Research Article

Invasion of the dark false mussel in shrimp farms in Venezuela: species identification and genetic analysis

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Abstract

An inspection was carried out at shrimp farms located South West of Lake Maracaibo (Zulia State, Venezuela), with high incidences of mussel bivalve. Morphological and genetic analysis helped to identify the species as the dark false mussel *Mytilopsis leucophaeata* Conrad, 1831, and this is the first record of the species from tropical waters of northern South America. The highest incidences of mussels were detected in ponds and channels but no live mussels were observed in the coastal intertidal area surrounding the entrances of the farms, although empty shells were detected there, suggesting their former presence. The environmental conditions of the artificial system of shrimp culture, is a niche suitable for the proliferation of the bivalve. The consequences of the presence of this bivalve in the production of shrimp are discussed.

Key words: Dreissenidae, invasive alien species, bivalve mollusks, shrimp culture

Introduction

In aquatic ecosystems, bivalve mollusks are one of the most invasive groups. They can cause environmental disturbances due mainly to their high filtering capacity, which can produce quantitative and qualitative changes in the plankton, causing an imbalance in the food chain and thus a high clearance of water. Bivalves also produce feces and pseudofeces that can alter the biogeochemical cycles and shells can change the physical properties of sediment (Strayer 1999; Ruesink et al. 2005).

Ponds and channels in shrimp farms are artificial ecosystems characterized by high loads of nutrients and organic matter in suspension, including phytoplankton. Optimal conditions for factors such as salinity, temperature, concentrations of dissolved oxygen, turbidity and phytoplankton, should be maintained for proper shrimp development

(Cuéllar-Anjel et al. 2010). However, optimal maintenance of balanced ecosystem conditions can be altered when invasive bivalves overcome these barriers and find favorable niches in the shrimp farms for colonization and establishment. This can lead to low yields of shrimp and significant economic losses (Aldridge et al. 2008).

This study resulted from the observation of low performance in shrimp production in cultivation ponds located in areas adjacent to Lake Maracaibo, in Zulia State, Western Venezuela, associated with the invasion of mussels (36% loss of normal production). The correct identification of the invasive species and its incidence in the shrimp farm allowed us to describe its potential impact on shrimp farming and to discuss possible invasion control measures.

Materials and methods

Location and characteristics of the shrimp farms

The inspected shrimp farms are contiguous and located in the South Western side of Lake Maracaibo (Zulia State, Venezuela), in the vicinity of Villa del Rosario de Perijá (~ 10°08′N; 71°57′W). The two farms are composed of several culture ponds that represent an area of approximately 2000 ha, with semi-intensive farming system (using densities of 8–25 shrimp/m^2) with productions close to 2000–3000 kg/ha per cycle, with 2.5 crop cycles per year. The area is characterized by mangroves in the coastal zone, and continental vegetation inland, mainly tropical dry to semi-dry forest. The rainy season, whose intensity and frequency is variable, often occurs between April and December. Farms have partial water recirculation systems and perform daily water exchange in the range of 5 to 7%; water is obtained from the lake, whose salinity ranges between 3 and 6 PSU, to replace losses through evaporation and seepage.

A monthly record (data supplied by the shrimp farms) was taken of the main environmental factors monitored during 2017 in more than 500 ponds, which were within the farms under study. Temperature and dissolved oxygen were monitored daily, both in the morning and in the afternoon, while turbidity, pH and nitrites were monitored weekly or biweekly.

Characterization of the invasive species

A visual inspection of ponds and water channels was performed, primarily detailing the slopes of the banks and the water inlets and outlets in the ponds (Figure 1). The density of mussel populations in the ponds was quantified by estimating their number from 20 quadrants of 25 × 25 cm, arranged randomly in four harvested ponds (of 3 ha each). The specimens of the invasive species were counted and their antero-posterior axis length was measured with a digital caliper (0.01 mm of precision), to determine the size-frequency structure of the established populations.

Figure 1. Establishment of the invasive mussel in the ponds and different structures in shrimp farms.

From the sampled mussels, subsamples (n = 60) were taken for morphological identification according to descriptions of Marelli and Gray (1983), Mackie and Claudi (2010) and the identification key of freshwater mussels of Williams et al. (2014). Also, visual observations of the gonad were made to estimate reproductive status of the organisms.

