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THE HELMINTHIC PARASITE FAUNA OF THE WORLD-WIDE INVADER *MICROPTERUS SALMOIDES* (LACEPÉDE, 1802) IN BRAZIL: A CASE OF CO-INTRODUCTION AND SPILLBACK

HELMINTOS PARÁSITOS DE *MICROPTERUS SALMOIDES* (LACEPÉDE, 1802) UNA ESPECIE INVASORA EN BRASIL: UN CASO DE CO-INTRODUCCIÓN Y SPILLBACK

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ABSTRACT

The largemouth bass, *Micropterus salmoides* (Lacepéde, 1802), is a centrarchid fish from North America that is now globally distributed because of wide-spread introductions for sport fishing. Its introduction in Brazil dates from the 1920's, primarily in southern regions. *Micropterus salmoides* is already known to have created a series of impacts in the ecosystems in which it is established. However, its parasite fauna in Brazil is unknown. This opens the possibility of new interactions and potential impacts. Therefore, the goal of the present study was to analyze the parasite fauna of *M. salmoides* in four reservoirs in southern Brazil, measuring the prevalence and mean abundance in each reservoir. A total of 59 individuals of *M. salmoides* were analyzed, 15 from each reservoir, except for the Capivari-Cachoeira Reservoir, with 14 individuals. Of the fish analyzed, 91.5% were parasitized by 1567 parasites belonging to four species, three nematodes: larval *Contracaecum* sp. (86.4%), *Procamallanus* (*Procamallanus*) *peraccuratus* Pinto, Fabio, Noronha & Rolas 1976 (6.7 %) and *Hysterothylacium brachyurum* Ward & Magath 1917 (6.7 %), and one species of monogenean flatworm: *Onchocleidus principalis* (Mizelle, 1936) (57.6 %). From these results we can conclude that the process of co-introduction and spillback is still in the early stages, mostly by the low diversity of parasites. Therefore, monitoring and control actions are highly recommended in order to both control the impacts of parasite infections as well as to promote mitigation of activities and prevention campaigns.

Keywords: Biological invasion – Largemouthbass – Neotropical Ichytioparasitology –Nonnative parasites

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RESUMEN

La perca americana, *Micropterus salmoides* (Lacepéde, 1802), es un pez centrarchido de América del Norte que ahora tiene una distribución global debido a introducciones extendidas por pesca deportiva. Su introducción en Brasil data de 1920, principalmente en las regiones del sur. Sabemos que *M. salmoides* ha generado una serie de impactos en los ecosistemas en que se ha establecido. Sin embargo, sus parásitos en Brasil son desconocidos. Esto genera la posibilidad de nuevas interacciones y potenciales impactos. Por tanto, el objetivo de este estudio fue analizar los parásitos de *M. salmoides* en cuatro reservorios de agua en el sur de Brasil, midiendo la prevalencia y la abundancia media en cada reservorio. Analizamos un total de 59 individuos de *M. salmoides*, 15 en cada reserva, con excepción de la reserva Capivari-Cachoeira, con 14 individuos. De los peces analizados, 91,5% estaban parasitados por 1567 parásitos pertenecientes a cuatro especies, tres nemátodos: *Contracaecum* sp. (86,4%) en larvas, *Procamallanus (Procamallanus) peraccuratus* Pinto, Fábio, Noronha y Rolas 1976 (6,7%) y *Hysterothylacium brachyurum* Ward & Magath 1917 (6,7%), y una especie de monogenoideo *Onchocleidus principalis* (Mizelle, 1936) (57,6 %). De estos resultados podemos concluir que el proceso de co-introducción y "spillback" aún está en etapas tempranas, en la mayoría de las veces por la baja diversidad de parásitos. Sin embargo, el monitoreo y las acciones de control son altamente recomendadas para controlar los impactos de infecciones por parásitos y promover actividades de mitigación y campañas de prevención.

Palabras clave: Parásitos no nativos – perca americana – ictioparasitología neotropical – Invasión biológica

INTRODUCTION

Biological invasions leads to novel interactions and negative impacts in native ecosystems (Elton, 1958; Lockwood *et al*., 2007). Globalization and its routes of transport and commerce plays a role as vectors and pathways in introduction events and large-scale dispersion of many invaders (Hulme, 2009). And this species never comes alone, bringing with them new parasites and pathogens. Which are responsible for changing the balance of the community interactions in its new environment. (Lockwood *et al*., 2007, 2009; Cassey *et al*., 2018). The process of parasites co-introduction is not yet contemplated in most of the invasion frameworks, what is justified by the fact that the forces which influence the invasion in free living organisms is different than for parasites (Taraschewski, 2006; Hulme, 2009; Carrete *et al*., 2012; Blackburn & Ewen, 2017). For example, the stages of introduction and establishment of parasites depends on the prevalence rate in the introduced host and the probability of finding suitable hosts to complete its life cycle in the environment (Hatcher *et al*., 2012).

