Could native predators help to control invasive fishes? Microcosm experiments with the Neotropical characid, *Brycon orbignyanus*


Abstract – The potential of the native characid, *Brycon orbignyanus*, to help to control some invasive fish species currently present in the Paraná River basin was addressed through microcosm experiments. Juveniles of three invasive (*Cichla piquiti*, *Oreochromis niloticus* and *Ictalurus punctatus*) and two native (*Astyanax altiparanae* and *Prochilodus lineatus*) fish species were offered as prey to *B. orbignyanus* in 300-l aquarium trials with three habitat complexity treatments (0%, 50% and 100% structure). Prey survival was much more variable among species (*I. punctatus* < *C. piquiti* < *O. niloticus* ~ *P. lineatus* ~ *A. altiparanae*) than through time, and *B. orbignyanus* predation was clearly mediated by habitat complexity, with lower prey survival in low-complexity treatments. The lower survival observed for *C. piquiti* could be partially explained by its less active behaviour and its preference for a surface microhabitat and less structured areas. Prey size also affected predation rates, as for the three less consumed species the largest individuals were significantly preyed on. Our results suggest that preserving and restoring the populations of *B. orbignyanus* might help to control some fish species introduced to the Paraná River basin, particularly *I. punctatus* and *C. piquiti*.

**Introduction**

Freshwater species and habitats are among the most imperilled in the world and introduction of non-native species is one of the leading threats to freshwater biodiversity (Saunders et al. 2002; Clavero & García-Berthou 2005). It is thus expected that conservation biologists will be challenged to manage and control invasive species in the next decades (Allendorf & Lundquist 2003). However, freshwater ecosystems have received little attention with respect to the control or eradication of successful invaders, especially in the case of invasive fishes (Wittenberg & Cock 2001; Simberloff 2003). Instead, the spread of invasive fish has been favoured by human activities, with most introductions succeeding in the Neotropical region and particularly in Brazil (Welcomme 1988), where several impacts on native species have been reported (Santos & Formagio 2000; Latini & Petrere 2004; Agostinho et al. 2006).

The Paraná River is the 10th largest river in the world and the second in South America, harbouring more than 250 freshwater fish species in its upper stretches (Agostinho et al. 2007). It also provides most of national hydroelectric energy supply and inland fishery catches (Agostinho et al. 2007). Nowadays, several medium- to large-sized invasive fishes (>300 mm total length (TL)) have become established in the Paraná River basin, as a result of misguided stocking programmes undertaken in the 1980s and aimed at mitigating the impacts of reservoirs on fisheries (Agostinho et al. 2006). Invasive species have been identified as one of the leading factors in the decline of its native fish richness, also contributing to
the reduction in the density of the formerly abundant migratory fishes (e.g., *Brycon orbignyanus*, *Salminus brasiliensis* and *Pseudoplatystoma corruscans*) (Agostinho et al. 2003).

The mechanisms that mediate the establishment and impact of invasive fish remain poorly understood (Alcaraz et al. 2008) and options to control or eradicate invasive fishes are virtually unknown (Strong & Pemberton 2000; Wittenberg & Cock 2001; Simberloff 2003). Although experiments to assess the role of prey morphology and behaviour and habitat complexity on predator–prey fish interactions have been widely used (Savino & Stein 1982; Juanes et al. 2002), most of the novel trophic interactions between native and invasive fishes are poorly understood (García-Berthou 2007) and we are not aware of any experimental study on the interactions of invasive and native Neotropical fishes.

In this study, the potential of *B. orbignyanus* to control some invasive species currently introduced to the Paraná River was experimentally addressed through microcosm trials. *Brycon orbignyanus* is a native, medium-sized (<500 mm TL) reophilic characid with historical importance to local fisheries. This species is currently endangered as a consequence of multiple anthropogenic impacts, such as hydrological alterations, pollution, habitat loss, overfishing and introduction of non-native species (Agostinho et al. 2003; Machado et al. 2007). The objectives of our study were: (i) to test whether *B. orbignyanus* piscivory affects the survival and behaviour of native and introduced fishes of the Paraná river basin; and (ii) to investigate how habitat complexity, predator and prey behaviour and prey size mediate these predator–prey relationships.

