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Rapid Communication

Non-native mud sleeper *Butis koilomatodon* (Bleecker, 1849) (Perciformes: Eleotridae) in Eastern Amazon Coastal region: an additional occurrence for the Brazilian coast and urgency for ecological assessment

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Abstract

Since 1989, specimens of the non-native mud sleeper *Butis koilomatodon* (Bleeker, 1849) (Perciformes: Eleotridae) have been collected off of the Brazilian coastline. The species has the potential to invade marine and estuarine systems, and outcompete native fish. The present study reports an additional occurrence of *B. koilomatodon* in the intertidal zone of Maranhão state, eastern Amazon coastal region of northeastern Brazil. Detailed environmental data was obtained at the site of specimen collection, and *B. koilomatodon* behavior *in situ* was documented. Ten individuals were sampled from three different mediolittoral tide pools at Panaquatira beach. Predominant substrate, percentage of algae coverage, surface area, tide pool volume and depth, roughness, pH, salinity, water temperature and dissolved oxygen were also documented. Ecological plasticity of analyzed individuals, associated with port handling behavior in neighboring bays of the studied area, suggest that the *B. koilomatodon* route of introduction was ship-mediated. High shipping intensity in this region may have favored introduction and possible invasive process of the species nearshore due to satisfactory propagule pressure. Nevertheless, more data are needed to comprehend the invasive potential of *B. koilomatodon* in Brazilian waters.

Key words: non-native marine fish, southwestern Atlantic, northeastern Brazil, intertidal zone, biological invasions, ballast water

Introduction

The Brazilian coast is among the most extensive (at about 8,600 km in length) and systems-diverse shorelines in the southern Atlantic. The area comprises three marine provinces (North Brazil Shelf, Tropical Southwestern Atlantic, and Warm Temperate Southwestern Atlantic) and eight marine ecoregions (Amazonia, São Pedro and São Paulo Islands, Fernando de Noronha and Atol das Rocas, Northeastern Brazil, Eastern Brazil, Trindade and Martin Vaz Islands, Southeastern Brazil, and Rio Grande) (Spalding et al. 2007), where mangroves, coral reefs, rocky shores, sand beaches and sea grass beds all can be found. However, researchers have only recently gained insight into marine benthic and nearshore diversity (MMA 2002; Miloslavich et al. 2011). Such research has started to fill in the gaps in local understanding of species distribution, endemic organisms and potential invaders (Ferreira et al. 2009; Ignacio et al. 2010). Nonetheless, this lack of knowledge is more evident in economically deprived regions, such as the state of Maranhão (northeastern Brazil) – which comprises more than 50% of the Brazilian Amazon coastal zone, and yet knowledge of

