**Live bait trade as a pathway to introduction of non-native species in Brazilian freshwater ecosystems**

*Diego Azevedo Zoccal Garcia1\*, Armando César Rodrigues Casimiro2, Iago Vinicios Geller2, João Daniel Ferraz2, Lucas Ribeiro Jarduli1,3 & Mário Luís Orsi1*

1Universidade Estadual de Londrina, Departamento d Biologia Animal e Vegetal, Laboratório de Ecologia de Peixe e Invasões Biológicas, Rodovia Celso Garcia Cid, CEP: 86057-970, Londrina, PR, Brazil.

2Universidade Estadual de Londrina, Departamento d Biologia Animal e Vegetal, Programa de Pós-Graduação em Ciências Biológicas, Rodovia Celso Garcia Cid, CEP: 86057-970, Londrina, PR, Brazil.

3Centro Universitário das Faculdades Integradas de Ourinhos, Rodovia BR 153, Km 338, Bairro Água do Cateto, CEP: 19909-100, Ourinhos, SP, Brazil.

E-mails: diegoazgarcia@hotmail.com (\*corresponding author); armandocesar82@yahoo.com.br; iagogeller@hotmail.com; jd\_ferraz@hotmail.com; lucasjarduli@gmail.com; orsi@uel.br

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**Abstract:** We report cases of introductions of live baits in Brazilian freshwater ecosystems and the first occurrence of the crab *Dilocarcinus pagei* in the Paranapanema River basin. The literature review revealed 16 species used as live bait across Brazil, the crab *D. pagei* and 15 fish species. During field samplings, we captured 10 individuals of *D. pagei*. Gymnotiformes were the largest number of fish species used as live bait. The basins that had introduction cases were Upper Paraná, Iguaçu, and Paraíba do Sul Rivers. The Paraguay-Lower Paraná River was the system that contributed with nine species to the most invaded ecoregion in Brazil, the Upper Paraná River basin. Anglers and live bait farmers should be aware of the risks and consequences of these constant introduction actions. In addition, the live bait trade must be monitored and controlled to occur only with species in their native range.

**Keywords:** biodiversity loss; conservation; propagule pressure; South America; vector.

The Amazon and Paraguay-Lower Paraná River basins are among the richest in freshwater fish in the world (Dagosta & Pinna 2019), with many medium and large species appreciated in sport fishing. Due to the great diversity of fish, biomes and watersheds, sport fishing has become increasingly popular (Britton & Orsi 2012). The fish richness and different environments attract tourist, and represent an opportunity for ecotourism and fishing tourism in Brazil (Embratur 2018).

The socioeconomic importance of recreational fishing has increased rapidly in several countries (Arlinghaus & Cooke 2009). According to the National Association of Ecology and Sport Fishing (ANEPE), Brazil receives 10 to 15 thousand international tourists for sport fishing per year and generates about 200 thousand jobs. In the United States, sport fishing generates around US$ 115 billion annually, while in Brazil, it moves only US$ 3 billion (Embratur 2018).

However, there is no technical cooperation to develop sport fishing tourism in Brazil, and important contributions are disregarded. Among them, is the translocation of species between watersheds (Freshwater Ecoregions), recognized worldwide as an imminent risk to freshwater ecosystems (Seebens et al. 2017). Besides, these environments are also threatened by damming (Agostinho *et al.* 2016), deforestation (Zeni *et al.* 2019), pollution, and some environmental laws (Brito *et al.* 2018).

The development of fishing tourism brings greater demand for live bait, which is any live animal used to attract and capture fish (Alho 2020). The use of live bait varies according to the fish required, and some professional anglers have specialized in catching fish, crustaceans and other invertebrates in the wild or create them to meet this demand. Live baits may be included in fishing tourism ‘packages’ sold to anglers (Catella *et al.* 2009). Thus, the increase of sport fishing influences the demand and capture of fish and crustaceans to be used as bait. However, at the end of fisheries, many anglers discard their bait to avoid sacrificing the animals, regardless of their native range (Kilian *et al.* 2012).

