maricá (~18 km²), Barra (~8 km²), Padre (~2 km²) and Guarapina (~6 km²) (Cruz et al. 1996). The system connects to the sea through the Ponta Negra canal, an artificial canal constructed in 1951 and which is often silted (Rodrigues et al. 2015), and with a minor drainage canal that ends at the Itaipuaçú Beach. Historically, the lagoon system had cycles of opening and closure of the sea connection through the site 'Barra da Emergência', usually regulated by anthropic means (Oliveira et al. 1955). The lagoon system shows a broad salinity variation according to weather conditions and the opening period of the Ponta Negra canal (Silvestre et al. 2017), with a mean of 18 ppt (Franco et al. 2019), although the inner waterbody (Maricá Lagoon) often shows salinity levels close to zero (Guerra et al. 2011, Laut et al. 2019). The lagoon system has great socioeconomic importance for the city of Maricá, due to several activities related to recreation, tourism and fishing. However, the growing urbanization at the margins of the lagoon system has increased sediment and sewage input, which, associated with the low depth levels and reduced water renovation, leads to its silting up and eutrophication (Cruz et al. 1996, Rodrigues et al. 2015, Silvestre et al. 2017, Laut et al. 2019, Toledo et al. 2021). Other near lagoons show different salinity levels, such as the euhaline Saquarema lagoon system (mean: 27–33 ppt) and the hyperhaline Araruama Lagoon (mean: 46–48 ppt) (Franco et al. 2019). Atypical years may greatly alter such conditions (Moreira-Turcq 2000) and differences in the salinity between dry and wet seasons might be high (Mendes & Soares-Gomes 2011, Dias et al. 2017).

2. *Sampling and laboratory procedures*

Fieldworks were conducted on seven events (without repetition of sites) between August/2021 and February/2022, in search of new areas for the establishment of *M. leucophaeata*. We mainly investigated hard substrata (natural, such as rocks and mangrove roots, or artificial, such as piers and decks) in estuaries and lagoons, with a careful visual inspection; empty valves were also searched in the sediment. Water temperature and salinity were measured through the equipment Hanna Instruments HI98319. When present, aggregates of *M. leucophaeata* were sampled (three replicates) by scraping the substratum with a spatula and a square sampler of 0.04 m², stored in plastic bags and kept in a freezer for posterior analyses. We used a 5 L Van Veen grab in the soft substratum in only one event (Guaratiba, 30/August/2021). At the same event, some agglomerates of the 'sururu' mussel *Mytella strigata* (Hanley, 1843) were collected in order to evaluate a possible presence of *M. leucophaeata* nested within it; the fauna associated to this mussel is shown in Appendix 1, regarding the few available data from this estuary.

At the laboratory, individuals of *M. leucophaeata* with soft parts were counted, and the shell length of each specimen was measured through a digital caliper rule (0.01 mm precision). The associated fauna was sorted, identified and stored in ethanol 70%, to be deposited in the scientific collections of Museu Nacional, Universidade Federal do Rio de Janeiro (MN/UFRJ) and Universidade do Estado do Rio de Janeiro (UERJ); the taxonomic identifications were confirmed by specialists (see 'Acknowledgments'). Photographs were conducted in a Leica DFC450 camera coupled to a stereoscopic Leica M205C. Mantle tissues were removed from eight specimens of *M. leucophaeata* and stored in 100% ethanol, for the genetic confirmation of the invasive lineage.

3. *Genetic procedures*

DNA was extracted with a Qiagen DNeasy Blood and Tissue kit, with the quality of the extraction measured through a Nanodrop 2000 spectrophotometer. We followed the procedures of Fernandes et al. (2021) for the partial amplification of the COI (cytochrome c oxidase subunit 1) gene, using primers HCO2198-LCO1490 (Folmer et al. 1994) and the thermocycling profile: initial denaturation at 95°C (5′); 37 cycles of denaturation at 95°C (45″), annealing at 48°C (45″), extension at 72°C (1′30″), followed by a final extension at 72°C (5′). Sequences were merged in *contigs* using the software MEGA 7, and aligned with MUSCLE. The eight new COI sequences were compared with 23 sequences of *M. leucophaeata* and three sequences of *Mytilopsis* cf. *sallei* (Récluz, 1849) retrieved from the GenBank, with a final alignment of 658 nt. Trees were generated by Bayesian Inference (MrBayes 3.2.7) and Maximum Likelihood (PhyML 3.0), following the procedures in Fernandes et al. (2021).