A set of mantle tissue samples were preserved in 80% ethanol until DNA barcoding analysis. Total DNA extraction was performed with the "E.Z.N.A. Mollusk DNA Kit" (Omega Bio-Tek). Partial COX1 gene PCR products were obtained with the LCO1490/HCO2198 barcoding primers set (Folmer et al. 1994), purified with ExoSAP-It kit (Affimetrix) and sequenced using the BigDye kit v3.1 and X terminator in an ABI 3500 equipment (Thermo Fisher Scientific).

Sequences were edited, aligned and distances were estimated with the software MEGA 7.0.26 (Tamura et al. 2013). BLAST (http://blast.ncbi.nlm.nhi.gov) and BOLD (www.barcodinglife.org) were used to search for available homologous sequences similar or identical to the detected haplotypes. The molecular-based taxonomy of analyzed samples was evaluated by their position within species-specific clades obtained by phylogenetic inference. Thus, an alignment of 137 COX1 partial sequences from *M. sallei* and *M. leucophaeata*, obtained in the present work and mined from GenBank (Table 1), was used to resolve evolutionary relationships involving these species lineages. A phylogenetic tree was inferred MEGA 7.0.26 (Tamura et al. 2013) using the neighbor-joining algorithm and based in Tamura-Nei distances. To visualize haplotype relationships and frequencies a Median-joining network (Bandelt et al. 1999) was constructed with PopArt 1.7 (Leigh and Bryant 2015).

Table 1. Location, GenBank accession number, and authority for COX1 *Mytilopsis leucophaeata* sequences analyzed in the present study.

Area	Location	Accession No.	Reference
North America	USA	MLU47649	Baldwin et al. 1996
	Maryland Chesapeake Bay, USA	KU905843	unpublished
	Maryland Chesapeake Bay, USA	KU905894	
	Maryland Chesapeake Bay, USA	KU905989	
	Maryland Chesapeake Bay, USA	KU905904	
	Maryland Chesapeake Bay, USA	KU906056	
Europe	Sevilla, Spain	MF401396	Clusa et al. 2017
	Antwerp, Belgium	EF414477	Albrecht et al. 2007
	Antwerp, Belgium	HM100251	Heiler et al. 2010
	Kiel Canal, Germany	HM100253	
	Kiel Canal, Germany	HM100254	
	Ukraine, Southern Bug,	HM100255	
Asia	Caspian Sea, Iran	HM100255	Heiler et al. 2010
		HM100257	
		HM100258	
		HM100259	
		HM100260	
South America	Rio de Janeiro, Brazil	MF139836–MF139866	Fernandes et al. 2018
	Lake Maracaibo, Venezuela	MK843806/VZ05	This Study
		MK843803/VZ06	
		MK843802/VZ07	
		MK843802/VZ09	
		MK843804/VZ23	
		MK843805/VZ24	

Results and discussion

Both shrimp farms showed a high incidence of mussels in the drainage channels, grow-out ponds and reservoirs. They were attached to various solid substrates (shells, concrete and metal structures, mesh, wood, plastic, stems of the grass *Brachiaria* sp., etc.) and particularly on concrete in inlet and outlet structures of ponds, as well as on aerator equipment inside the ponds (Figure 1). The highest incidence of mussels was observed in ponds and channels. In the intertidal coastal zone, next to the farm's entrance, no live mussels were observed, although there were empty shells, suggesting their former presence in this area.

Taxonomic identification

The invading species was identified as *M. leucophaeata*, based on the ratio of the maximum antero-posterior to dorso-ventral length of the shell smaller than 1.3 mm (population mean of 1.27 ± 0.097 mm), which gives an elongated appearance and short in height. This feature, together with the absence of dorsal curvature of the shell, presenting the *septum* dorsally curved and with clefts, as well as the apophysis associated to *septum* and wide and long muscle scar in the shell (Figure 2), defined morphologically the studied specimens as dark false mussel, *M. leucophaeata* (Marelli and Gray 1983). The morphological-based species identification was corroborated by genetic analysis. We obtained a set of 6 partial COX1 sequence 658 bp
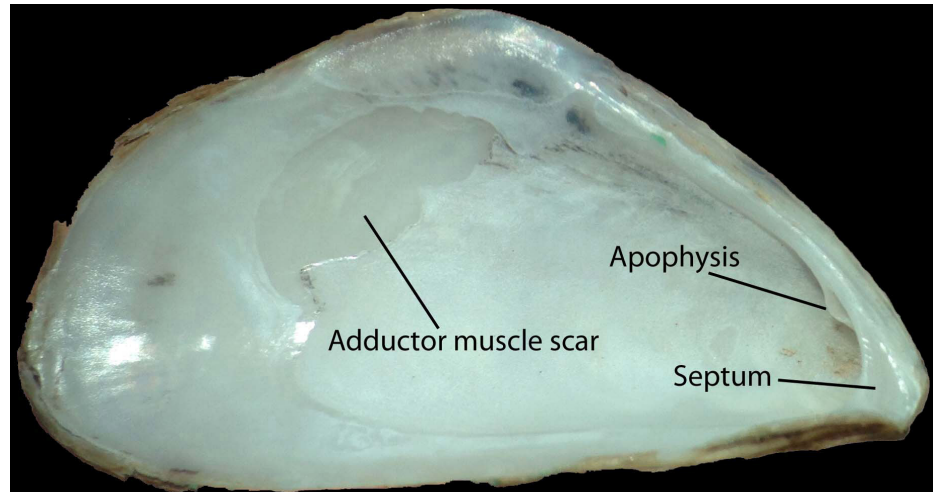
Figure 2. Shell of *Mytilopsis leucophaeata* from this study, showing the main morphological characteristics for its identification.