**Methods**

**Experimental trials**

Two native (*Astyanax altiparanae* and *Prochilodus lineatus*) and three invasive (*Cichla piquiti*, *Oreochromis niloticus* and *Ictalurus punctatus*) species were offered as prey to juvenile *B. orbignyanus*, an endangered fish native to the Paraná River basin (Machado et al. 2007). Native prey species were chosen because of their relatively high abundance and wide distribution in the Paraná River (Agostinho et al. 1997), whereas invasive species were selected because of their presence in many South American ecosystems, its likely impacts on native taxa (Agostinho et al. 2006), and the increasing use of *O. niloticus* and *I. punctatus* in Brazilian aquaculture (Vitule et al. 2009). All the fishes used in the experiments came from local fish farms, from which they were acquired in January 2006.

Predation trials were performed in microcosms (300-l glass aquaria, 100 × 50 × 60 cm). To simulate submersed macrophytes, we used plastic filaments, extending from the bottom to the surface of the aquaria, with a density of ca. 250 filaments per m², following Savino & Stein (1982). We used three treatments of habitat complexity: 0%, no plastic filaments; 50%, intermediate complexity, with plastic filaments covering one half of the aquarium surface; 100%, high complexity, with plastic filaments covering the entire aquarium.

All fish were acclimated to aquarium conditions 15 days before performing the trials, and treated with fungicide and bactericide solution to prevent infections. Fish were anaesthetised with Eugenol (5 ml per 20 l of water) 48 h prior to the experiments and measured (TL and total weight) to assess size-selective predation (see below). The nine individuals of *B. orbignyanus* used in the trials averaged 133.9 mm (SE = 0.12) in TL, and 31.3 g (SE = 1.17) in weight, whereas prey individuals (*N* = 540, including control treatments) measured 38.9 mm (SE = 0.34) TL and 0.68 g (SE = 0.02) on average. To mimic natural conditions and to reduce the opportunistic predation over disoriented prey, all aquaria were first stocked with prey and the predator was stocked 3 h later. Only one *B. orbignyanus* was stocked in each aquarium with six individuals of each prey species (i.e., a total of 30 prey individuals per aquarium). The fish density used in the treatments is similar to natural densities of juvenile fish in the lagoons and littoral areas of the Paraná River (Pelicce et al. 2005; Bulla 2006). Control groups (same prey density, no predator) were also used to test prey survival and behaviour in the absence of predation risk.

Three replicates were performed for each treatment and all trials, carried out within 4 days, were exposed to a 12D:12L light regime. The number of surviving prey and the behaviour of both predator and prey were recorded at 4-h time intervals (at 9:00, 13:00, 17:00 and 21:00 hours). Fish behaviour was assessed directly through visual inspection, and each visual census took ca. 5 min per aquarium.

Three behavioural traits were measured for *B. orbignyanus*: (i) microhabitat use (surface, water column or bottom); (ii) refuge use (sheltered, hiding within submersed structures; partially sheltered, using the interface between structured and unstructured areas; or not-sheltered, occupying open water areas); and (iii) activity (immobile, swimming, pursuing or attacking). For each prey species, four behavioural traits were measured (as for predators, except when specified): microhabitat use; refuge use; schooling (yes/no); and activity (immobile, swimming, or escaping). Subsequent to the first census of the fifth day, all aquaria were emptied and the length of the remaining fishes was measured to compare it with the
length of the fish stocked and so to test for size-selectivity in predation.