The transport of non-native species used as bait in sport fishing increases the probability that they will be discarded in water bodies, resulting in the entry of species that can successfully colonize new environments. Therefore, the practice of ‘discard’ live bait is an important pathway of aquatic species introduction that allows transposition between different ecoregions (Garcia *et al.* 2015, Ortega *et al.* 2015). Therefore, we aim to: (i) record the presence of the crab *Dilocarcinus pagei* (Decapoda, Trichodactylidae) in the Paranapanema River basin, southern Brazil; (ii) inventory freshwater species are among the most commercialized as live bait, as well as their occurrence in Brazilian watersheds.

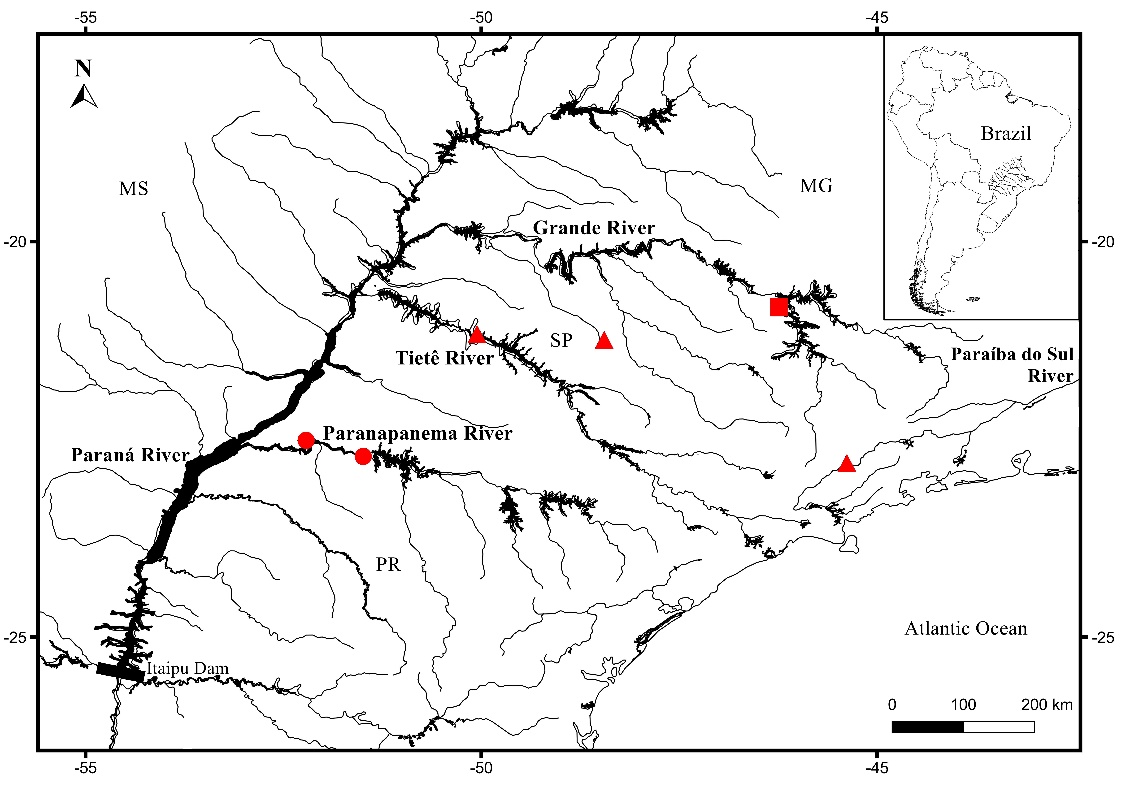
During fish fauna monitoring within the Paranapanema River basin (Projects Nº 3224/2012 and Nº 11218/2018), specimens of the crab *D. pagei* were unexpectedly captured. The samplings were seasonal between September 2012 and April 2016, and between July 2018 and July 2019. The crabs were caught together with fish, using trawls and sieves operated by four people during one hour on the margins of the transition stretch of the Rosana and Taquaruçu reservoirs. Traps with baits (pieces of fish, liver and bread) were also used, which were installed at dusk and removed at dawn.