Host-parasite interaction can influence community structure in several ways (Price *et al*., 1986; Dunn & Hatcher, 2015; Calhoun *et al*., 2018). A parasite can interconnect a series of trophic levels through its life cycle, modulating the population growth of the host species and the apparent level of competition in the ecosystem (Prenter *et al*., 2004). When successfully co-introduced, a parasite species can spillover to native hosts in the new ecosystem, which may lead to a highly pathogenic interaction due to the lack of co-evolutionary history between the parasite and the new host. Besides that, a nonnative host can also be infected by native parasites, *i.e.* spillback, which can make this new host a reservoir for the native parasite species, increasing its prevalence in the ecosystem (Kelly *et al*., 2009; Dunn, 2009; Dunn & Hatcher, 2015). These two possible impacts are influenced by the encounter between susceptible and infected hosts (Telfer & Brown, 2012).

The fish *Micropterus salmoides* (Lacépède 1802) (Percifomes: Centrarchidae) is a species of great importance in sport fishing because of its characteristics as a top predator, including its large size and voracity (Jackson, 2002; Brown *et al*., 2009; Estes *et al*., 2011). The species is native to North America but is already established in more than 50 countries, and is responsible for changes in ecosystems around the world (Brown, 2009; Van Der Walt *et al*., 2016; Froese & Pauly, 2016). The impacts caused by *M. salmoides* have led its inclusion in the list of the 100 worst invasive species in the world, which makes its management and control a priority (Lowe *et al*., 2000). In Brazil, it was introduced in the 1920's and was originally established in the southern region (Schulz & Leal, 2005; Ribeiro *et al*., 2015; Frehse *et al.*, 2016).

The parasite fauna of *M. salmoides* is well known in its native area, although there are few studies where it has been introduced. Of those, none was concerning the ecological impacts of co-introduced parasites in the new environment (Costa *et al.*, 2018). Information concerning the possible direct and indirect impacts of *M. salmoides* in Brazil's native communities is essential to ensure effective management and control. The fact that *M. salmoides* is well established and can be found in great abundance in the south of Brazil increases the probability of co-introduction and spillover of parasites, leading to possible secondary impacts related to the invasion by *M. salmoides* (Taraschewski, 2006). Therefore, the present study aimed to provide an evaluation of the helminth parasite fauna of *M. salmoides* in Brazil and the possible impacts it may have on the invaded ecosystems.

MATERIAL AND METHODS

We sampled four reservoirs in South Brazil, on the metropolitan region of Curitiba, Paraná, Brazil. The reservoirs were chosen primarily by its abiotic similarities, are in the same basin, and have a small distance between each other. In the environmental matter they are all categorized as moderately degraded and are localized in the same basin with a maximum distance of 78 km by each other (Rodrigues *et al*., 2005; Brunkow *et al*., 2009; Xavier *et al*., 2009; Seara, 2010; Da Conceição *et al*., 2014).

The reservoirs sampled were: Piraquara I (25°29'48.1"S 49°01'05.0"W), Passaúna (25°27'41.2"S 49°22'58.4"W), Capivari-cachoeira (25°11'39.1"S 48°52'35.2"W) and Vossoroca (25°50'32.6"S 49°04'31.8"W).

The sampling effort was thought in a way that maintained the same number of hosts (15 hosts by reservoir) with similar average life stage, all adults. Samples were all collected in the spring of 2015, to decrease the influence of seasonality on the parasite diversity between the reservoirs. All individuals of *M. salmoides* were captured using rod, hook, and artificial baits, in the period necessary to reach the number of samples previous stipulated (Parana, 2005). The fishes sampled were anesthetized, killed by spinal section and taken to the Zoology Laboratory of the Federal University of Parana (Underwood *et al.*, 2013). There, they were numbered, measured (cm) (total and standard length) and weighed (g). The gastrointestinal tract and viscera were collect for endoparasite samples and gills were separated for ectoparasite analyzes, both were fixed in formol 5%. The parasites were collected, quantified and preserved in ethyl alcohol 70% and prepared for identification following the methods and protocols for which taxa described in Eiras *et al.* (2006). The species of parasites were identified using the classical studies of Margolis & Kabata (1984), Moravec (1998) and Hoffman (1999).

