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Ornamental aquaculture as a pathway for the introduction of non-native fish in coastal drainage of northeastern Brazil

Aquicultura ornamental como caminho para a introdução de peixes não nativos na drenagem costeira do nordeste do Brasil

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Abstract: Aim: We present the first record of the introduction of three non-native ornamental fish species, resulting from ornamental aquaculture escapes on the Northeast Mata Atlântica hydrographic ecoregion, with information on the reproductive activity. **Methods:** The specimens were collected bimonthly between 2018 and 2019 using seine and sieve nets, and analyzed for determination of reproductive phases. **Results:** A total of 830 specimens were collected, corresponding to the species *Hyphessobrycon eques* (N= 311), *Poecilia reticulata* (N= 500), and *Xiphophorus maculatus* (N= 19). We used 250 specimens for reproductive analysis. *Hyphessobrycon eques* and *P. reticulata* were reproductively active throughout the sampling period. Among the species, the smallest reproductive female measured 22.26 mm (*H. eques*), 12.53 mm (*P. reticulata*), and 17.58 mm (*X. maculatus*). The smallest males measured 20.64 mm (*H. eques*), 10.71 mm (*P. reticulata*), and 17.17 mm (*X. maculatus*). **Conclusions:** The presence of reproductive specimens of *H. eques* and *P. reticulata* throughout the year supports the hypothesis of their establishment. We hypothesize that the introduction of *X. maculatus* is a recent event.

Keywords: accidental escapes; exotic species; invasiveness; dispersion; plasticity.

Resumo: Objetivo: Apresentamos o primeiro registro da introdução de três espécies de peixes ornamentais não nativas, decorrente de escapes de aquicultura ornamental para a ecorregião hidrográfica Mata Atlântica Nordeste, com informações sobre a atividade reprodutiva. **Métodos:** Os exemplares foram coletados bimestralmente entre 2018 e 2019 usando redes de arrasto e peneira, e analisados para determinação das fases reprodutivas. **Resultados:** Um total de 830 espécimes foram coletados, correspondentes às espécies *Hyphessobrycon eques* (N= 311), *Poecilia reticulata* (N= 500) e *Xiphophorus maculatus* (N= 19). Foram utilizados 250 exemplares para análise reprodutiva. *Hyphessobrycon eques* e *P. reticulata* estiveram em atividade reprodutiva durante todo o período amostral. Entre as espécies, as menores fêmeas reprodutivas mediram 22.26 mm (*H. eques*), 12.53 mm (*P. reticulata*), e 17.58 mm (*X. maculatus*). Os menores machos reprodutivos mediram 20.64 mm (*H. eques*), 10.71 mm (*P. reticulata*), e 17.17 mm (*X. maculatus*). **Conclusões:** A presença de espécimes reprodutivos de *H. eques* e *P. reticulata* durante todo o ano suporta a hipótese de que estão estabelecidas. Hipotetizamos que a introdução de *X. maculatus* é um evento recente.

Palavras-chave: escapes acidentais; espécies exóticas; invasividade; dispersão; plasticidade.

Biological invasions are increasingly considered a biodiversity threat (Magalhães et al., 2021). In the past, these events occurred slowly, but currently, they appear at higher frequency and intensity, due to multiple human actions that can collaborate (Lockwood et al., 2019). The rising global ornamental fish trade is one of the main routes of introduction (Magalhães et al., 2021). The high demand for novelties in this market has a negative effect, as it increases the possibility of new introductions (Olden et al., 2021).

One of the main pathways related to fish introduction is escape from ornamental aquaculture ponds, which can occur due to missteps during the filtering, harvesting, and cleaning processes (Ortega et al., 2015). The introduction of ornamental fish as means of biological control is an old practice that is still widespread (Bajer et al., 2019), as well as aquarium dumping (Magalhães et al., 2020b). Unfortunately, many of these introductions are silent and difficult to record. Recently, the use of social media has become an important source for tracking these introductions (Magalhães et al., 2021; Tutman et al., 2021).

Although introducing non-native species is common in Brazilian river basins (Garcia et al., 2018), studies involving non-native fish in streams are still restricted to a few basins (Magalhães et al., 2019, 2020b; Garcia et al., 2021). According to Bueno et al. (2021), the southeast region gathers the highest number of introduced non-native ornamental fish, mainly in the upper Paraná and Paraíba do Sul river basins. Many introductions in these basins are probably consequences of the expansion of the aquarium trade (Magalhães et al., 2019).