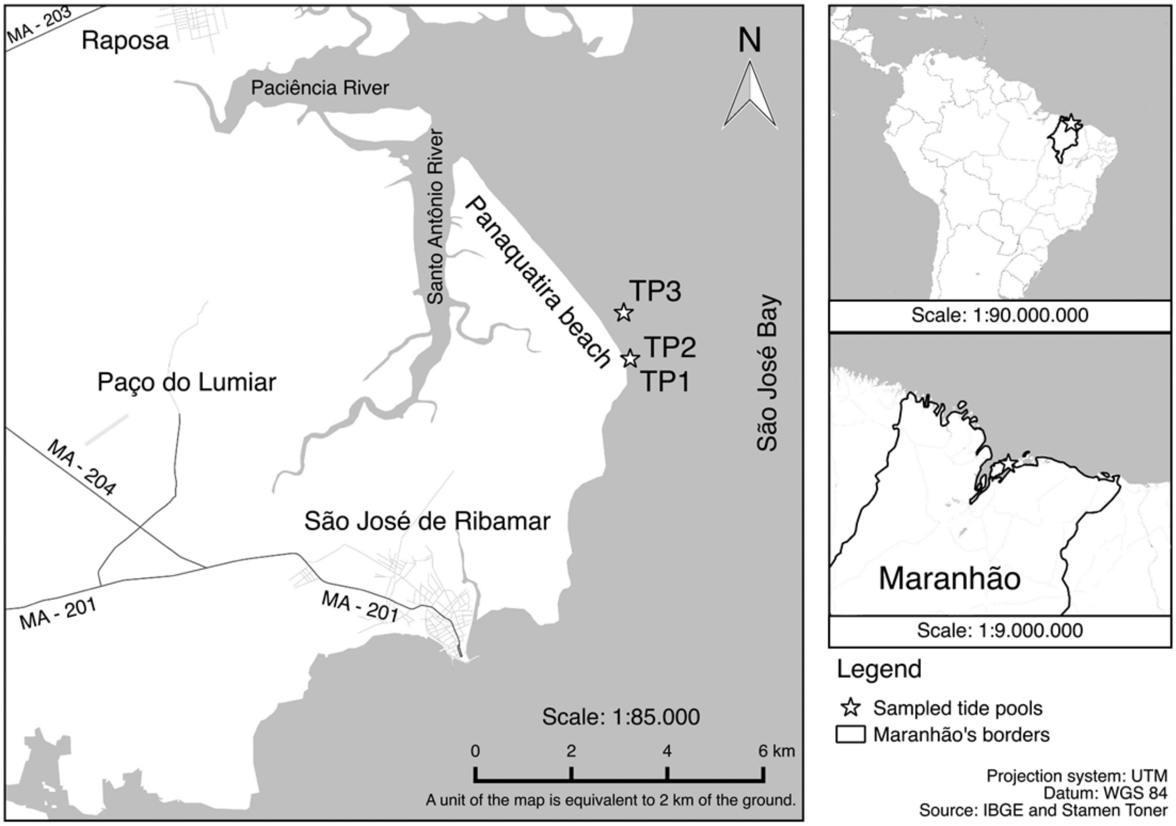

Figure 1. Location of three tide pools (TP 1, TP 2 and TP 3) where specimens of non-native mud sleeper *Butis koilomatodon* were found in intertidal zone in state of Maranhão, Brazilian Amazon coastal zone**.**

marine diversity is poor compared to other regions of the Brazilian coast. As a consequence of the combination of low interest in conducting surveys, low study effort, and accumulation of unpublished data (Miloslavich et al. 2011), the presence of non-native species is frequently overlooked (Marques 2011) and detection of introduced species in this region remains a challenge (Loebmann et al. 2010; Lasso-Alcalá et al. 2011).

Global shipping is a key driver of human-mediated dispersal of marine species across biogeographical barriers, oftentimes contributing to biological invasions and homogenization of coastal systems (Ruiz et al. 2000). Organisms can be accidentally captured during ballasting operations and/or get attached to the hull of ships, which then serve as vectors. Thus, vessels can significantly contribute to propagule supply of non-natives elsewhere, directly increasing the likelihood of invasions, especially in frequently visited ports (Drake and Lodge 2004) such as the port complex of Maranhão. The port complex of Maranhão (formed by Itaqui port, and private maritime terminals of Ponta da Madeira and Alumar) is the second largest site of cargo operations in Brazil, annually handling more than 126,000,000 tonnages of cargo, most of which is for international export (ANTAQ 2013). Given its economic importance and potential contributions for ship-borne biological invasions in coastal waters, it would be expected that Maranhão have a consistent monitoring system to look for non-native biota. In fact, it does not; only a handful of studies have attempted to investigate the presence of organisms in port waters and ballast tanks (Costa et al. 2014) or to look for new occurrences of potential marine invaders on the coast of Maranhão (Loebmann et al. 2010; Lasso-Alcalá et al. 2011).

The mud sleeper *Butis koilomatodon* (Bleeker, 1849) (Perciformes: Eleotridae) was first detected in the southwestern Atlantic in 2012 (Macieira et al. 2012). This species is thought to be native to the Indo-Pacific estuarine region, and individuals are able

Figure 2. Lateral and dorsal view of preserved specimen of mud sleeper *Butis koilomatodon* found in tide pools in state of Maranhão, Brazilian Amazon coastal zone. (Photographs by Nivaldo Piorski).

to live in environments with a wide range of water temperatures (26–36 °C) and salinity (3.8–37.0) (Miller et al. 1989; Contente et al. 2016). Previous studies showed the mud sleeper were common in the tide pools in the intertidal zone; they have limited capacity for continuous swimming, a sedentary life style, and a carnivorous diet (Contente et al. 2016). Small size and wide environmental tolerances may enable the fish to survive in ballast tanks during transoceanic voyages, and they may subsequently establish populations in new environments where introduced (Soares et al. 2012). Specimens of *B. koilomatodon* have been collected off of the Brazilian coastline (Macieira et al. 2012) and it is expected that it has the potential to invade coastal habitats and outcompete (for food and habitat) native fish, such as *Bathygobius soporator* (Valenciennes, 1837) (Contente et al. 2016).