The captured crabs were euthanized by exposure to clove oil. Afterward, they were fixed in 10% formalin for 48 hours, and then preserved in 70% alcohol. *Dilocarcinus pagei* has a convex carapace in the anteroposterior direction and is usually red in live specimens, with six or seven teeth on the anterolateral margins. They are identified by a distinct transversal carina along the anterior margin of the third abdominal somite (Magalhães *et al.* 2005). Voucher specimens were added to the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL 508, 509), and a specialist confirmed the identification (Dr. G. M. Teixeira, Universidade Estadual de Londrina). The collection license is Nº 16578, and the Animal Ethics Committee authorized field sampling (CEUA Nº 30992.2014.33).

A species was considered non-native if suggested in the literature that should not have naturally occurred in the Brazilian Freshwater Ecoregions due to biogeographical factors. Fish considered non-native included species from other South American Ecoregions, for example Amazon, Paraguay and Lower Paraná ecoregions.

The literature review was carried out to investigate the origin of the introduction of *D. pagei*, as well as to carry out an inventory of non-native aquatic species. The review was based on searches in Web of Science, Scopus, Scielo, and Google Scholar, starting with ‘live bait’ and ‘Brazil’ in ‘title’ searches, and then using these within Boolean logic search terms with words including ‘*Dilocarcinus pagei’*, ‘*Dilocarcinus*’, ‘bait’, ‘knifefish’, ‘sporting fish’, ‘non-native’, ‘introduced’, ‘release’. These searches provided articles that resulted in a list of species used as live bait in Brazil. In addition to articles, literature was also obtained from books and documents. Where taxonomic and native origin information was not available, then it was collected from other literature sources: Reis *et al.* (2003), Britski *et al.* (2007), and Eschmeyer *et al.* (2019).

Field samplings resulted in the first record of the crab *D. pagei* at two sites in the Paranapanema River basin. Ten individuals were captured, one female in the Rosana Reservoir (22º36’02.11”S; 52º09’56.67”O); and two juveniles, one female and six males in the Taquaruçu Reservoir (22º39’37.00”S; 51º37’49.06”O). The new records were added to the complementary bibliographic survey, making it possible to map the distribution of this species in the Upper Paraná and Paraíba do Sul River basins (Figure 1).



**Figure 1.** Distribution of *Dilocarcinus pagei* in the Upper Paraná and Paraíba do Sul River basins. Triangles: Magalhães *et al.* (2005); square: Azevedo-Santos & Lima-Stripari (2010); circle: present study. MS: state of Mato Grosso do Sul; MG: state of Minas Gerais; SP: state of São Paulo; PR: state of Paraná.

From the literature source, 15 fish species were introduced in different Brazilian basins (Table 1). The basins that presented cases of introduction by live baits release were the Upper Paraná River (*D. pagei* and 10 fish species), the Iguaçu River (seven fish species), and the Paraíba do Sul River (*D. pagei*). Gymnotiformes (knifefishes) showed greater richness (eight species), being represented mainly by the genus *Gymnotus* (four species). The most invaded ecoregion in Brazil (Upper Paraná River basin) received nine species only from the Paraguay-Lower Paraná.