Northeast Brazil has approximately 30 introduced fish species (Instituto Hórus, 2021), of which the fish stock for food production is the primary driver (Brito et al., 2020). In addition, recent findings have shown increased number of ornamental fish introduced through deliberate releases (Ramos et al., 2020) and for biological control (Instituto Hórus, 2021). Herein, we present the first record for three ornamental fish *Hyphessobrycon eques* (Steindachner, 1882), *Poecilia reticulata* Peters, 1859 and *Xiphophorus maculatus* (Günther, 1866), by escapes of ornamental aquaculture tanks in the Northeast Mata Atlântica Freshwater Ecoregion - NMAFE (Abell et al., 2008), with information on their reproductive activity.

Sampling expeditions were carried out bi-monthly from March/2018 to January/2019 in a stretch of Tabocas stream (11°2’16.77”S, 37°22’0.05”W), Piauí river basin, Sergipe State. The sampling area was in the vicinity of man-made ornamental aquaculture ponds. The substrate of the Tabocas stream was composed of a bed of trunks and leaf litter in the backwaters, sandy bottom in the rapids, and sandy/muddy bottom in the lentic stretch. The average depth reached 1 m and the width ranged about 4 m. Seine (10 m long, 5 mm mesh) and sieve (60 cm x 40 cm, 5 mm mesh) nets were used along a 50 m sampling stretch. The fish were anesthetized with eugenol (100mg/L) (Sladky et al., 2001), preserved in a 10% formalin solution, and stored in 70% ethanol (Uieda & Castro, 1999). Vouchers were deposited in the Coleção de Ictiologia da Universidade Federal de Sergipe (CIUFS) under catalog numbers CIUFS 2704 (*H. eques*, Figure 1A), CIUFS 2705 (*P. reticulata*, Figure 1B), and CIUFS 2706 (*X. maculatus*, Figure 1C).

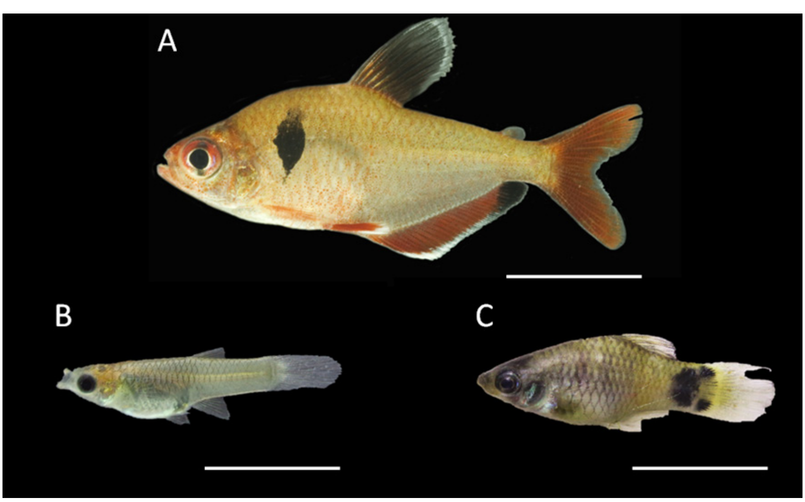

Figure 1. Non-native ornamental fish species sampled in the Tabocas stream: *Hyphessobrycon eques* (A), *Poecilia reticulata* (B) and *Xiphophorus maculatus* (C). Scale bar = 1 cm.

Specimens were identified, with standard length (SL, 0.01 mm) and body mass (BM, 0.01 g) recorded with a digital caliper and semi-analytical precision scale, respectively. The reproductive analysis consisted of a maximum of 20 specimens per species for each sampling event. Ovaries and testes of *H. eques* were macroscopically determined by reproductive phase through morphological characteristics following Brown-Peterson et al. (2011): (1) Immature; (2) Developing; (3) Spawning Capable; (4) Regressing; (5) Regenerating. Magalhães & Jacobi (2017) protocol was applied for the Poeciliidae *(P. reticulata* and *X. maculatus*) and adapted for classifying adult females according to Winemiller (1993) and Haynes (1995): Nonpregnant (NP); Yolk eggs (YE); Embryos with eyes (EE); and Mature embryos (ME). Reproductive phases for males were determined as Immature (IM) or Spawning Capable (SC), according to the presence of the gonopodium (Gómez-Márquez et al., 2016). Chi-square distribution (χ^2) for the sex ratio and Sturges' rule (Sturges, 1926) were analyzed per sampling event to obtain the size class intervals of *H. eques* and *P. reticulata*.

Sampling efforts resulted in a total of 830 specimens collected, corresponding to the species *H. eques* (N= 311) (Figure 2A), *P. reticulata* (N= 500) (Figure 2E), and *X. maculatus* (N= 19). Unfortunately, *X. maculatus* was only recorded during the last sampling event in January/2019.