length and 5 distinct haplotypes. Two haplotypes (VZ05:MK843806, VZ07/VZ09:MK843802) were identical to haplotypes assigned to *M. leucophaeata* species, recently deposited in the GenBank/BOLD database and sampled at Rio de Janeiro (Brazil) (Fernandes et al. 2018). The other 3 distinct haplotypes showed pairwise identity values > 99.7% to *M. leucophaeata* sequences, a typical value expected from intraspecific comparisons. The mean genetic *p*-distance among these sequences was low, only 0.005 (S.E. = 0.001).

A single invasive haplotype has been detected in North America (Hudson River) (Baldwin et al. 1996), Belgium (Antwerp) (Albrecht et al. 2007), Germany (Baltic and North Sea), Moldova, Ukraine, Russia (Black sea), Iran, Russia, Kazakhtan (Caspian Sea) (Zhulidov et al. 2018) and Spain (Clusa et al. 2017). Its mean pairwise identity respect to Venezuela (this study) and Brazil (Fernandes et al. 2018) haplotypes (Table 1) was 97.5% and the net *p*-distance between the two haplotype clusters was 0.021 (S.E. = 0.001).

The mean intra-specific distance from all available data within *M. leucophaeata* was 1.2%, a value close to the mean intra-specific value (0.96%) estimated from 2,801 sequences in 569 species of Pacific mollusks (Sun et al. 2016). In contrast, within the congeneric *Mytilopsis sallei*, the mean intra-specific value was higher (5%) with the presence of particularly divergent lineages (*p*-distance = 0.1).

