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Original Article

Inventory reveals non-native species and variation in spatial-temporal dynamics of fish community in a Brazilian protected area

Inventário revela espécies não nativas e variação na dinâmica espaço-temporal da comunidade de peixes em uma área protegida brasileira

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Abstract

The increase in the number of Brazilian protected areas has been progressive and, although it is essential for the conservation of biodiversity, it is important to monitor and properly manage these areas, as they present several cases of biological invasions. The Lençóis Maranhenses constitute the peculiar delta of the Americas and are under the consequences of the bioinvasion of tilapias and peacock bass. Collections were carried out in the Lençóis Maranhenses National Park from March/2016 to November/2020, with the aid of gill nets and cast nets. The species were identified with the help of specialized literature and a historical comparison with previous works was carried out. Cytochrome oxidase subunit I was sequenced to confirm identification of non-native species. We recorded the expansion of the occurrence of *Oreochromis niloticus*, and the first record of the species *Oreochromis mossambicus* and *Cichla monoculus.* A total of 31 species belonging to eight orders, eighteen families and twenty-nine genera were identified, indicating a lag in the diversity of species found in relation to previous studies. After 20 years of the first record of invasive fish, there is an expansion of bioinvasion and new cases that indicate a lack of monitoring and containment measures for the species, indicating the fragility of conservation in the area

Keywords: National Park, Cichlidae, neotropical freshwater fish, species richness.

Resumo

O aumento do número de áreas protegidas brasileiras tem sido progressivo e, embora seja essencial para a conservação da biodiversidade, é importante o monitoramento e o manejo adequado dessas áreas, já que apresentam diversos casos de invasões biológicas. Os Lençóis Maranhenses constituem o peculiar delta das Américas e estão sob as consequências da bioinvasão de tilápias e tucunarés. Foram realizadas coletas no Parque Nacional dos Lençóis Maranhenses no período de março/2016 a novembro/2020, com o auxílio de redes de emalhe e tarrafas. As especies foram identificadas com o auxilio de literatura especializada e uma comparacao historica com trabalhos anteriores foi realizada. O Citocromo oxidase subunidade I foi sequenciado para confirmar a identificação das espécies não nativas. Registramos a expansão da ocorrência de *Oreochromis niloticus*, e o primeiro registro das espécies *Oreochromis mossambicus* e *Cichla monoculus*. Um total de 31 espécies pertencentes a oito ordens, dezoito famílias e vinte e nove gêneros foram identificadas, indicando uma defasagem na diversidade de espécies encontradas em relação a estudos anteriores. Após 20 anos do primeiro registro de peixes invasores, constata-se a expansão da bioinvasão e novos casos que assinalam ausência de monitoramento e de medidas de contenção para as espécies indicando a fragilidade na conservação da área

Palavras-chave: Parque Nacional, Cichlidae, peixes neotropicais, riqueza de espécies.

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1. Introduction

Maintaining ecosystem services is the global strategic plan for biodiversity until 2050 (CDB, 2019). And to achieve this goal, indirect and direct factors of biodiversity loss and its consequences for humanity must be considered, in addition to the concern with the effectiveness of protected areas, which are essential for the maintenance of species, populations and ecosystems (UNEP, 2018).

However, the global panorama shows that the fulfillment of these objectives is still far from what was proposed (Azevedo-Santos et al., 2019; Mormul et al., 2022). In Brazil, despite advances related to the creation of protected areas, preventing biological invasion has become a main challenge, and around $104.33 billion was spent due to damage and loss caused by trespassers in the period 1984 to 2019 (Adelino et al., 2021). Biological invasion is known to cause the loss of 50% of the world's native fish species, and 11 non-native fish species are registered in the protected areas of the Brazilian federation (Sampaio and Schmidt, 2014).

As in the whole globe, several fish species have been introduced in Brazil (Frehse et al., 2016; Ziller et al., 2020; Bueno et al., 2021), such as African Cichlids (Forneck et al., 2016), which are among the most cultivated species in aquaculture and active predators that have been translocated by sport fishing (Fugi et al., 2008) in the country. Among these cichlids, we highlight the representatives of the genera: *Cichla* (Schneider, 1801), endemic to the Amazon region with 15 described species (Kullander and Ferreira, 2006) and *Oreochromis* Gunther with 32 native African species.