In this study, we report an additional occurrence of *B. koilomatodon* on the Brazilian coast and first on the state of Maranhão, supported by detailed environmental data and *in* situ observations at the intertidal zone of Maranhão state, eastern Amazon coastal zone. Such expansion of potentially introduced range highlights that community and population assessment of this species in local marine ecosystems is urgently needed. These are important in determining population persistence in a species' invaded range, thus having profound implications for the management of non-native species, including the containment of spread and prevention of future introductions (Lasso-Alcalá et al. 2011; Luiz et al. 2013).

Methods

Specimens of *B. koilomatodon* were collected in August 2011 during an ichthyofaunal survey of the intertidal zone in the state of Maranhão. Sampling was carried out in tide pools (TP) on Panaquatira beach (São José de Ribamar city, north coast of Maranhão, Brazil) using a non-destructive method based on the application of an anesthetic solution of menthol and ethanol (40 $g.L^{-1}$) in pool waters to facilitate the manual capturing process (Nunes et al. 2011). The anesthetic solution was administered to three tide pools (TP 1: 2º30′27.61″S; 44º1′23.91″W; TP 2: 2º30′27.61″S; 44º1′23.91″W; TP 3: 2º29′56.60″S; 44º1′28.22″W) (Figure 1). *In situ*, environmental conditions were assessed to ascertain an accurate habitat description. Characterization of tide pools was carried out based on: sampling location within the intertidal zone; visual assessments of dominant substrates (based on type of sediment and grain size); estimates of surface area (3 × 1 m-grid of 10 × 10 cm squares placed over the pool, as in Macieira and Joyeux 2011); mean depth (distance to the bottom of 10 randomly chosen intersections of the grid); tide pool volume (surface area × mean depth); percentage of algae coverage (percentage of occupied area by algae in similar method to surface area estimates); roughness (calculated based on the chain-and-tape technique, Wilding et al. 2010); measurements of pH, salinity, water temperature and dissolved oxygen (multiparameter meter–HI 98194, Hanna instruments).

Captured individuals were tagged, standard length measured with calipers (precision of 0.1 mm) and

Table 1. Characteristics of three tide pools on Panaquatira beach where *Butis koilomatodon* specimens were found. (*) = average; TP = tide pool; TPL = tide pool location on intertidal zone; PS = predominant substrata; Alg = percentage of algae coverage (%); SA = surface area (m²); Vol = volume (m³); Dp = depth (cm); R = roughness; WTemp = water temperature (°C); Sal = salinity (g. kg^{-1}); pH and DO = dissolved oxygen (mg. L^{-1}).

	TPL	PS	Alg	SA	Vol	Dp	R	WTemp	Sal	pH	DO
TP 1	Upper Mediolittoral	Rock	50	6.5	0.17	8.7	1.1	29.9	30.8	8.3	6.5
TP 2	Upper Mediolittoral	Rock	40	11.8	0.83	11.8	1.0	29.3	31.6	8.1	7.3
TP 3	Middle Mediolittoral	Rock	80	4.2	0.70	12.3	1.1	30.3	30.8	8.2	9.1

weighed (precision of 0.1 g). Specimens were initially preserved in 4% formaldehyde solution, photographed (Figure 2), and identified to species level. Taxonomic keys and specialized literature were consulted for species identification (Miller et al. 1989, Macieira et al. 2012). Subsequently, animals were transferred to vials containing 70% ethanol solution and deposited in the Fish Collection at the Federal University of Maranhão (Universidade Federal do Maranhão), Brazil.