4. *Ecological analyses*

The species richness associated to the agglomerates of *M. leucophaeata*, as well as diversity (Shannon-Weaver, H') and equitability (Pielou, J') of the associated fauna were compared between the three sites where agglomerates were sampled. The density of *M. leucophaeata* specimens per class size was calculated for each of the three sites where agglomerates were sampled. The spatial abundance of *M. leucophaeata* was interpolated (Inverse Distance Weighted – IDW) regarding the distance to sampled sites, in a map elaborated in a GIS software.

Results

The single estuary in which *Mytilopsis leucophaeata* was found is the Maricá-Guarapina lagoon system, more specifically at the Maricá and Barra lagoons, with a few live individuals found near the connection between Barra and Padre lagoons (Figures 1–2; Appendix 2). At the Padre Lagoon, we found only empty valves of *M. leucophaeata*, whereas at the Guarapina Lagoon there were no traces of the invader. The maximum salinity measured at this lagoon system during the sampling event was 17.3 ppt, with a minimum of 2.2 ppt (Appendix 2). Live specimens of *M. leucophaeata* were observed in different habitats in the lagoon system, such as bridge pillars, wood and PVC (polyvinyl chloride) decks, rocks and even at the soft sediment (Figure 3). At stations 16–17, where live specimens of *M. leucophaeata* were observed in the soft sediment, they were originally adhered to small pieces of pebble and empty valves of *Anomalocardia flexuosa* (Linnaeus, 1767) (Figure 3F-G), with a subsequent colonization and formation of agglomerates above the small nucleus of *M. leucophaeata*. Owing to the scarcity of mangrove trees in this area, we could not search specimens of *M. leucophaeata* in this substratum.

The invasive bivalves at the Maricá-Guarapina lagoon system reached a maximum shell length of 28.1 mm, near the record of this

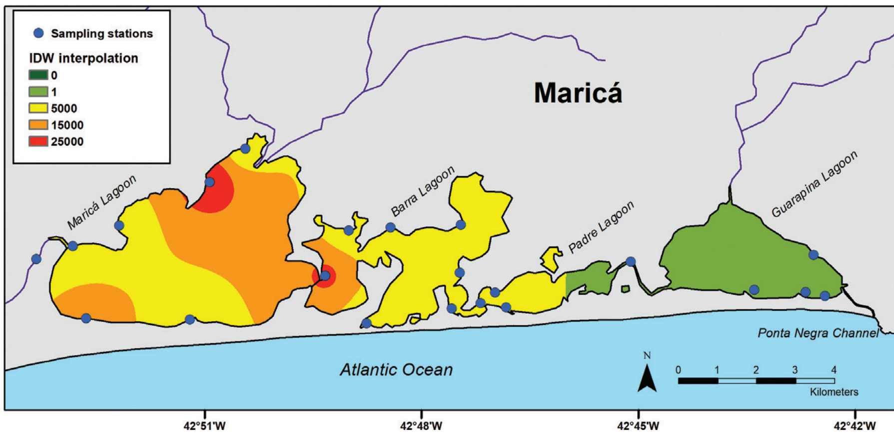

Figure 2. Interpolated density of *Mytilopsis leucophaeata* in the Maricá-Guarapina lagoon system.