In this context, we highlight the Lençóis Maranhenses National Park, created by decree No. 86060 (June 2, 1981), in the Lençóis region with an area of 155,000 hectares (Brasil, 2003). The Management Plan of PNLM presents data on freshwater fish fauna, such as the list of species. However, these data refer to studies conducted in the year 1999 reporting the occurrence of *Oreochromis* sp. as a non-native species (Brasil, 2003). Additionally, Brito et al. (2019) increased the occurrence of other native species and reinforced the occurrence of *Oreochromis* sp. in the region.

Therefore, this paper provides a current list of freshwater fish species in the Lençóis Maranhenses National Park, as well as the first record of *Cichla monoculus* and *Oreochromis mossambicus*, adding the spatial expansion of the occurrence of *Oreochromis niloticus*. In addition to a historical comparison with data on richness and spatial dynamics, contributing to updating the record of native and alien species and subsidizing actions for the management and conservation of species in the region.

2. Methods

2.1. Study area

The state of Maranhão, located on the North Equatorial Coast of Brazil, corresponds to an ecotone between the Amazon, Cerrado and Caatinga biomes. It houses the Lençóis Maranhenses National Park (PNLM), an international tourist destination peculiar to the delta of the Americas, crucial for global biodiversity (Figure 1).

The region has a megathermal climate, very hot and ranging from humid to sub-humid, with annual precipitation of 1,600.00 to 1,800.00 mm and temperatures ranging from 26 °C to 38 °C (Brasil, 2003).

The Maranhão sheets are made up of areas of free and fixed dunes, in addition to being a mosaic of ecosystems such as mangroves, cerrado and restinga (Brasil, 2003). Several water bodies are present in the park, rivers, creeks, streams, lakes, ponds, lagoons, supplied by the Periá and Preguiças rivers (Silva, 2008)

With an area of 155,000 ha, located between the municipalities of Primeira Cruz and Barreirinhas (IBAMA, 1989), the Lençóis Maranhenses National Park moves around 150,000 tourists per year (ICMBIO, 2020) and presents conflicts such as artisanal fishing versus industrial

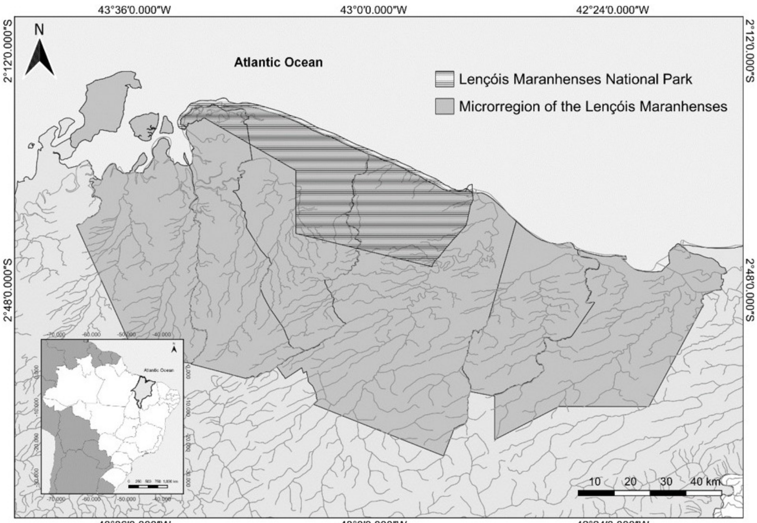

Figure 1. Map of the location of the Lençóis Maranhenses National Park and region on the equatorial coast of Brazil.

fishing, mangrove cutting, extractivism, hunting, rally, irregular occupation and public use activities as bathing, camping, hiking, boat trips, surfing and windsurfing (Brasil, 2003).

The limnological characteristics were obtained from the park management plan (Brasil, 2003).

2.2. Sampling

All collections for this study were done with authorization from the Brazilian Institute of the Environment and Non-Renewable Natural Resources (SISBIO-Number 53224-1). The collections were taken from March/2016, March and July/2017, July/2019 and November/2020 in twenty sampled points among rivers, creek, lakes, ponds and lagoons (Figure 2), in the four campaigns distributed in dry and rainy seasons (Supplementary Material).