Statistical analyses

Prey survival and behavioural traits of both predator and prey were compared among treatments (predator presence and habitat complexity) through time with repeated-measures analysis of variance (rm-ANOVA). All the factors were considered as of fixed effects in the rm-ANOVAs. In addition to P-values (statistical significance), we also report partial eta squared (η²), as a measure of effect size (i.e., biological significance or importance of factors). Partial η² is the proportion of variation explained for a certain effect [effect sum-of-squares (SS)/(effect SS + error SS)] but is better than conventional η² (effect SS/total SS) because it does not depend on the number of sources of variation in the ANOVA design used, as it does not use the total SS as the denominator (Tabachnick & Fidell 2001). These measures of effect size have the advantage over P-values that do not rely on arbitrary significant/non-significant dichotomy and allow the proper comparison of treatments in contrast to P-values (e.g., a very low P-value does not necessarily mean that a factor has a strong/stronger effect). Proportions (prey survival) were arcsine-transformed (arcsine ½x) for statistical analyses because normality and homoscedasticity were clearly improved. A conventional two-way ANOVA (with habitat complexity and before/after predation as factors) was performed for each prey species to compare the sizes (TL) of the surviving individuals and those initially stocked in each aquarium (i.e., to compare the sizes of prey fish before and after the B. orbignyanus predation treatment and so to assess size selectivity). All statistical analyses were performed with spss 15® (SPSS Inc., Chicago, IL, USA).

Results

Prey survival

The presence of B. orbignyanus significantly influenced prey survival (Table 1, Fig. 1), which was 8–32% higher in predator-free trials. The effect of predator presence varied significantly among prey species (predator × prey species interaction, Table 1) and the invasive I. punctatus and C. piquiti were the most consumed prey species during the entire trial period (Table 1, Fig. 2), whereas the invasive O. niloticus and the native A. altiparanae and P. lineatus were the least consumed. Habitat complexity significantly affected prey survival (Table 1), with higher predator success in trials with low habitat complexity (0% and 50% of cover) (Fig. 1). The three-way and prey species × habitat complexity interactions were not significant, indicating that the effect of habitat complexity on prey survival was similar for the different prey species. Overall, predator and prey species were the most important sources of variation followed by the predator × prey species interaction (η², Table 1).

Predator and prey behaviour

Activity of the predator changed significantly with habitat complexity (rm-ANOVA: \( F_{2,96} = 18.6, P < 0.01 \)), with a constant swimming activity in 0% structure treatment, a motionless behaviour in 50% structure, and intermediate in 100% structure treatments. Significant temporal variation in predator behaviour was only recorded for microhabitat use (rm-ANOVA: \( F_{16,96} = 2.6, P < 0.01 \)), with B. orbignyanus occupying preferentially the surface areas in the first 52 h of the experimental trial and the bottom areas afterwards. Significant habitat complexity × time interaction was also observed for microhabitat use.
For the microhabitat use of prey, predator presence × habitat complexity interaction was significant for *C. piquiti* and *P. lineatus* and habitat complexity was the most important source of variation (Table 2). *Cichla piquiti* remained primarily at the surface in all treatments but increased the use of bottom areas in 50% structure treatments and of surface areas in 0% and 100% treatments in the presence of *B. orbignyanus*. *Prochilodus lineatus* preferred the bottom areas and the water column in all treatments, except in structure-less ones in which it increased the use of surface areas in predator presence (Fig. 3). Microhabitat use of other species did not vary with habitat complexity and predator presence, with *I. punctatus* and *O. niloticus* mainly using bottom areas and *A. altiparanae* the water column (Fig. 3).

Because unavailability of shelter in 0% structure treatments and of open water areas in 100% treatments, refuge use preferences in the absence of a predator can only be assessed in 50% treatments. In these treatments, *C. piquiti* and *I. punctatus* selected sheltered areas, whereas *O. niloticus*, *A. altiparanae* and *P. lineatus* used both open water and sheltered areas (Fig. 4). Refuge use changed significantly with predator presence for *C. piquiti*, *A. altiparanae* and *P. lineatus* (Table 2). *Cichla piquiti* used mainly structure-less areas whereas *A. altiparanae* and *P. lineatus* used more structured areas in the presence of *B. orbignyanus* (Fig. 4). Significant predator presence × habitat complexity interaction in refuge use was detected for *C. piquiti*, which used more open areas, and for *A. altiparanae*, which preferred more covered areas in 50% structure treatments in predator presence (Fig. 4).

For schooling behaviour, predator presence × habitat complexity interaction was always the most
important source of variation and was significant for all prey species except *C. piquiti* (Table 2). This interaction reflects that all species rather schooled in predator’s absence, whereas predator’s presence increased prey schooling behaviour in the 100% cover treatments but decreased it in the low habitat complexity treatments (Fig. 5).