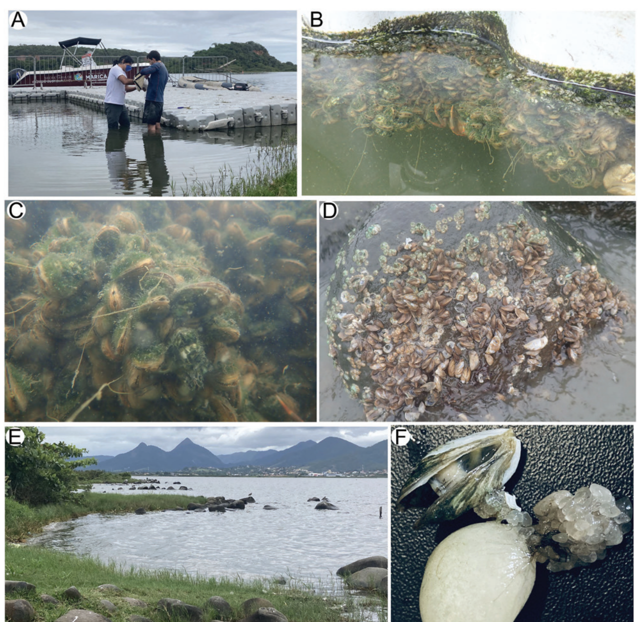

Figure 3. Sites of occurrence of *Mytilopsis leucophaeata* at the Maricá-Guarapina lagoon system; PVC deck – sta. 14 (A-C), rocks – sta. 21 (D-E), soft sediment – sta. 17 (F-H).

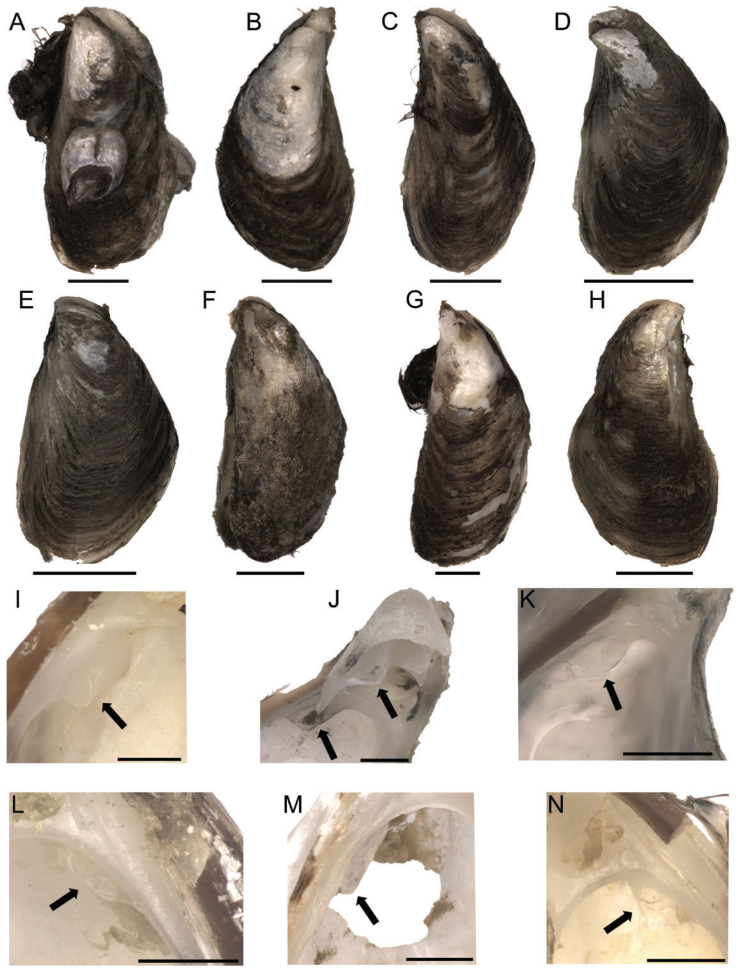

Figure 4. A-H. Specimens of *Mytilopsis leucophaeata* with DNA sequenced, from the Maricá-Guarapina lagoon system; sta. 17 (A-C), sta. 19 (D-E), sta. 21 (F-H). I-N. Apophysis (indicated by black arrows) from specimens illustrated in Figures A-B, D, F-H, respectively. Scale bars: A-H, 5 mm; I-N, 1 mm.