Captures were developed through passive fishing using gillnets with spacing of 10 and 20 mm between nodes, during the day, twilight and night, with an average permanence time of 4 hours and review every two hours. It was then followed by active fishing using gear such as trawl nets with spacing between opposing nodes of 1.5mm, cast nets with 50mm between nodes and sieves with an average effort of 15 minutes per gear, adapted from Magnusson et al. (2005).

The identifications were carried out from the specialized literature for each group: Fowler (1954); Mago-Leccia (1994), Piorski et al. (2017) and taxonomic review articles.

Figure 2. Heterogeneous sampled points in Lençóis Maranhenses. (A) and (B) Pond in Paulino Neves, sandy substrate; (C) Tutóia, Delta das Américas, sandy substrate; (D) Lago de Santo Amaro, muddy substrate; (E) Prainha do amor, Barreirinhas, muddy substrate; (F) Tamacão Creek, Tutóia, muddy substrate.

The vouchers of identified fishes were deposited in the Tissue and DNA Collection of Maranhenses Fauna (CoFauMA) of the State University of Maranhão, Brazil. The identification of specimens obtained in this work followed the classification of Fricke et al. (2022).

2.3. Data analysis

For historical verification of diversity, we accessed published data from the work carried out in the 2000s for the Management Plan of the Lençóis Maranhenses National Park (Brasil, 2003) and from the work carried out in 2017 (Brito et al., 2019). Considering the same sampling points, we noted the collection methodologies in order to compare the species richness, as well as the absence/presence of native and non-native species recorded over the last 20 years.

2.4. Molecular procedures

For non-native species, total genomic DNA was extracted from individuals by the salting out method based on proteinase K digestion, followed by sodium chloride extraction and ethanol precipitation (Aljanabi and Martinez, 1997). A fragment (635 bp) of the mitochondrial cytochrome oxidase I subunit (COI) locus was amplified from six specimens (MW694823-MW694824 and MW692108 to MW692111).

The fragment was amplified using two pairs of universal primers FishF1 and FishR1, described by Ward et al. (2005). For amplification, 0.4 µL of DNA, 0.1 µL of each primer, 1.0 µL of buffer (10X), 0.4 µL of $MgCl_2$ (50 nM), 1.6 µL of dNTP, 0 were used in each sample., 1 µL of Taq DNA polymerase and 6.3 µL of ultrapure water to complete the reaction.

The PCR reaction followed an initial denaturation at 94 °C for 5 min followed by 35 cycles of 1 min of denaturation at 94 °C, 30s of hybridization at 56 °C and 1 min of extension at 72 °C, in addition to a final extension of 7 min at 72 °C. Amplified fragments were purified using the Wizad/Promega Purification Kit following the manufacturer's protocol and recommendations. The samples sequencing by the company ACTGene Analytical Moleculars Ltda. (Biotechnology Center, UFRGS, Porto Alegre, RS) using the automatic sequencer ABI-PRISM 3100 Genetic Analyzer armed with 50 cm capillaries.

COI sequences were visually checked and manually corrected. The statistical method chosen for the phylogenetic analyzes and tree assembly was neighbor-joining (K2P) which is recommended as a standard methodology. Additionally, the identification tool available in the Barcode of Life Data System was used, considering as an identification criterion, the similarity above 98% within the same species (BOLD, 2023).

3. Results

In this work, we obtained a total of 1010 specimens collected in the Lençóis Maranhenses National Park, distributed in 08 orders, 18 families, 29 genera and 31 species (Supplementary Material).

The most representative Orders were Characiformes (n=12), Cichliformes (n=8) and Siluriformes (n=5), followed by the orders Clupeiformes, Mugiliformes, Pleuronectiformes and Synbranchiformes, Gymnotiformes (n=1).

In our records, the most abundant species were *Oreochromis niloticus* (Linnaeus, 1758) (n= 148), *Bryconops* sp. and *Astyanax* sp. (n= 139). We also highlight that 16.4% of the total captured correspond to the occurrence of non-native species: *Oreochromis niloticus, Oreochromis mossambicus* (Peters, 1852) and *Cichla monoculus* Spix & Agassiz 1831 (Figure 3).

The identification of these taxa was confirmed by the similarity of the COI gene sequences with the data available in GenBank. The COI gene cluster for the species found, as proposed by Hebert et al. (2003), presents a similarity superior to 98% (Figure 4).

We also observed that in the Lençóis Maranhenses National Park *Oreochromis* spp. was found in 94.11% of the sampled points and in all campaigns.