**Table 1.** Species introduced as live bait in Brazil. Native Freshwater Ecoregion according to Reis *et al.* (2003).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Situation | Locality of introduction (basin or system) | References | Native Freshwater Ecoregion |
| Crustacea |  |  |  |  |
| Decapoda |  |  |  |  |
| Trichodactylidae |  |  |  |  |
| *Dilocarcinus pagei* Stimpson 1861 | Introduced as live bait | Upper Paraná and Paraíba do Sul River basins | Magalhães *et al.* (2005), Azevedo-Santos & Lima-Stripari (2010), Latini *et al.* (2016) | Amazon, Paraguay and Lower Paraná River basins |
| Actinopterygii |  |  |  |  |
| Characiformes |  |  |  |  |
| Curimatidae (Toothless characiforms) |  |  |  |  |
| *Steindachnerina brevipinna* (Eigenmann & Eigenmann 1889) | Introduced as live bait | Iguaçu River basin | Daga *et al.* (2016) | Uruguay, Paraguay and Lower Paraná River basins |
| Erythrinidae (Trahiras) |  |  |  |  |
| *Erythrinus erythrinus* (Bloch & Schneider 1801) | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná River basin | Langeani *et al.* (2007), Graça & Pavanelli (2007), Júlio Júnior *et al.* (2009), Ortega *et al.* (2015), Ota *et al.* (2018) | Orinoco, Guianas, Amazon, Paraguay and Lower Paraná River basins |
| *Hoplerythrinus unitaeniatus* (Agassiz 1829) | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná River basin | Langeani *et al.* (2007), Graça & Pavanelli (2007), Júlio Júnior *et al.* (2009), Ortega *et al.* (2015), Ota *et al.* (2018) | Amazon, Paraguay, Lower Paraná, Orinoco, São Francisco, and Magdalena River basins, and coastal rivers in Guyana, Suriname, and French Guiana. |
| Triportheidae |  |  |  |  |
| *Triportheus angulatus* (Spix & Agassiz 1829) | Introduced as live bait | Upper Paraná River basin | Ortega *et al.* (2015) | Amazon River basin |
| Siluriformes |  |  |  |  |
| Callichthyidae (armored catfishes) |  |  |  |  |
| *Callichthys callichthys* (Linnaeus 1758) | Introduced as live bait | Iguaçu River basin | Baumgartner *et al.* (2012), Daga & Gubiani (2012), Ortega *et al.* (2015) | Most Cis-Andean South American river drainages north of Buenos Aires. |
| *Hoplosternum littorale* (Hancock 1828) | Introduced as live bait | Iguaçu River basin | Baumgartner *et al.* (2012), Daga & Gubiani (2012), Ortega *et al.* (2015) | Most Cis-Andean South American river drainages north of Buenos Aires. |
| Gymnotiformes |  |  |  |  |
| Gymnotidae (Naked-back knifefishes) |  |  |  |  |
| *Gymnotus inaequilabiatus* (Valenciennes 1839) | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná and Iguaçu River basins | Baumgartner *et al.* (2006), Graça & Pavanelli (2007), Baumgartner *et al.* (2012), Daga & Gubiani (2012), Ortega *et al.* (2015) | Paraguay and Lower Paraná River basins and some coastal drainages |
| *Gymnotus pantanal* Fernandes et al. 2005 | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná River basin | Graça & Pavanelli (2007), Margarido *et al.* (2007), Ota *et al.* (2018) | Paraguay and Lower Paraná River basins |
| *Gymnotus paraguensis* Albert & Crampton 2003 | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná River basin | Graça & Pavanelli (2007), Ota *et al.* (2018) | Paraguay and Lower Paraná River basins |
| *Gymnotus sylvius* Albert & Fernandes-Matioli 1999 | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná and Iguaçu River basins | Agostinho *et al.* (2002), Baumgartner *et al.* (2006), Graça & Pavanelli (2007), Baumgartner *et al.* (2012), Daga & Gubiani (2012), Ortega *et al.* (2015) | Ribeira de Iguape, Paraíba do Sul and Pardo River basins |
| Rhamphichthyidae (Sand knifefishes) |  |  |  |  |
| *Gymnorhamphichthys britskii* Carvalho, Ramos & Albert 2011 | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná River basin | Ota *et al.* (2018) | Paraguay and Lower Paraná River basins |
| *Rhamphichthys hahni* (Meiken 1937) | Introduced as live bait or transposition after Itaipu Dam | Upper Paraná River basin | Ota *et al.* (2018) | Paraguay and Lower Paraná River basins |
| Hypopomidae (Bluntnose knifefishes) |  |  |  |  |
| *Brachyhypopomus gauderio* Giora & Malabarba 2009 | Introduced as live bait | Upper Paraná River basin | Langeani *et al.* (2007), Graça & Pavanelli (2007), Ortega *et al.* (2015), Ota *et al.* (2018) | Orinoco, Guianas, Amazon, Uruguay, Paraguay and Lower Paraná River basins |
| Apteronotidae (Ghost knifefishes) |  |  |  |  |
| *Apteronotus ellisi* (Alonso de Arámburu 1957) | Introduced as live bait | Iguaçu River basin | Agostinho *et al.* (2002) | Paraguay and Lower Paraná River basins |
| Synbranchiformes |  |  |  |  |
| Synbranchidae (Swamp-eel) |  |  |  |  |
| *Synbranchus marmoratus* Block 1795 | Introduced as live bait | Iguaçu River basin | Baumgartner *et al.* (2012) | Mexico to northern Argentina |