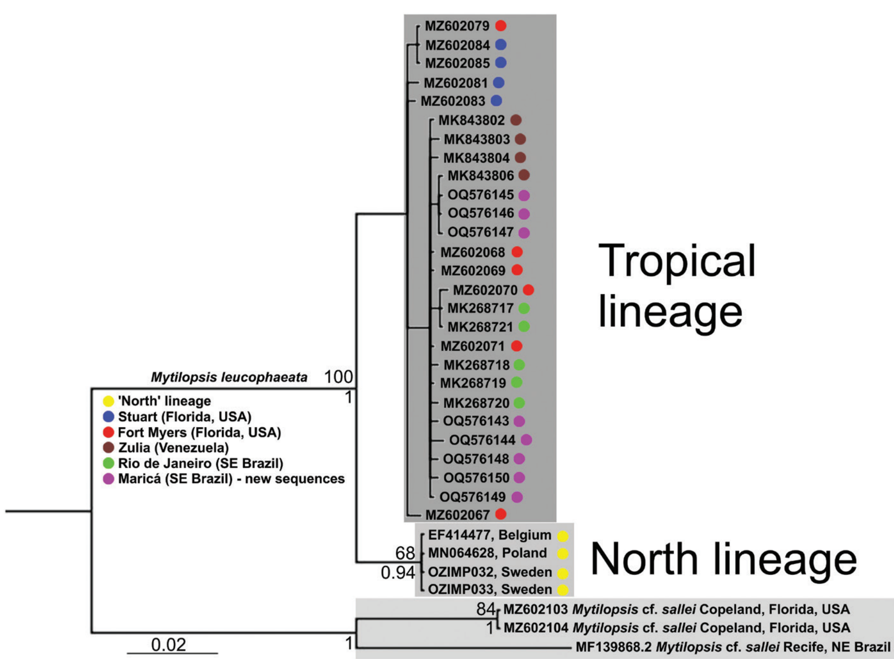

Figure 5. Tree generated by the Bayesian Inference, based on COI sequences. Bootstrap and posterior probability indexes situated respectively above and below nodes.

species, 31.7 mm (Maia-Neto et al. 2020). Shells usually had a nearly rectilinear profile (Figure 4), but some shells had a slightly curvilinear profile, broad, which is somewhat similar to the pattern of *Mytilopsis* cf. *sallei* (Fernandes et al. 2018). Juveniles of *M. leucophaeata* show a typical zigzag pattern in the shell (Fernandes et al. 2020), whereas older specimens become brownish or whitish due to the loss of the periostracum (Figure 4). The apophysis had different morphologies, such as oblong or pointed (Figure 4I-N).

The genetic investigation corroborated the identification of the eight specimens from the Maricá-Guarapina lagoon system as *M. leucophaeata* (Figure 5). Such sequences nested within the 'tropical lineage', together with the native populations from Florida (USA) and the invasive ones from South America (Venezuela and Rio de Janeiro), but apart from the 'North lineage', which shows a single COI haplotype present from NE USA, Europe and Caspian Sea (Fernandes et al. 2018, 2021).

The benthic macrofauna associated with the agglomerates of *M. leucophaeata* in the Maricá-Guarapina lagoon system was composed of 3,413 specimens belonging to seven taxa (Figure 6; Table 1): the cryptogenic *Amphibalanus improvisus* (Darwin, 1854) (Cirripedia), *Sinelobus stanfordi* (Richardson, 1901) (Tanaidacea) and *Alitta succinea* (Leuckart, 1847) (Polychaeta); and the native *Cassidinidea fluminensis* (Mañe-Garzón, 1944) (Isopoda), *Neohelice granulata* (Dana, 1851) (Brachyura), *Melita* cf. *lagunae* (Oliveira, 1953) (Amphipoda) and *Heleobia australis* (d'Orbigny, 1835) (Gastropoda).

Most taxa were found in the three sampling stations (Table 1). The station 14 (PVC deck) had the highest number of associated species, however most of them with very low abundances, except *Heleobia australis*, which on the other hand was absent from station 21 (rocks). In addition to this snail, the barnacle *Amphibalanus improvisus* also showed high abundances overall, except at the station 14 (PVC deck) (Table 1; Figure 7). Only one species (*Sinelobus stanfordi*) appeared in a single station. Station 14 had the lowest diversity and equitability values, whereas station 21 had the highest values.

Table 1. Abundance of the associated fauna within agglomerates of *Mytilopsis leucophaeata* at Maricá-Guarapina lagoon system, considering three replicates per station, as well as diversity (H'), equitability (J') and richness.