As for the record of *Cichla monoculus*, it initially occurred in a lotic environment of the Preguiça River near the mouth in 2016, however, the occurrence in lentic environments was also recorded in the following years (Supplementary Material).

In our comparison of the distribution and occurrence of invasive species in two distinct moments: the occasion of the elaboration of the management plan, in 1999 and the collections carried out in this work, we verified the

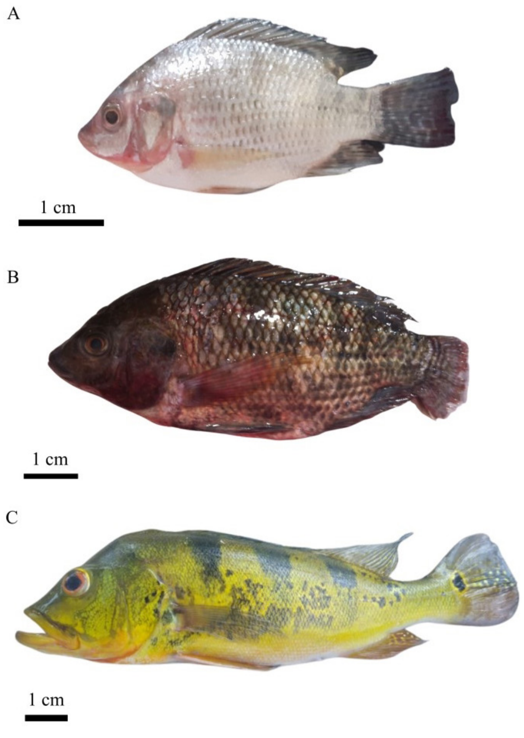

Figure 3. Non-native specimens collected in Lençóis Maranhenses National Park: (A) *Oreochromis niloticus*; (B) *Oreochromis mossambicus*; (C) *Cichla monoculus*.

spatial expansion of *Oreochromis niloticus* in the Park and in the adjacent areas (Figure 5).

In addition to the increase in the number of non-native species, we found a gap in native fish species in Lençóis Maranhenses, as can be seen in the comparison of species captured in the work of the Brasil (2003) and Brito et al. (2019) (Figure 6).

According to the Brasil (2003), in six expeditions in 2000, carried out preferably during the day with trawls and sieves, 43 species of fish were captured, with the first record of Oreochromis sp. While in the work by Brito et al. (2019) 49 species were collected with two trawl nets (20 m long, 2.5 m high, 10 mm mesh; and 4 m long, 2 m high, 5 mm mesh), cast nets (2 m high, 15 mm mesh), gill nets of various mesh sizes (15, 25, 35, 45 and 55 mm) and dip nets (5 and 10 mm mesh) in three expeditions, also indicating the occurrence of *Oreochromis* sp.

Five families registered in 2000, with the representatives *Awaous tajasica* (Lichtenstein 1822), *Polydactylus virginicus* (Linnaeus 1758), *Lycengraulis batesii* (Günther 1868), *Mugil curema* Valenciennes, 1836 e *Eucinostomus argenteus* Baird & Girard 1855, have a marine habit and were not found in this survey.

Other species such as: *Brachychalcinus parnaibae* Reis, 1989, *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa, Carvalho-Costa & Ottoni 2018, *Hemigrammus* spp., *Serrapinus* sp., *Steindachnerina notonota* (Miranda Ribeiro, 1937), *Aequidens tetramerus* (Heckel, 1840), *Poecilia*