For prey activity, predator × habitat complexity interaction was significant for *A. altiparanae* and *P. lineatus*, which were less active mostly in the 50% structure treatment with predator presence (Table 2). Overall, all prey species exhibited less active behaviour in the presence of *B. orbignyanus*. Habitat complexity did not significantly affect the activity level of *C. piquiti* and *O. niloticus* (Fig. 6).

**Prey size**

The comparison of the sizes of fish stocked in each aquarium with those of fish surviving at the end of the experiment (Fig. 7) showed that mean size of surviving prey was significantly smaller for *P. lineatus*, *O. niloticus* and *A. altiparanae* (two-way ANOVAs: $F_{1,73–78} \geq 6.2, \ P \leq 0.02$), with no significant
differences for *I. punctatus* and *C. piquiti* (ANOVA: $F_{1,56-64} < 1.9$, $P \geq 0.17$). In contrast, there were not significant effects of habitat complexity ($F_{2,56-78} \leq 2.6$, $P \geq 0.06$) or before–after × habitat complexity interaction (ANOVA: $F_{1.56-78} \leq 2.7$, $P \geq 0.08$) in the size of any species. Therefore, *B. orbignyanus* significantly affected the size structure of *O. niloticus*, *A. altiparanae* and *P. lineatus*, preying preferentially on larger individuals. Moreover, prey species per cent survival was well correlated (Spearman’s $r = 0.88$; $P < 0.05$; $N = 5$ species) with the effect of predation on size structure (mean TL of survivors – mean TL of initial stocking). Therefore, *I. punctatus* and *C. piquiti* were the most consumed prey with few individuals surviving in the predation treatments, confirming that size was less important for these species; conversely, for the other less consumed species, larger individuals were more vulnerable to predation.

**Discussion**

Potential of *B. orbignyanus* to control invasive fish species

*Ictalurus punctatus* and *C. piquiti* were by far the prey species most consumed by *B. orbignyanus,*
respectivey surviving 45% and 25% less on average than the other three prey species. Of the 54 individuals of each prey species initially stocked in all treatments with B. orbignyanus, only six I. punctatus (11.1%) and 14 C. piquiti (25.9%) survived, corresponding to an overall intake of 1.3 I. punctatus and 1.1 C. piquiti per predator per day. We are not aware of any similar study in the literature but our findings strongly contrast those from an experiment (A.F.G.N. Santos, unpublished data) in which juvenile S. brasiliensis was tested as native predator. With the same experimental conditions, the indigenous P. lineatus was the most consumed prey by S. brasiliensis, whereas I. punctatus was the least consumed. The only similarity between both studies is that C. piquiti was the second most consumed prey by both B. orbignyanus and S. brasiliensis.

As juvenile B. orbignyanus was more effective than S. brasiliensis in preying on the same invasive fish species, the former species is apparently a better candidate to control the populations of I. punctatus and C. piquiti introduced into the Paraná River basin. Furthermore, our experimental results lead to further predictions about the predation risk of the other three less consumed prey species. First, the potential of B. orbignyanus to control O. niloticus in natural systems is expected to be weak, as this species displayed the greatest survival at the end of the experiments. Second, as the vulnerability of A. altiparancae and P. lineatus to B. orbignyanus piscivory was also low, probably because of their shared evolutionary history in the Paraná River, no adverse effects of B. orbignyanus predation would be expected on these native prey species in the natural systems.

Brycon orbignyanus piscivory was clearly mediated by habitat complexity, with greater predator effectiveness in low-complexity treatments (0% and 50% structure). When accounting for the differential effects of habitat complexity, the consumption of the invasive prey species was even greater, as no I. punctatus survived in 0% and 50% structure treatments, and only three C. piquiti survived at 50% structure treatments (e.g., all C. piquiti were eaten in 0% structure). Habitat complexity has often been addressed to investigate predator-prey interactions and the lower efficiency of B. orbignyanus to predate on more structurally complex habitats might be attributed to a decline in its swimming performance and in maintaining visual contact with prey as well as to a greater ability of prey to take refuge in the artificial vegetation. These results agree well with the usual patterns of higher predation rates in low-complexity habitats lacking prey refuges (e.g., Savino & Stein 1982; Bettolli et al. 1992). However, they strongly contrast with the findings of the companion experiment with juvenile S. brasiliensis (A.F.G.N. Santos, unpublished data), which was more effective in high-complexity treatments. Brycon orbignyanus and S. brasiliensis are two closely related characids, which share similar ecological niches when juveniles (Agostinho et al. 2003). However, their broad deviations on prey species preferences and prey consumption rates with habitat complexity indicate that Neotropical piscivores have an interesting potential to control invasive species but their effectiveness is highly variable, depending on species-specific life-history traits.