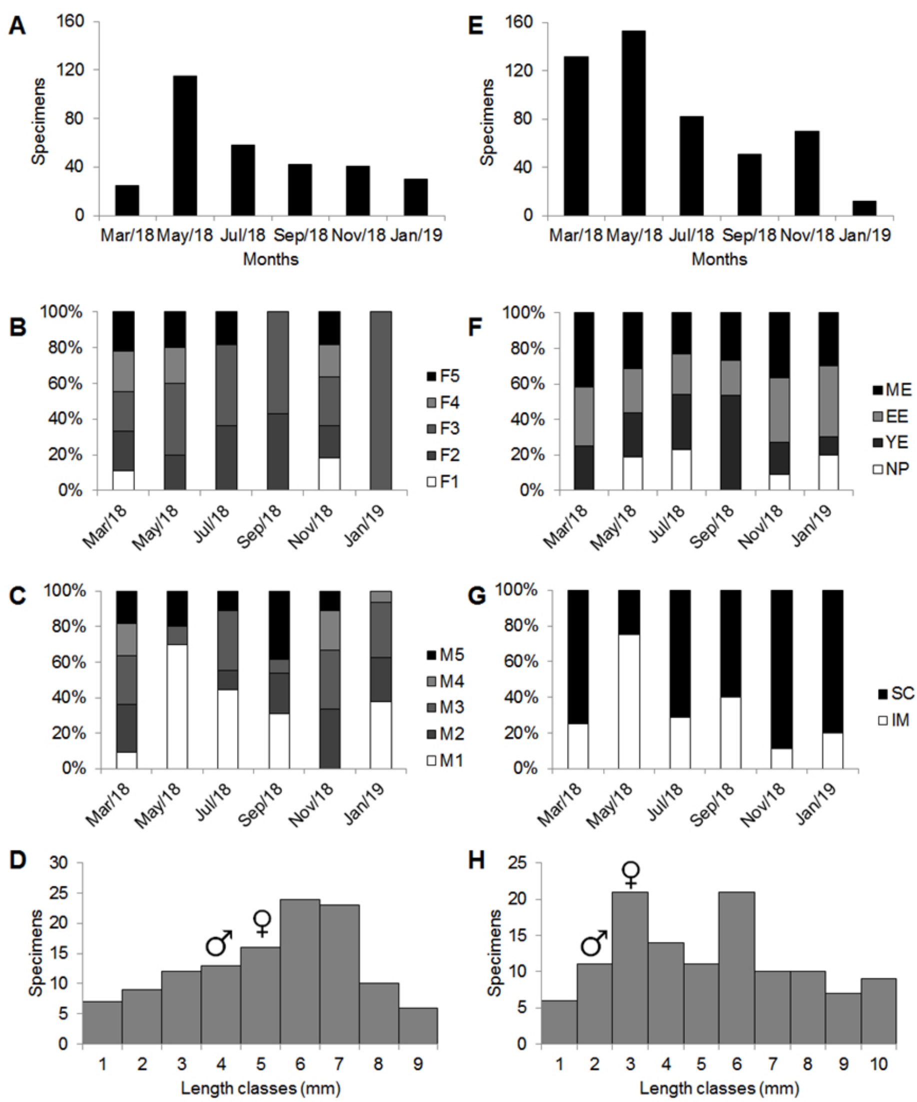

Figure 2. Distribution of specimens throughout the sampling period (A – *Hyphessobrycon eques*, E – *Poecilia reticulata*); Reproductive phases of *H. eques* (B – Females, C – Males) and *P. reticulata* (F – Females, G – Males); Class sizes (D – *H. eques*, H – *P. reticulata*), including smallest reproductive males (♂) and females (♀).

From this total, 250 specimens were used for reproductive analysis.

Males were more frequent than females in *H. eques* (68:52; χ^2= 2.13, p> 0.05) and *X. maculatus* (7:3; χ^2= 1.6, p> 0.05), while for *P. reticulata* females (43:77; χ^2= 9.63, p< 0.05) were more frequent. We registered *H. eques* and *P. reticulata* females in reproductive activity during the study period (Figure 2B, 2F), recoding all gonadal/embryonic development stages. The same reproductive tendency was observed for males throughout the year for both species (Figure 2C, 2G). Ten *X. maculatus* specimens were analyzed, in which both females and males were reproductively active. The smallest reproductive female of *H. eques* measured 22.26 mm, with the smallest pregnant females measuring 12.53 mm for *P. reticulata* and 17.58 mm for *X. maculatus*. The smallest reproductive males measured 20.64 mm for *H. eques*, 10.71 mm for *P. reticulata*, and 17.17 mm for *X. maculatus*.

Size class distribution revealed nine classes for *H. eques* with intervals of 1.69 mm (Figure 2D) ranging from 12.71–29.73 mm, while for *P. reticulata* were observed 10 classes with intervals of 1.25 mm (Figure 2H) varying from 10.65–19.24 mm. Given the lower number of specimens of *X. maculatus*, available information on the analyzed sample provided SL ranges from 17.17–24.44 mm. Juveniles of *H. eques* were more prevalent when compared to *P. reticulata*, whereas the latter presented a greater number of adults.