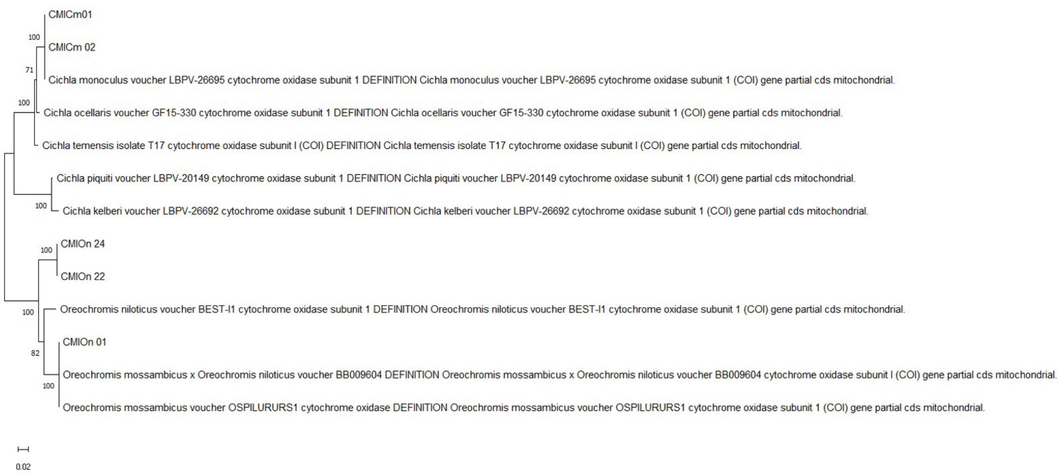

Figure 4. Phenogram generated by the MEGA X program for the COI gene sequences, by the neighbor joining method with 1000 replicates to support the clusters, demonstrating the identification of *Cichla monoculus, Oreochromis niloticus* and *Oreochromis mossambicus*.

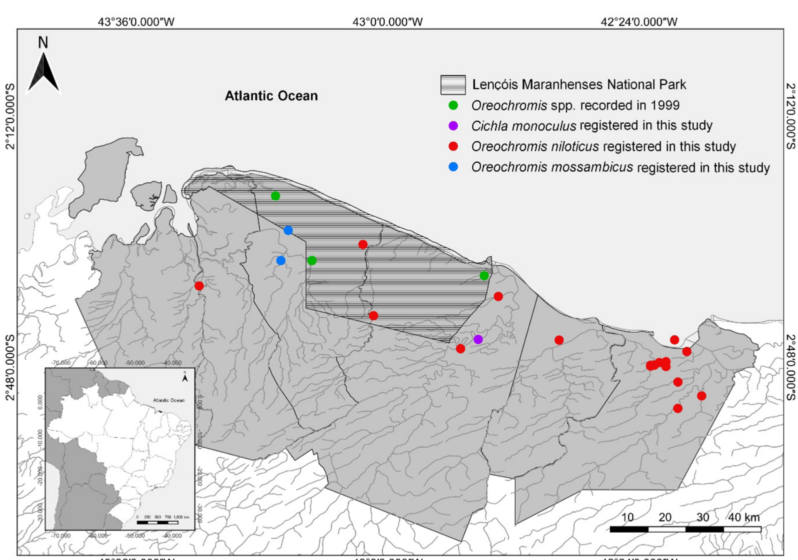

Figure 5. Distribution of allochthonous species *Oreochromis* spp. and *Cichla* spp. collected in Lençóis Maranhenses National Park recorded in 1999 and this work.