The neighbor-joining tree supported the monophyly of *M. leucophaeata* (99% bootstrapping value) and exhibited two intra-specific clades, one of them represented by the single invasive haplotype (95%), and the other including samples from Brazil and Venezuela (68%) (Figure 3A). The haplotype network displays a star-conformation for the Brazil/Venezuela clade, suggesting a recent demographic expansion, and shared common haplotypes for the Brazilian and Venezuelan samples (Figure 3B). These results likely suggest a common origin for the invasive colonization at
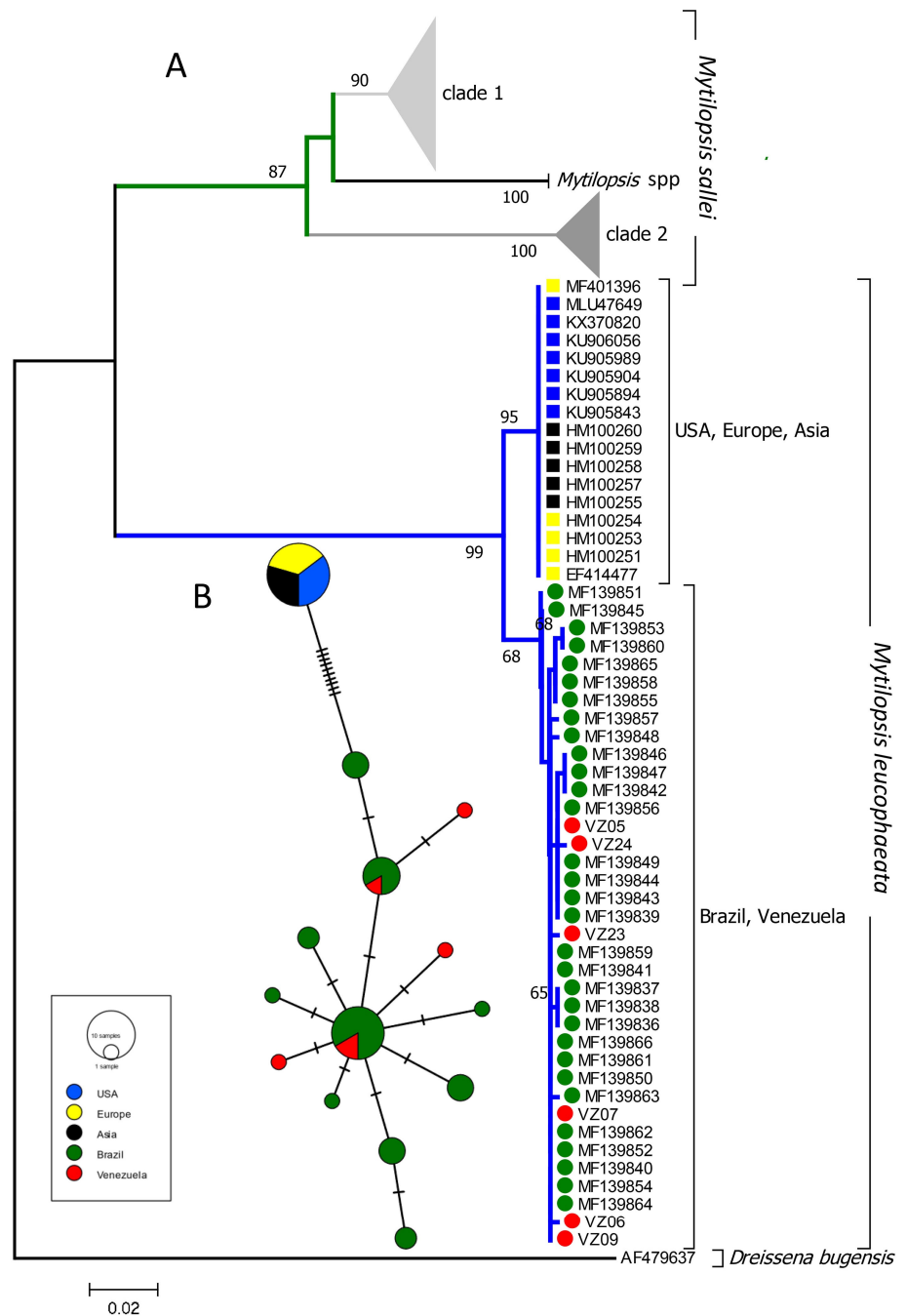
Figure 3. A. Neighbor-joining tree for *Mytilopsis sallei* and *Mytilopsis leucophaeata* estimated from a 439 bp alignment of available COX1 sequence data mined from Genbank and sequences generated here from Venezuela. It was based in Tamura-Nei (1993) distances, with a shape parameter of the gamma distribution = 0.39. Clade support was estimated by bootstrapping (2000 replicates) and are shown (if > 65%) next to the nodes. As an outgroup *Dreissena bugensis* was included. Location specific colors are detailed in legend, including red color coding for present study samples. B. Haplotype network using a median joining algorithm from the *Mytilopsis leucophaeata* COX1 sequence alignment. Each geographically color-coded circle represents a single haplotype, whose area is proportional to the haplotype frequency. The hatch marks in the connecting lines indicate the number of mutational steps between haplotypes.

Rio de Janeiro and Maracaibo at the south of its native range and the disassociation from those invasive events involving the northern haplotype.