The main vectors responsible for the introduction of freshwater organisms in Brazil are aquaculture escapes (Ortega *et al.* 2015), the release of sport fish (Britton & Orsi 2012) and live bait (Garcia *et al.* 2015), the aquarium trade (Magalhães & Vitule 2013), and the use of fish as a biological control agent (Ortega *et al.* 2015). In Brazil, there are 22 freshwater ecoregions (Abell *et al.* 2008) and in many there are species translocation between them (Vitule *et al.* 2019). The release of live bait at the end of fisheries is a pathway of introducing species that receive little attention from inspection, should be considered due to the fishing tourism increase in the country.

*Dilocarcinus pagei* was the only introduced crustacean used as live bait (Catella *et al.* 2009, Latini *et al.* 2016). Native from the Paraguay and Lower Paraná River basins, *D. pagei* may have reached the Upper Paraná after filling the Itaipu Reservoir (Magalhães *et al.* 2005). Nevertheless, this is a species often created and used as bait to fish ‘piapara’ *Megaleporinus obtusidens* and ‘piavuçu’ *Megaleporinus macrocephalus*, and widely used as bait in Pantanal (Mussato *et al.* 2019).

Some species of shrimp, for example, *Macrobrachium rosenbergii* (native from the Indo-Pacific region), *Macrobrachium amazonicum* and *Macrobrachium jelskii* (both from the Orinoco, Amazon and Paraguay-Lower Paraná River basins) have been widely used in aquaculture (Magalhães *et al.* 2005). In addition, shrimp are also used as live bait (personal observation). Despite this, we did not find in the literature the record of introducing shrimp used as live bait in Brazil, but we warn that they may be translocated between basins due to this practice.

Fish used as live bait generally have accessory breathing, which allows good survival and is one of the reasons for its wide use in fishing. The low cost of the baits facilitates the acquisition, and anglers buy in large quantities from the lighters and release the baits remaining at the end of the fisheries into the rivers (Garcia *et al.* 2015, Ortega *et al.* 2015). In the Pantanal Mato-Grossense, the live bait trade is a source of income for the riverside community. The state of Mato Grosso do Sul applies the State Law 2,898/2004, which refers to the capture, transport, storage, marketing and culture of live baits (Catella *et al.* 2017). However, the export of live baits from the state of Mato Grosso do Sul, central-west Brazil (municipalities of Corumbá, Miranda and Porto Murtinho), to other regions of the country, such as the states of Paraná, Goiás and Santa Catarina, may introduce species (Catella *et al.* 2009). These states receive baits without the correct identification and inspection. In the Upper Paraná River basin, at least four species of *Gymnotus* may have been introduced by the release by anglers (Graça & Pavanelli 2007). This pathway introduced *Gymnotus pantanal* and *G. paraguensis* in the Upper Paraná Basin (Graça & Pavanelli 2007). Genetic studies have correctly identified *Gymnotus*, and provide information for conservation, fishing, trade regulation and monitoring of introduced species strategies (Orsi *et al.* 2016, Sousa *et al.* 2017, Faria-Pereira *et al.* 2019). Studies like these should be performed with all species used as live bait.