Species	Sta. 14 (PVC deck)	Sta. 17 (sediment)	Sta. 21 (rocks)
Sinelobus stanfordi	0	0	173
Amphibalanus improvisus	7	803	545
Alitta succinea	5	0	31
Heleobia australis	539	291	0
Melita cf. *lagunae*	7	84	290
Neohelice granulata	1	1	0
Cassidinidea fluminensis	1	53	25
Diversity (H')	0.2155	0.9457	1.185
Equitability (J')	0.1203	0.5876	0.7365
Richness	6	5	5

The highest mean density of *M. leucophaeata* was recorded at the station 14 (PVC deck), reaching 43,375 ± 46,537 specimens/m^2 (one replicate with up to 95,225 specimens/m^2), contrasting with 2,833 ± 823 specimens/m^2 and 5,925 ± 2,606 specimens/m^2 from stations 17 (soft

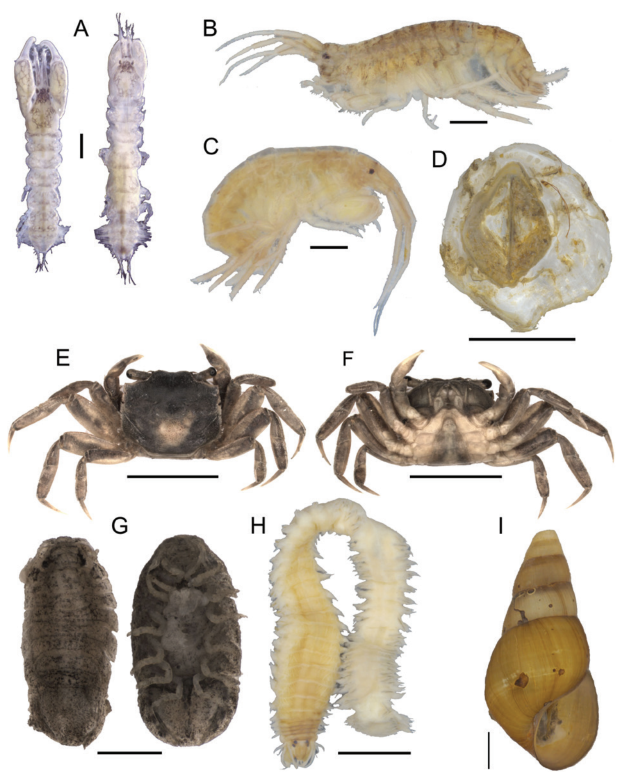

Figure 6. Benthic macrofauna associated with the agglomerates of *M. leucophaeata* in the Maricá-Guarapina lagoon system. A. *Sinelobus stanfordi*. B. *Melita* cf. *lagunae* (female). C. *Melita* cf. *lagunae* (male). D. *Amphibalanus improvisus.* E-F. *Neohelice granulata.* G. *Cassidinidea fluminensis*. H. *Alitta succinea.* I. *Heleobia australis*. Scale bars: A-C, G, I, 1 mm; D, H, 5 mm; E-F, 1 cm.

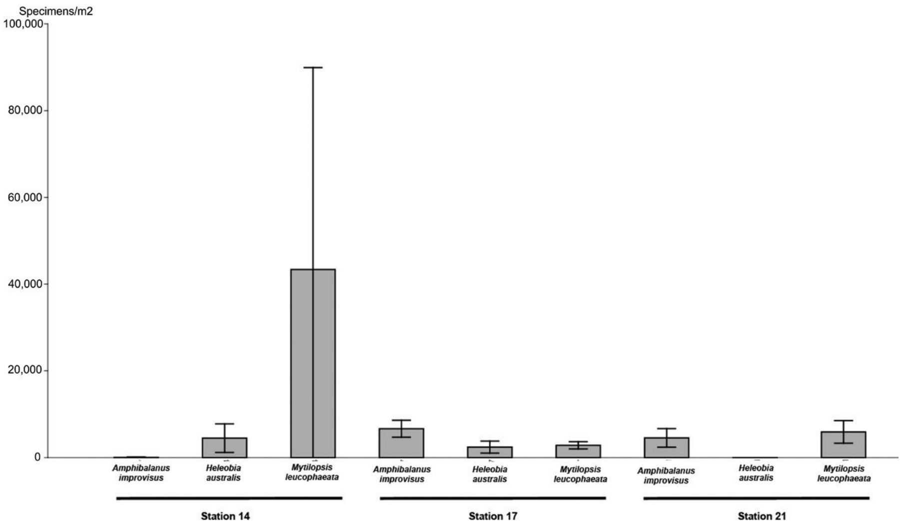

Figure 7. Density of *Mytilopsis leucophaeata, Amphibalanus improvisus* and *Heleobia australis* at the three stations in which agglomerates of the invasive bivalve were sampled in the Maricá-Guarapina lagoon system.