A main drawback of the evolutionary analysis of detected clades is the absence of sequence data from the native geographic range suggested for
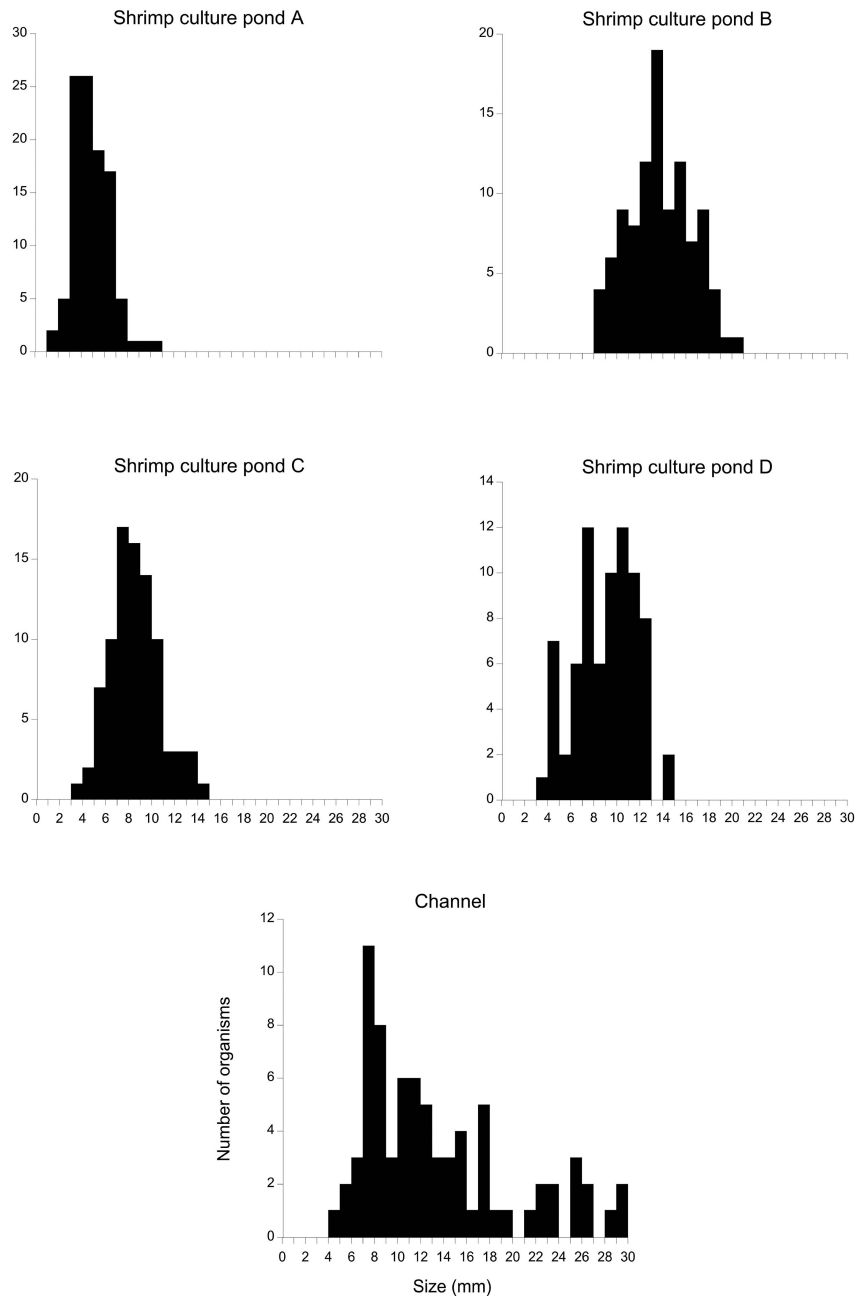
Figure 4. Size structure (antero-posterior length, mm) of the invasive mussel in samples from the water supply channel and 4 different ponds of the shrimp farm (Ponds A to D).

M. leucophaeata. Nevertheless, the false mussels found here in aqueous systems of Lake Maracaibo shrimp farms belong to *M. leucophaeata* and therefore this constitutes the first record of the species in waters of northern South America.

Density, size distribution and onset of mussel reproduction in shrimp ponds

In ponds analyzed, a coverage of the mussel aggregations of 10–30% was estimated, and population density in the aggregations averaged 13,200 ± 1,905 individuals/m^2, with densities that exceeded 20,000 individuals/m^2.

The distribution of sizes in the different places where mussels were collected in the shrimp farms (Figure 4), showed several modes, suggesting

Table 2. Monthly average (± SD) of physical and chemical parameters from water in cultivation ponds during 2017. Transparency was estimated with Secchi disc depth.