The parasite variables calculated were the parasite richness of each reservoir class. Abundance, prevalence and mean abundance of which parasite species followed by Bush *et al*. (1997).

Ethic aspects: The authors point out that they fulfilled all national and international ethical aspects.

RESULTS

A total of 59 individuals of *M. salmoides* were analyzed, 15 from each reservoir, except for Capivari-Cachoeira Reservoir, with 14 individuals. The mean and SD of the hosts total length analyzed was 31.41 ±3.50 cm in Passaúna reservoir, 28.7 ±7.03 cm in Piraquara I reservoir, 31.75 ±2.58 cm in Vossoroca reservoir and 31.94 ±7.19 cm in Capivari-cachoeira reservoir. All the reservoirs sampled had 100% of the fishes parasitized by some species of helminth, with exception of Capivari-Cachoeira reservoir, that had only 66.67 % of infected hosts. The parasites sampled belong to four species, three nematodes: *Contracaecum* sp. in their larval stage according to Moravec, Kohn & Fernandes, 1993 found encysted in the stomach external wall; *Procamallanus* (*Procamallanus*) *peraccuratus* Pinto, Fabio, Noronha and Rolas, 1976, and *Hysterothylacium brachyurum* Ward & Magath, 1917, both found inside the intestine of the hosts; and one species of monogenean infecting the host's gills: *Onchocleidus principalis* (Mizelle, 1936). Of these, *Contracaecum* sp. and *O. principalis* were recorded in all the reservoirs sampled, while *P. peraccuratus* and *H. branchiurum* were found in only one reservoir each. All the parasite variables of prevalence, mean abundance, number of parasite sampled by reservoir and number of infected fish can be seen in Table 1.

Table 1. Helminth parasites of *Micropterus salmoides* by reservoir sampled, all located in the metropolitan region of Curitiba, Paraná, Brazil. IF: Number of Infected Fish; NP: Number of Parasites; P%: Prevalence; MA±SD: Mean Abundance ± Standard Deviation.

Reservoir	Parasites	IF	NP	P%	MA ±SD
Passaúna (N=15)	*P. peraccuratus*	1	1	6.7	0.07 ±0.3
	O. principalis	2	5	13.3	0.33 ±0.9
	Contracaecum sp.	15	149	100	9.9 ±11.8
Piraquara (N=15)	*H. branchiurum*	1	1	6.7	0.07 ±0.3
	O. principalis	15	237	100	15.8 ±15.3
	Contracaecum sp.	15	394	100	26.7 ±15.2
Capivari-cachoeira (N=14)	*O. principalis*	2	4	14	0.3 ±0.8
	Contracaecum sp.	7	70	50	5 ±11.07
Vossoroca (=15)	*Contracaecum* sp.	14	387	93	21.7 ±19.7
	O. principalis	15	319	100	25.8 ±14.2
Total	*H. branchiurum*	1	1	6.7	0.07 ±0.3
	P. peraccuratus	1	1	6.7	0.07 ±0.3
	O. principalis.	34	565	57.6	16.6 ±15.18
	Contracaecum sp	51	1000	86.4	19.6 ±16.8

DISCUSSION

The invasion of *M. salmoides* is responsible for several disturbs in places where it was introduced, although the impacts related to its parasite community in Brazil were not known until the present study (Ribeiro *et al*., 2015; Costa *et al*., 2018). The native helminth fauna of the fish *M. salmoides* is well studied, with more than 50 species of parasites recorded in its native region (Hoffman, 1999; Costa *et al*., 2018). The nematodes *Contracaecum* sp. and *H. branchiurum* observed during the present study have also been noted parasitizing *M. salmoides* in its native environment (Hoffman, 1999; Tavakol *et al*., 2015). The same is true for the monogenean *O. principalis* (Galaviz-Silva *et al*., 2016). However, the nematode *P. peraccuratus* is a parasite native to South America, which makes its infection in *M. salmoides* a possible instance of parasite spillback (Azevedo *et al*., 2006; Takemoto *et al*., 2009).