All *Gymnotus* species (electric fish) are often used as live baits, as they are considered efficient in catching several fish species in different ecoregions, facilitating their introduction due to disposal by ill-informed anglers who can translocate them between the basins. In the Pantanal of the state of Mato Grosso do Sul, *Gymnotus* spp. are the main live bait, accounting for more than three-quarters of sales (Sousa *et al.* 2017). These fish have high survival rate, withstanding poor maintenance conditions. Part of this is due to its ability to breathe atmospheric air through its swimming bladder system (Alho 2020).

The number of species introduced from the live bait release in the Upper Paraná River basin may be greater than our survey. For some species of Gymnotidae, Rhamphichthyidae and Erythrinidae there are uncertainties as to their origin, as they may also have been introduced by the transpose of the geographical barrier of the Saltos de Sete Quedas after the construction of the Itaipu Dam (Júlio Júnior *et al.* 2009) (Table 1). In addition, the Upper Paraná Basin is a donor of species that invaded the Iguaçu River Ecoregion, as the case of *Callichthys callichthys* and *Hoplosternum littorale* (armored catfishes). None of the Gymnotiformes species is native to the Iguaçu River: *G. inaequilabiatus*, *G. sylvius* and *Apteronotus ellisi*. The occurrence of *Synbranchus marmoratus* (swamp-eel) and *Steindachnerina brevipinna* (toothless characiform) in the Iguaçu River is also attributed to the bait release (Baumgartner *et al.* 2012, Daga *et al.* 2016).

The ecological risk of the live bait trade depends on the source of the bait (capture in wild or culture), the type of fishing (commercial or recreational), and the context of release (Drake & Mandrak 2014). Fish health and pathogen certification programs undertaken by live bait farmers are an important step in reducing the possibility of release. Surviving individuals, when released, can alter the food web and increase the habitat eutrophication (Gallardo *et al.* 2016).

We emphasize the importance of using native species as live baits. The lambari fish *Deuterodon iguape* is considered as bait on the southeastern coast of Brazil (Henriques *et al.* 2018). In the Upper Paraná Basin, several *Astyanax* species may be used as bait. However, care must be taken with fish to avoid genetic pollution (Porto-Foresti *et al.* 2001).

The income of bait anglers (‘isqueiros’) and their families depend on the development of the activity. Many of them farm baits without the correct aquaculture techniques and sell without supervision. This can lead to the release of individuals with low genetic variability or introgression of harmful genes, in addition to possible hybridism (Do Prado *et al.* 2012). Therefore, revisions to the legislation and a management plan for live baits are necessary (Sousa *et al.* 2017). Moreover, it is essential to carry out studies on biology, ecology and genetics to support the legislation, guarantee the sustainability of the activity and allow bait anglers to continue to access this natural resource.

The practice of release non-native live baits in different watersheds is evident, but it still needs studies to measure it at various scales. Thus, there is an urgent need to raise awareness among sectors related to commercial and sport fishing of this impact factor on aquatic biotas. The following should be encouraged: (i) divulgation programs to inform anglers and live bait farmers about the biological risks arising from release; (ii) environmental education programs on the social, economic and environmental problems of invasive species; (iii) creation of live baits only from native species to the hydrographic basin itself.

According to the National Environment Policy (Law Nº 6,938/1981), pollution is the degradation of environmental quality, which is an adverse change in the characteristics of the environment. Therefore, urgent awareness and inspection actions must be taken to avoid further impact on Brazilian freshwater ecosystems.

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