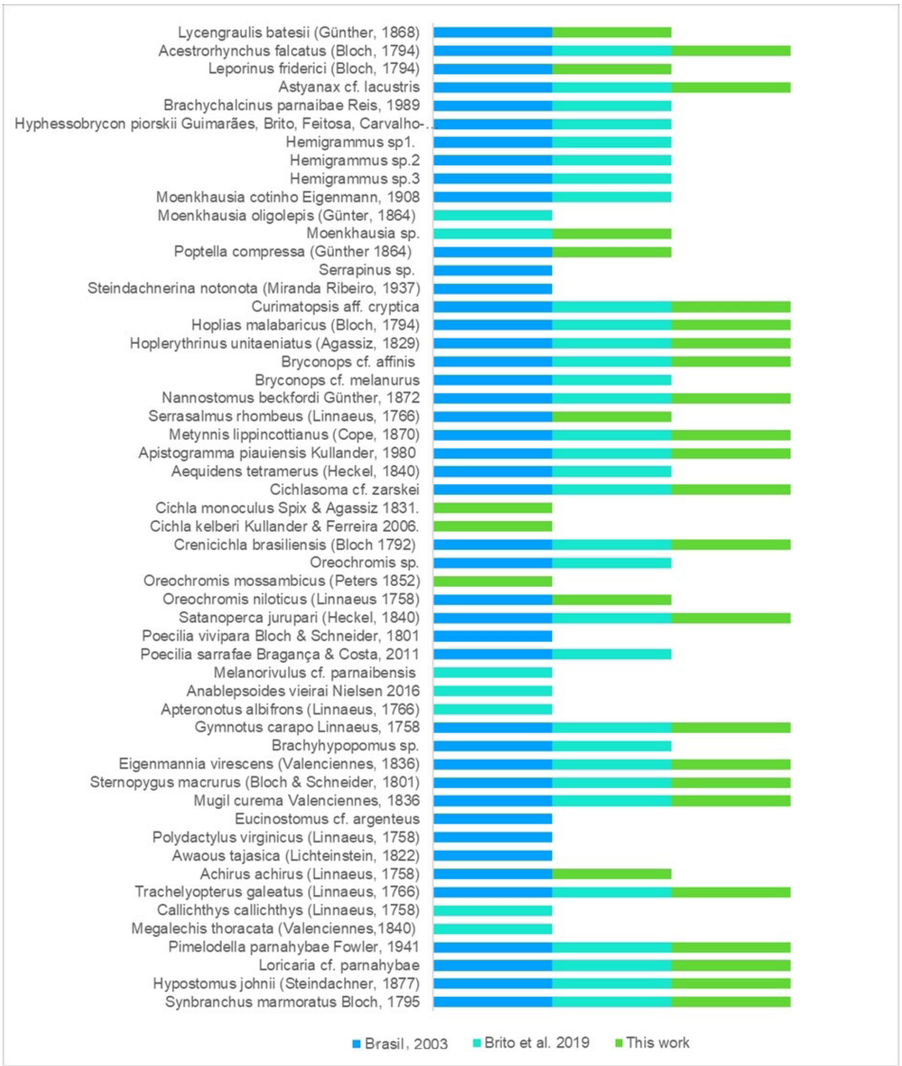

Figure 6. Graph of the historical comparison of the species registrated of freshwater fish collected in the Maranhão wetlands by the authors Brasil (2003), Brito et al. (2019) and this work.

vivipara Bloch & Schneider, 1801, *Poecilia sarrafae* Bragança & Costa, 2011, *Melanorivulus* cf. *parnaibensis*, *Anablepsoides vieirai* Nielsen 2016, *Apteronotus albifrons* (Linnaeus, 1766), *Brachyhypopomus* sp., *Megalechis thoracata* (Valenciennes, 1840) and *Callichthys callichthys* (Linnaeus, 1758) were not recorded in our work.

On the other hand, the Cichliformes had an increase in representativeness, due to the capture of representatives such as *Oreochromis mossambicus* and *Cichla monoculus*.

4. Discussion

The representativeness pattern of the taxa recorded in this study, despite being consistent with what was expected for semi-arid areas of the Neotropical region (Reis et al., 2016) and similar to the patterns of the next ones: Parnaíba, Itapecuru and Tocantins (Melo et al., 2016; Barbosa et al., 2017; Ramos et al., 2014), reveals the presence of three non-native species: *Oreochromis niloticus, Oreochromis mossambicus* and *Cichla monoculus*.

Oreochromis niloticus was introduced in Brazil in 1953 in lakes in the Northeast region (Oliveira et al., 2007; Leão et al., 2011) and *Oreochromis mossambicus,* the red tilapia, a modified strain was introduced in 1981 (Oliveira et al., 2007). In 1940, *Cichla monoculus*, *Cichla kelberi* Kullander & Ferreira 2006, *Cichla piquiti* Kullander & Ferreira 2006 and *Cichla temensis* Humboldt 1821 were introduced to control invasive species and sport fishing in the northeast region with the first record by Peixoto (1954).

The introduction of tilapia in the PNLM was recorded in 1999, at the time of the rapid ecological assessment for the management plan, while peacock bass is probably recent, as there is no record in surveys of the local ichthyofauna (Brasil, 2003; Brito et al., 2019).

In the park region, the main factors for the occurrence and expansion of these species were fish farming and sport fishing. And the success of these introductions is due to the increase and intensity of human activity acting as propagation pressure for these bioinvasions, corroborating with other cases cited by Lima Júnior et al. (2018), Latini et al. (2016) and Magalhães et al. (2017). The high propagation pressures are attributed to the size of the propagule in the case of tilapia and the frequency in the case of peacock bass, making the environment more unstable and susceptible to new invasions (Ricciardi, 2007).

Due to the ecological characteristics of the environments in the Lençóis Maranhenses region, such as the presence of lakes, rivers and small bodies of water (Brasil, 2003), the occurrence of these species is favored and, associated with phenotypic, feeding and reproductive plasticity, culminates in the establishment of these organisms, such as Kovalenko et al. (2010) and Diamante et al. (2017) report for other regions.