Table 2. Densities of *Butis koilomatodon* and other tide pool fish species on Panaquatira beach, State of Maranhão, Brazilian Amazon coastal zone.

	Tide pool	Individuals	Abundance (individuals.m^{-2})
Butis koilomatodon	TP1	1	0.15
	TP2	8	0.68
	TP3	1	0.24
Other species	TP1	25	3.85
	TP2	50	4.24
	TP3	80	19.05

Results

Ten individuals of *B. koilomatodon* were collected from the three tide pools on the same date (TP 1 – 1 specimen; TP 2 – 8 specimens; TP 3 – 1 specimen). All tide pools were located on the mediolittoral zone. TP 2 had the highest salinity, lowest temperature, greatest volume (Table 1), and highest density of *B. koilomatodon* individuals (0.67 ind.m^{-2}) and of other analyzed fish species (4.23 ind.m^{-2}) (Table 2). The average (± SD) standard length of the 10 specimens was 54.7± 14.1 mm with a mean (± SD) weight of 2.18 ± 1.47 g.

Discussion

The presence of *B. koilomatodon* in tide pools of the Maranhão coast could be a result of physiological adaptations to a wide range of water temperatures and salinity. Temperature in the pools reached from 29.0 and 33.3 °C, and salinity was between 30.8 and 31.6. This variation was similar to that reported in other regions of Brazil (Soares et al. 2013) and the world (Dawson 1973; Miller et al. 1989) in a variety of systems where *B. koilomatodon* has been found. The ability to tolerate a range of environmental conditions is a feature of invasiveness that allows for a good invasion performance. The mud sleeper's tolerance to salinity variation suggests that individuals could withstand long periods of adverse conditions, such as during transportation in ballast tanks, and quick adapt to different habitats, such as estuaries, mangroves, and pools in the intertidal zone. Thus, apparent environmental mismatching between the mud sleeper's native and introduced ranges may not constitute a barrier for species invasion and spread, including along the Maranhão coast.

A detailed habitat description is of critical importance to understand survival constraints of a given species, especially in intertidal communities (Cunha et al. 2008; Godinho and Lotufo 2010; Macieira and Joyeux 2011; Soares et al. 2013). Considering pools' characteristics presented here, we highlight the predominantly rocky substrate, suggesting usage by *B. koilomatodon* of small fissures in the rocks to escape predation or ameliorate effects of other environmental stressors, such as desiccation (Andrades et al. 2016). This cryptic behavior is common among small-sized intertidal fishes in Maranhão (Nunes et al. 2011; Soares et al. 2013) and may limit fish species presence and abundance in these systems due to competition for habitat usage. Based on this and other associated studies on the coast of Brazil, we believe *B. koilomatodon* has the potential to compete with native fish for habitat and prey, and perhaps change community composition and functioning.

The use of anesthetics during fish sampling reduces fish mobility, potentially trapping individuals in crevices in the rocks. Thus our abundance estimates (n = 10 specimens) likely are underestimates. Accurate assessments of population size would require additional active sampling that involves inspection of crevices in rocks (i.e. refuges) and, of course, considerably expanded survey area if studies are to be representative of the whole coastal region.

Specimens of *B. koilomatodon* were first collected in Brazilian waters in 1989 but remained unidentified for over 20 y (Macieira et al. 2012). Once the taxonomic resolution of the species became clearer and more specialists conducted ichthyofaunal surveys locally, the mud sleeper was reported in five states (Pará, Rio Grande do Norte, Sergipe, Bahia, Espírito Santo and São Paulo) within the same year (Macieira et al. 2012; Soares et al. 2012). This study fills in the large spatial gap that previously existed in species introduced range in northern Brazil - between Pará and Rio Grande do Norte - and raises the possibility that mud sleepers may also be present in many other sites in Amazon coastal region, besides Maranhão. We think the lag time to being reported and gaps in spatial distribution are not necessarily related to the species' establishment process, but are mainly due to undersampling, and logistic issues. Thus, if investigated thoroughly, the invasive history of *B. koilomatodon* might provide invaluable insights into the mechanisms by which the species was introduced and whether the populations are presently contained or spreading.