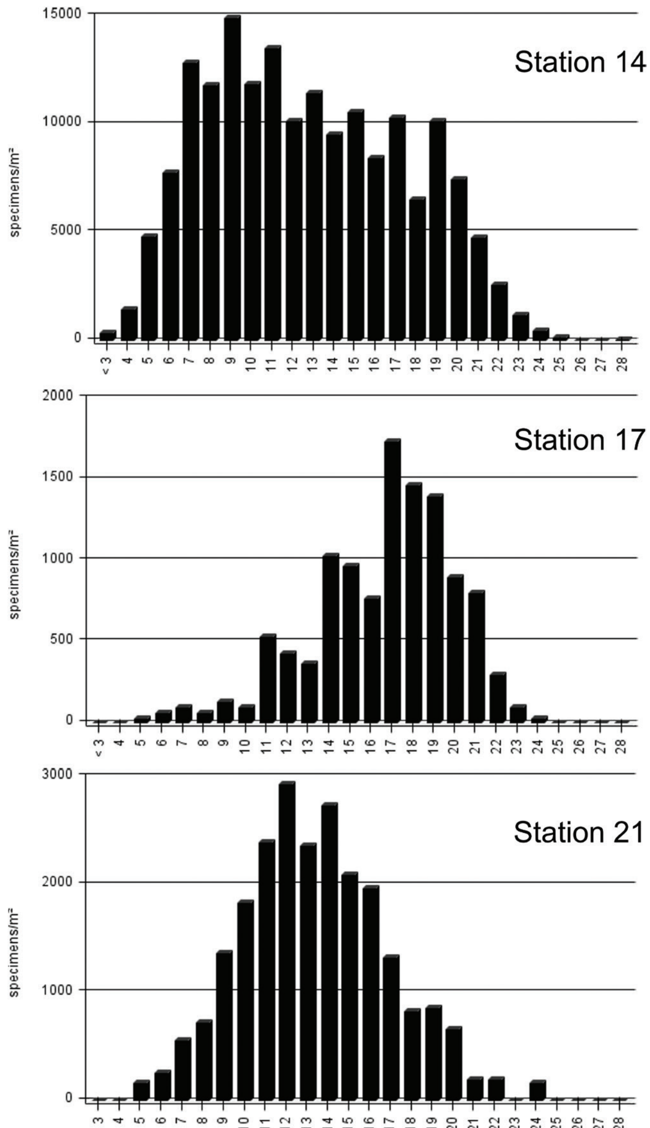

Figure 8. Density of *Mytilopsis leucophaeata* per size class (mm) at three sampling stations in the Maricá-Guarapina lagoon system.

sediment) and 21 (rocks), respectively (Figure 7). There is a large proportion of juveniles at station 14, instead of more adults and few juveniles at station 17 (Figure 8), regarding the size of 10 mm as conservative for the beginning of the reproductive stage for the 'North lineage' (Van der Gaag et al. 2020) and yet unknown for the 'tropical lineage'.