Determining the minimum reproductive size of an invasive species can help understand the evolution of establishment in new habitats. This can provide management strategies to control the invasive species and minimize the impact on native biota and local ecosystems. The sexual precocity of invasive fish relative to native fish can generate a competitive advantage over native species by reproducing more quickly and occupying ecological niches that previously belonged to native species. This can reduce species diversity and disturb the ecological balance in aquatic ecosystems (Winemiller & Rose, 1992; Pikitch et al., 2004).

The presence of the three nonnative species registered herein relates to the accidental escapes from man-made ornamental aquaculture ponds on the Tabocas stream. Escapes may be derived from the inefficiency of screens (e.g., mesh size larger than fish), lack of maintenance (e.g., holes), or during routine protocols in which the tanks are drained for cleaning and the effluent is discarded directly into the nearest water body without treatment or biosecurity measures. Another possibility can be related to the overflow of the tanks during intense rainfall in a short period, a common occurrence in the study area. Moreover, advances implementing new ornamental aquaculture grounds in the region call for attention (Santos et al., 2019), possibly boosting new introductions.

Hyphessobrycon eques showed a reproductive pattern [Opportunistic, sensu Winemiller (1989)] similar to their native range, with continuous reproduction and fractionated-type spawning (Santana et al., 2019). However, the reproductive plasticity verified in *H. eques* allows its reproduction to occur seasonally in places outside its native distribution (Gonçalves et al., 2013). This certifies the high invasiveness, which can adapt its reproductive responses to local requirements, and adjust its diet (Carvalho & Del-Claro, 2004) to the availability of resources in new areas.

Likewise, *P. reticulata* has high plasticity to the establishment in lotic and lentic environments due to particular survival strategies, such as living in hypoxic waters, extreme variations in tolerance in water temperatures, pH, salinity, and aquatic pollution (Carvalho et al., 2019). In addition, viviparity and generalist diet contributed to the success of colonization and the high adaptability of this species in diverse environments (Rosenthal et al., 2021). Such strategies represent the evolutionary success of fundamental importance for establishment and dispersion.

The presence of reproductive specimens throughout the year and specimens in different size classes for both *H. eques* and *P. reticulata* supports the hypothesis that they are established in the Tabocas stream in NMAFE. This assumption can be supported by the evidence that analyzed specimens of *P. reticulata* showed regression in their wild pattern of coloration and reduction of the caudal fin in males, suggesting that the introduction took place in past generations. Our findings indicate that the *X. maculatus* population is in the introduction phase and is likely a recent event because of the few specimens captured during the last sampling expedition.

The establishment of these species in the Piauí river basin is alarming since, once established, they can disperse and colonize new areas. In addition, the Poeciliidae have a high degree of euryhalinity and can survive under mesohaline conditions for extended periods (Pol & Kujawa, 2011), using the brackish waters of estuaries as dispersal vectors to other environments (Nekrasova et al., 2021). In addition to the dispersal potential, the presence of phylogenetically close native fish in the Tabocas stream, such as congeners *Hyphessobrycon parvellus* Ellis, 1911 and *Poecilia hollandi* (Henn, 1916) may result in competition for resources and the possibility of hybridization. Studies have shown that genetically and morphologically close species can hybridize, overlap niches, compete for resources with native species, and even lead these populations to decline (Magalhães & Jacobi, 2017; Freitas-Souza et al., 2022).

Due to the presence and establishment of at least two species in the Tabocas stream, as well as the risk of dispersion and adverse ecological impacts, it is recommended that, to avoid new introductions, awareness-raising actions be carried out through widely accessible media to disseminate environmental legislation, ensuring control and enforcement actions. According to Casimiro et al. (2018), Magalhães et al. (2020a, 2021), and Garcia et al. (2021), solutions may include: (i) Environmental education about the risks of biological invasions for students, aquarium shop owners, aquarists, fishermen, fish farmers, and decision-makers; (ii) Practical application of the precautionary principle or the 'polluter-pays' principle to minimize the risk of fish escapes; (iii) Increasing the height of fish farm tanks and ponds to prevent fish escapes during stream floods; (iv) Installation of containment structures such as nylon screens that prevent non-native species from escaping into the natural environment; (v) The strict control of tank fishing; (vi) Quarantine of new broodstock and frequent sanitary control of the fish stock.

Implementing these measures makes it possible to reduce the number of introductions, as well as the negative impacts that invasive species can bring to the native species. It is important to highlight that prevention is the best approach to address the problem of invasive species, with public awareness and education being essential measures to prevent new introductions in the aquatic environment.

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