The way in which *B. koilomatodon* reached the Brazilian coast is unknown (Lopes 2009). However, the fact that most specimens have been captured near ports suggests that their transport may be ship-mediated (Macieira et al. 2012). In 2012, more than 900 million t of cargo passed through Brazilian ports. Indeed, Brazil is one of the largest exporters of ore and oil in the world. More than 14% of the export tonnage was handled in the state of Maranhão (ANTAQ 2013), which mostly receives ballasted vessels that discharge content of the tanks on the shore. Port complex of Maranhão is the second largest of the country and an important harbor hub for Latin America and other countries overseas (e.g. China). Patterns of port handling behavior noted here can be used as case study for ship-borne invasions not only in the Brazilian coast, but also to other port areas in the world.

As a first assessment to determine harbor behavior and potential contributions of non-native ship-mediated introductions, we briefly explored shipping traffic patterns in port complex of Maranhão. Using governmental and public databases (PORTOSMA 2014; ANTAQ 2016) and official reports from local maritime authorities, we queried port handling information for the last full report available (2013). Considering number of arrivals per vessel class (bulk carrier, oil tanker or container ship), port activity type (receiving or shipping cargo), source location (defined by last port of call) and mean volume of ballast tanks, we estimate that over 50 million m^3 of ballast water were discharged along the Maranhão coast that year. From these records, about 78% of arrivals were trips coming from other continents, indicating a high possibility of transportation of non-native organisms.

The high shipping intensity in Maranhão ports means ships can serve as vectors for marine introductions (Wonham et al. 2000), providing large and consistent releases of propagules that enable populations to overcome establishment problems related to small population size (e.g. stochasticity, demographic effects, low genetic diversity) (Lockwood et al. 2005). Brazilian maritime laws are quite strong and not all introduced organisms may thrive. However, considering the propagule pressure hypothesis for marine invasions (Lockwood et al. 2005), the high number of ship arrivals (high propagule size) discharging large quantities of ballast water (high propagule number) could have contributed for *B. koilomatodon* and other unwanted marine organisms introduction in Maranhão and Brazilian shelf (Feres and de Andrade Santos 2007; Ferreira et al. 2009; Loebmann et al. 2010). In this sense, there is a clear threat offered by Maranhão ports to ship-borne invasions on the entire northern coast. We suggest that further research on potential invasive species focus: on the ichthyoplankton association with hull fouling communities; DNA barcoding for rapid identification of eggs and larvae in ballast water, and bottom sediment samples from ballast tanks; as well as investigate the mud sleeper's population size in local waters and variation of population growth in controlled systems.

At present, no detailed studies on the biology (i.e. reproductive biology, feeding) and ecology (i.e. population structure, spatial distribution) of *B. koilomatodon* in Brazilian waters have been developed. This species might constitute threat not only just to the state of Maranhão, but also could be spreading along the entire coast of South America where suitable water temperatures exist. Moreover, the state of taxonomic expertise is such that it may already be present in many places as a cryptic invader. This study can serve as an alert, and patterns noted here for Maranhão state, can be used to deepen knowledge regarding routes/pathways and vehicles of introduction of mud sleepers, as well as survival in novel environments. Furthermore, more detailed and accurate investigation of invasive species is warranted in a country with such a large coastline

(Rocha et al. 2013). To generate reliable criteria to determine the degree of marine invasions in the Brazilian coast and shelf areas (Lopes 2009), more data concerning population and community ecology are clearly needed.

Conclusion

Specimens of *B. koilomatodon* likely reached the Maranhão coast via ballast water discharge from large cargo ships. However, its non-native status and routes of introduction remain speculative. Environmental conditions of the Maranhão coast and the adaptive characteristics inherent to the species possibly supported the survival of the mud sleeper once introduced. In addition, its presence might interfere with the ecology of native species and the structure and functioning of intertidal ecosystems. A review of fish specimens stored in state fish collections to identify additional individuals and locations is needed, alongside further research aiming to better understand the biology, ecology and vehicle of introduction of *B. koilomatodon* in Brazil. More specifically, it is crucial to investigate if the mud sleeper has established reproductive populations and recruitment in Brazil, especially considering its potential spread to adjacent waters in southern Atlantic.

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