Discussion

In our studied area, some localities in the Rio de Janeiro state showed proper salinity levels for the establishment of *Mytilopsis leucophaeata*; however, this species was only found in the Maricá-Guarapina lagoon system, as a new record in addition to Rodrigo de Freitas Lagoon and Marapendi Lagoon (Rizzo et al. 2014, Fernandes et al. 2020). The measured salinities at Saquarema lagoon system and Jaconé Lagoon were respectively 7.7–20.4 and 2.2–5.4, apparently proper for the bivalve, i.e., below 18–21 ppt (Kennedy 2011, Fernandes et al. 2018, Maia-Neto et al. 2020). However, the Saquarema lagoon system may show remarkably different salinities according to the years, reaching a mean salinity of up to 33 ppt (Franco et al. 2019), which is influenced by the opening of the connection with the sea. Both estuaries do not have frequent circulation of boats from the Rio de Janeiro city (José Manoel P. Rebouças, pers. comm. – president of the Z-13 fishermen colony), especially the innermost Jaconé Lagoon. As expected, the freshwater condition of Jacarepiá Lagoon was inadequate for an introduction of *M. leucophaeata*. At the Guaratiba mangrove, the salinity varies between 27.3–29.1 ppt (Silva 2011) and 31.8–33.6 ppt (Neves et al. 2006) in the main canal and in Araçatiba, although we measured 19.9–27.4 ppt in the region (including rivers). The visited sites in Guandu River, Itaguaí and Angra dos Reis were mostly in the downstream portion of rivers, which show a higher hydrodynamics than lagoons, and might hamper the introduction of *M. leucophaeata* despite the frequent circulation of boats or even ships. In addition, salinity levels at these sampling sites were often inadequate for the bivalve (Appendix 2), and further fieldworks in more proper sites may be required.

At the Maricá-Guarapina lagoon system, there are no continuous records of salinity levels, and comparisons based on sporadic records are prone to variation in tides, seasonality, sites (e.g., close or away from rivers) and especially the opening of the main connection with the sea (Ponta Negra canal). We measured salinity levels of 16.5–17.3 ppt in Guarapina Lagoon (19 ppt in Guerra et al. 2011; 20–21 ppt in Ricevuto et al. 2013; 10–20 ppt in Laut et al. 2019), 12.9–13.8 ppt in Padres Lagoon (8 ppt in Laut et al. 2019), 10.8–13.8 ppt in Barra Lagoon (5–9 ppt in Guerra et al. 2011; 0.5–3.1 ppt in Laut et al. 2019) and 4.5–9.1 ppt in Maricá Lagoon (0 ppt in Guerra et al. 2011; 6–10 ppt in Ricevuto et al. 2013; 0.1–1 ppt in Laut et al. 2019); Franco et al. (2019) measured 8–25 ppt (winter) and 8–34 ppt (summer) in the entire lagoon system. From the recorded salinities, Guarapina Lagoon is indeed too close to the upper limit of tolerance of *M. leucophaeata*, despite considerable reductions in some events (Laut et al. 2019). The salinities measured at Padres Lagoon seem proper for the colonization of *M. leucophaeata*, as the existence of empty valves indicate such presence, but no live specimens were found. Live specimens were only found at Barra and Maricá lagoons, which showed ideal salinity levels at the sampling event, but Maricá Lagoon showed an inadequate freshwater condition in other events (Guerra et al. 2011, Laut et al. 2019). The single sampling station in the Brejo da Costa canal (a drainage canal connecting to Maricá Lagoon) had no invasive bivalves, with a reduced salinity of 2.2 ppt (Appendix 2) and few available hard substrata.

With the exceptions of stations 9 and especially 14, all other stations in Barra Lagoon showed only 0–3 live specimens of *M. leucophaeata* and hundreds of empty valves (Appendix 2). The abundance of dead specimens with articulated valves suggests the mortality events were recent. This might have been caused by sporadic openings of the Barra da Emergência canal, which was opened in 2010 and 2016 due to heavy storms, and systematically in February/March of 2019, 2020 and 2021 in order to increase the oxygen levels of the lagoon system (Maricá 2021), but also increasing salinity levels. According to local fishermen, this bivalve is common in the lagoon system since at least 2019, which might be related to the opening of this canal. There is a considerable flow of fishermen boats between the Maricá-Guarapina lagoon system and the invaded sites of *M. leucophaeata* in Rio de Janeiro city (José Manoel P. Rebouças, pers. comm.), i.e., the lagoons Marapendi and Rodrigo de Freitas, which are suggested to be the source of this secondary dispersion to the lagoon system. The distance by the sea between Rodrigo de Freitas Lagoon and Maricá Lagoon is only of ~41 km (Barra da Emergência canal) and ~53 km (Ponta Negra canal), which are covered in a few hours by a small boat.