Month	Temperature a.m. (°C)	Temperature p.m. (°C)	Oxygen a.m. (mg/L)	Oxygen p.m. (mg/L)	Transparency (cm)
January	27.8 ± 0.8	29.7 ± 1.2	3.5 ± 0.3	9.5 ± 0.5	44.5 ± 37.0
February	27.9 ± 0.7	29.9 ± 1.1	3.6 ± 0.4	9.5 ± 0.5	31.2 ± 16.2
March	27.9 ± 0.8	29.7 ± 1.0	3.5 ± 0.3	9.5 ± 0.6	32.6 ± 15.3
April	29.4 ± 0.9	31.3 ± 1.5	3.4 ± 0.3	9.4 ± 0.6	35.5 ± 16.9
May	30.3 ± 0.8	32.1 ± 1.4	3.4 ± 0.4	9.2 ± 0.8	32.8 ± 15.1
June	30.2 ± 0.6	31.8 ± 1.2	3.4 ± 0.4	9.1 ± 0.9	34.9 ± 18.1
July	30.6 ± 0.6	32.3 ± 1.4	3.5 ± 0.5	9.2 ± 0.8	36.5 ± 16.8
August	30.6 ± 0.6	32.3 ± 1.3	3.5 ± 0.4	9.1 ± 0.8	35.4 ± 15.0
September	30.5 ± 0.6	32.3 ± 1.4	3.4 ± 0.6	9.1 ± 0.7	28.4 ± 10.0
October	30.1 ± 0.8	31.8 ± 1.5	3.5 ± 0.4	9.1 ± 0.8	23.5 ± 6.30
November	30.1 ± 0.8	31.9 ± 1.5	3.5 ± 0.4	9.1 ± 0.8	23.5 ± 6.3
December	28.7 ± 0.8	30.8 ± 1.3	3.5 ± 0.4	9.5 ± 0.9	25.0 ± 7.7

different times of growth and invasion. The channel showed a wider population structure indicating different recruitments. Unfortunately, for this analysis there were no records of the time when the ponds were filled up; only pond A, which had been in operation for 36 days. This pond showed a unimodal distribution of small size mussels with an average length of 4.9 ± 1.53 mm. The larval development of the species takes place within 6–8 days (Siddall 1980), estimating about 30 additional days to reach a length of 4 mm, which suggests the larvae invaded the ponds at the beginning of pond seeding with shrimp. The first size of organisms forming mature gonads is 8–10 mm (Mackie and Claudi 2010), which could be reached in about 50 days, demonstrating that the mussel can reproduce several times within the culture pond, because the shrimp culture cycle is between 90 and 120 days.

The formation of reproductive tissue in small-sized individuals was verified; gonad tissue was observed in mussels of 14–16 mm. Most individuals had little or no reproductive tissue, indicating low reproductive activity at the time of sampling.

Environmental factors in shrimp farms

Salinity is not recorded on a regular basis in the sampled shrimp farms, as are the other factors listed in Table 2. However, values on average of 4.8 PSU were observed, within an interval of 3 to 6.2 PSU during 2017. The technicians of the shrimp farm suggest an association of the presence of dark false mussels with consecutive droughts, which could have allowed a higher salinity in the shrimp farms, facilitating the proliferation of the invasive species, which can support from freshwater (< 0.5 PSU) to marine conditions (Siddall 1980; Therriault et al. 2004). However, their populations normally occur in oligohaline to mesohalines conditions, in general ranges of 0.5–10 PSU (Kennedy 2011), which coincides with the normal salinity recorded in the evaluated shrimp farms and in Lake Maracaibo (annual range of 2–8 PSU; José-Bracho et al. 2016). Measurements of temperature, oxygen, and turbidity found in ponds (Table 2) were consistent with those

known to be appropriate systems for the proliferation of *M. leucophaeata* (Kennedy 2011).

The geographic distribution of *M. leucophaeata* (both native and invaded) ranges from locations of cold temperature in New England, USA (Hall 1964), to tropical areas such as estuarine systems in the State of Pernambuco in Brazil (de Souza et al. 2005; Farrapeira et al. 2007). According to these data and studies on temperature tolerance, the species can be considered an eurytherm (Kennedy 2011).

Conclusion and recommendations

The presence of *M. leucophaeata* reported in this study represents the first record of the species in tropical waters of northern South America. The species is an invader that can produce multiple effects on pond systems, and may contribute to decreases in the production of farmed shrimp. Studies on the biological cycle, population dynamics, reproduction, and growth, in association with environmental biotic and abiotic factors; as well as physiological effects of molluscicides or the use of biocontrols and possible mussel-shrimp interactions are recommended. In terms of management and operation of the shrimp farms, prevention and rapid control is desired, hence the early monitoring of larvae in the water and visualization of the invaders on substrates is recommended, particularly at the beginning of each growing cycle. Species identification with the help of molecular tools could raise the ability to detect, even at a larval stage, an incipient early invasion that may improve the chances of quick responses to eradicate or contain the spread of the invasive species.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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