In our temporal analysis, we verified a variation in the number of native and non-native species recorded. An increase in non-native species and a reduction in the occurrence of native species. Like species that were cited in historical data (Brito et al., 2019; Brasil, 2003) *Hemigrammus* spp. and *Hyphessobrycon* spp. and were not recorded in our expeditions. Both have opportunistic habits, being omnivores with a preference for insects, microcrustaceans and filamentous algae (Barreto et al., 2018), a factor that may be associated with competition with *O. niloticus*.

A scenario similar to that reported by Attayde et al. (2007), in which Nile tilapia causes a reduction in the abundance of certain planktonic microcrustaceans, an increase in the biomass of nanoplanktonic algae and a reduction in water transparency. These effects of Nile tilapia can negatively affect the recruitment of other fish species that feed essentially on zooplankton in the juvenile stage and are visually oriented to locate and capture their prey (Attayde et al., 2007).

Furthermore, the presence of tucunaré may be associated with the non-registration of these species, since this invader hunts and devours whole prey (Ellis et al., 2011; Sales et al., 2018; Bajer et al., 2019; Santos et al., 2019). Also considering that most of the species not recorded in this study are small Characids and Cyprinids, corroborating Pelicice and Agostinho (2009) when they found a reduction in small fish, mainly Characiformes, such as *Hemigrammus* spp. and *Hyphessobrycon* spp. in environments invaded by peacock bass.

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Another bias to be considered is the impact of climate change such as the implications of increased temperature and its effect on reducing the oxygen content of aquatic systems (Jenny et al., 2016; Blaszczak et al., 2019), compromising the species most sensitive to abiotic changes. The climate is also associated with the increase, loss or changes in areas suitable for various species of fish, being a factor that can directly interfere with the maintenance of aquatic biota (Heino et al., 2009) impacting the increase in organic matter and pollution (MacNeil et al., 2004) can generate differential effects in bioinvasions (Dickey et al., 2021).

Additionally, one should take into account the growing impact of tourism in the region, which in 2022 was visited by 367,000 people (Brasil, 2003), the construction of roads and facilitation of access contributes to transport of species and increased impact on the local ichthyofauna due to effects in synergy with the aspects mentioned above due to the complexity of the study in this scenario.

Given the potential and environmental dynamics of Lençóis Maranhenses, it is necessary to establish monitoring of populations of *Oreochromis niloticus, O. mossambicus* and *Cichla monoculus* and together with native species, mainly associated with the endemicities of *Anablepsoides vieirai* Nielsen 2016, *Apistogramma piauienses* Kullander 1980, *Hyphessobrycon worstskii* Guimarães, Brito, Feitosa, Carvalho-Costa and Ottoni 2018, *Hypostomus johnii* (Steindachner, 1877), *Poecilia sarrafae* Bragança e Costa 2011 and *Pimelodella parnahybae* Fowler, 1941 registered by Brito et al. (2019) in the region.

Considering the management plan as a tool to protect the protected area, the information has not been sufficient to contain the advance of non-native species (Monroe et al., 2021). This fact contributes to intensifying the impacts on the park's biodiversity, which, although it stands out for having more severe restrictions and controls, should bring greater visibility to policies and practices in this protected area (Pressey et al., 2015).

Mainly in a territory highlighted by areas of endemism and biodiversity hotspots, with approximately 20% of its ichthyofauna endemic (Abell et al., 2008), it is necessary to advance in relation to the maintenance of this diversity, given the lack of studies, with data considered underestimated for the area by some authors (Dagosta and Pinna, 2019; Brito et al., 2019). We also indicate environmental education actions for the local and tourist population, in order to sensitize social actors to the consequences of these invasions. In addition to constant vigilance to avoid the release and exchange of species between the park and adjacent areas, considering that Brazilian legislation establishes the need for a routine of eradication, containment, control and monitoring of invasions.

Acknowledgements

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Details of surveys conducted in 2016/2020 in Lençóis Maranhenses National Park: location name and coordinates, habitat features, presence of non native species and habitat features.

Table S2. Comparison of species richness between this study, Brasil (2003) and Brito et al. (2019).

Table S3. Details of collection campains conducted in 2016/2020 in Lençóis Maranhenses region: Campain and date of capture.

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