The artificial substrate at station 14 (a mobile PVC deck) had by far the highest abundance of *M. leucophaeata* in the lagoon system (Figure 7). According to the Maricá prefecture (pers. comm.), this structure is used during studies of water quality in the region; however, we could not obtain records of when the deck was settled and whether it was allocated in other sites of the lagoon system (or outside of it). This high abundance confirms the preference of this species for hard and wide surfaces situated considerably far from the sediment; because they are filter feeders, the proximity with the soft sediment may be harmful (Fernandes et al. 2020). Station 14 showed a large proportion of juveniles (Figure 8), which indicate that the gregarious behavior of larvae are still acting even in such dense agglomerates. The scarce abundances of the associated species in this station (except by the snail *H. australis*) and the consequent reduced diversity/equitability raise suspicious whether the PVC deck was recently moved and colonized by the associated fauna. The agglomerates of *M. leucophaeata* in the soft sediment of Maricá Lagoon were sparse and did not form a reef, such as observed in Marapendi Lagoon (Fernandes et al. 2020); the bivalves at station 17 were mainly adults, with few cohorts of juveniles, suggesting these agglomerates may not prosper in this inadequate habitat.

Another factor that may influence the local density of *M. leucophaeata* in the Maricá-Guarapina lagoon system is organic pollution, which is considered high or much high in the estuary and rivers that discharge in it (INEA 2021b; using data from 2012–2021). For example, we are unaware whether the scarcity or even absence of live specimens of *M. leucophaeata* at stations 20 and 22 were caused by excessive pollution (the former situated near a sewage exit, the latter at the mouth of a polluted river), or whether the availability of hard substrata in both sites is too recent for the colonization of *M. leucophaeata*.

The fauna associated to the agglomerates of *M. leucophaeata* in the Maricá-Guarapina lagoon system is similar to those obtained in the agglomerates from Marapendi and Rodrigo de Freitas lagoons (Fernandes et al. 2020, Rodrigues et al. 2021), with the shared presence of *Alitta succinea*, *Amphibalanus improvisus*, *Melita* spp. and *Heleobia*

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spp. The mud crab *Eurypanopeus dissimilis* (Benedict & Rathbun, 1891) identified from these two estuaries was absent from the agglomerates in Maricá-Guarapina, which otherwise housed two specimens of *Neohelice granulata*. Similarly to Marapendi Lagoon and contrary to Rodrigo de Freitas Lagoon, there were no native mussels *Brachidontes darwinianus* (d'Orbigny, 1842) in Maricá-Guarapina. Similarly to Rodrigo de Freitas Lagoon and contrary to Marapendi Lagoon, there were no shell-boring polychaetes *Polydora* sp. in the agglomerates from Maricá-Guarapina, but there were *Cassidinidea fluminensis* and *Sinelobus stanfordi*.

Together with the proper salinity gradient, the anthropic pressure at Maricá-Guarapina is the main cause for the well-succeeded invasion of *M. leucophaeata* there due to the environmental fragility and reduced biological competition in this estuary, adequate for new invasions. The constant investigation about the expansion range of invasive species and their interactions with the local fauna is essential to understand the impacts in the ecosystem. More rigor in the surveillance of invasive species and a better interlocution between public, private and nonprofit agencies are required.

Supplementary Material

The following online material is available for this article:
Appendix 1.
Appendix 2.

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Author Contribution

Clarisse Duarte da Rocha: conducted the fieldworks; sorted, counted and photographed the specimens at laboratory; created figures and drafted the initial version of the manuscript; contributed to the final version of the manuscript.

Maurício Romulo Fernandes: designed the project; conducted the fieldworks; sorted, counted and photographed the specimens at laboratory; performed the genetic procedures; created figures and drafted the initial version of the manuscript; contributed to the final version of the manuscript.

Igor Christo Miyahira: designed the project; conducted the fieldworks; created maps (Figures 1–2); provided financial support; contributed to the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data availability

DNA sequences are stored in the GenBank database under codes OQ576143-OQ576150. The fauna associated to 'sururu' (*Mytella strigata*) mussels in Guaratiba (Appendix 1) and data related to fieldwork (Appendix 2) are available at the Biota Neotropica Dataverse, respectively under the links: https://doi.org/10.48331/scielodata.9UCE2U and https://doi.org/10.48331/scielodata.USSCCU.

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