The helminth parasite fauna observed in the present study showed low species diversity in comparison with studies conducted in the native region (Costa *et al*., 2018). It is known that most of the parasite community are introduced with it host, although is probably lost on the initial stages of introduction. Mainly by its difficulty of adaptation in the nonnative environment (MacLeod *et al*., 2010; Carrete *et al*., 2012; Lymbery *et al*., 2014, Blackburn & Ewen, 2017). Yet, highly stress situation in the capture and transportation of nonnative hosts can influence its immunologic conditions, selecting more resistant propagules (Carrete *et al*., 2012). The introduction of *M. salmoides* in the reservoirs sampled during the present investigation is still recent (the reservoirs were constructed from the 1960's to the 1980's). So, once *M. salmoides* are constantly introduced in reservoir by multiple fonts (mostly fishermen) of several distinguish life stage and local fonts, the richness and prevalence of nonnative parasite may increase with time, as its chances to establish in a nonnative environment (e.g. Vitule *et al*., 2009; Ribeiro *et al*., 2015).

In the present study only one specimen of both *H. brachyurum* and *P. peraccuratus* was found. The nematode *H. brachyurum* is commonly found parasitizing the genus *Micropterus*. It has been registered previously in the species *M. dolomieu*, in Michigan, and in *M. salmoides*, also in the USA (Amin & Minckley, 1996; Gopar-Merino *et al*., 2005); this paper represents the first record of the species in Brazil. As for *P. peraccuratus,* it has only been recorded in Brazil, parasiting primarily cichlids (Moravec *et al.*, 1993; Azevedo *et al*., 2006; Takemoto *et al*., 2009). According to standard practice, a host-parasite relationship is only considered to be effective if it results in at least one other case of parasite infection, *i.e.* when the rate of the parasite reproduction in the new host is greater than one (Hatcher *et al*., 2012, Blackburn & Ewen, 2017), or if the prevalence of infection is greater than 10% (Bush *et al*., 1990). Although more studies need to be done to confirm both infections, we cannot discard the possibility of a co- introduction event in the case of *H. brachyurum* and a spillback event for *P. peraccuratus* (Kelly *et al*., 2009). In relation to *Contracaecum* sp., its larval stage is very generalist and are globally distributed, once its final host are mainly piscivorous birds (Madi & Silva, 2005; Takemoto *et al*., 2009; Tavakol *et al*., 2015). This parasite has a complex life cycle, and *M. salmoides*, among other intermediary host, has its infection influenced by its trophic level; *i.e.* top predators typically have a higher probability of parasite infection (Lafferty & Morris, 1996; Poulin & Leung, 2011; Chen *et al.*, 2008).

The species *O. principalis* has a high level of specificity to the genus *Micropterus*, what makes it presence an event of co-introduction (Maitland & Price, 1969; Margolis & Kabata, 1984; Collins & Janovy, 2003). Although, over the several monogenean species that parasite *M. salmoides* (Hoffman, 1999; Galaviz-Silva *et al*., 2016; Costa *et al*., 2018), only one was found in this study. This shows a clear example of enemy release, still the high abundance of *O. principalis* in two of the reservoirs sampled could be a compensation of the poor parasite richness (Colautti *et al*., 2004; Roche *et al*., 2010).

This study shows only a preliminary sample of the parasite community of *M. salmoides* in Brazil, what may increase in studies in other regions that include seasonal samples. This allied to a constant monitoring of the impacts made by these parasites on the nonnative environments of *M. salmoides*. However, we presented important information for the introduction management and control for nonnative hosts and its parasites. The parasite fauna of *M. salmoides* can lead to multiple scenarios of indirect impact. For example, the increase in the prevalence of native parasites caused by *M. salmoides* serving as a reservoir for infection can facilitate the invasion success of the host. The presence of *M. salmoides* can also lead to a decrease in the prevalence and intensity of native parasites in the cases where *M. salmoides* does not serve as a proper host that can be included in the parasite's life cycle. Furthermore, the co-introduction of parasites may lead to emergent diseases in the new environment because of the lack of co-evolutionnary history in the host-parasite relation, or because of the occurrence of apparent competition, which may decrease the population of native fishes in the ecosystem (Strauss *et al*., 2012; Blackburn & Ewen, 2017; Young *et al*., 2016). Finally, more than that, the parasite community dynamics in a nonnative host may suffer temporal variations (tend to increase its richness over time), increasing the links of connectance and nestedness in trophic networks, or changing its patterns of predation and competition; primordially in the early stages of the host introduction. The current paper represents a first record of the *M. salmoides* parasite fauna in Brazil. From the results cited we can conclude that the process of co-introduction and spillback is still in the early stages and that management actions are highly recommended in order to both control the impacts of parasite infections as well as to promote mitigation activities.

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