Species-specific vulnerability to predation

Apart from the effects of habitat complexity, prey vulnerability to B. orbignyanus piscivory was highly variable among prey species (I. punctatus > C. piquiti > O. niloticus ~ P. lineatus ~ A. altiparancae) rather than through time. Predation vulnerability seemed related, at least in part, to species-specific behavioural and morphological features, as the two most consumed species (I. punctatus and C. piquiti) displayed a suite of characteristics quite different to the others species. In the low-complexity treatments (0% and 50% structures) with predator presence, C. piquiti reduced its activity, preferentially occupying the surface and structure-less areas. Brycon orbignyanus predation was greater in low-complexity treatments and Savino & Stein (1982) observed a high vulnerability to Micropterus salmoides predation by nonschooling immobile bluegill, Lepomis macrochirus, occupying the top edges of the pool. Therefore, the conjunction of behavioural traits displayed by C. piquiti, which was not shared by the other four prey species, could be the primary cause of its high predation vulnerability.

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Fig. 7. Total length (mm) of prey species before (□ = initial stocking) and after (■ = surviving individuals) predation effects of Brycon orbignyanus. Vertical lines correspond to standard errors.
Despite also remaining inactive in the low complexity treatments, \textit{I. punctatus} behaved quite differently to \textit{C. piquiti} in other aspects. \textit{Brycon orbignyanus} attacked \textit{I. punctatus} in a very aggressive way, pursuing and masticating them several times, and finally swallowing them only 30–90 s afterwards (A.F.G.N. Santos, personal observation). \textit{Ictalurus punctatus} preferentially used bottom and structured areas, persisting in a scattering behaviour in predator’s presence. These behavioural patterns cannot clearly explain the greater vulnerability of \textit{I. punctatus} to \textit{B. orbignyanus} predation, as they were also present in the three least consumed species. The only observed behavioural difference from \textit{I. punctatus} was the higher swimming activity of \textit{A. altiparanae} and \textit{P. lineatus} in predator’s presence. As swimming performance can crucially affect prey fish survival (Juanes et al. 2002; Wolter & Arlinghaus 2003), the more active swimming of \textit{A. altiparanae} and \textit{P. lineatus} might have provided them some advantages against \textit{B. orbignyanus} predation. We also hypothesise that the stridulatory sounds produced by catfish with their pectoral spines (Fine & Ladich 2003) might lead to opposite results than for their native predators, triggering the attacks of \textit{B. orbignyanus} instead of reducing predation rates.

The predation by \textit{B. orbignyanus} was also mediated by prey size: for the three least consumed prey species, the predator selected the largest individuals, whereas there was no significant effect on predation on size structure for the most vulnerable species (\textit{C. piquiti} and \textit{I. punctatus}; respectively, the smallest and largest prey used in the experiments).

Management implications

Our results suggest that juvenile \textit{B. orbignyanus} is an interesting candidate to help to control some fish species introduced to the Paraná River basin, particularly \textit{I. punctatus} and \textit{C. piquiti}. \textit{Ictalurus punctatus} is not widespread in the Paraná River basin (Agostinho & Gomes 1997) but increasingly used and escaping from aquaculture facilities (Vitule et al. 2009), so enhancing the populations of \textit{B. orbignyanus} might help to limit its spread. \textit{C. piquiti} was first recorded in the Paraná River basin in the early 1980s (Agostinho et al. 2006, 2007) and is presently widespread, with stable populations in numerous reservoirs, marginal lagoons and rivers of this huge ecosystem. Given the predation rates observed for this species in the experimental trials and that young \textit{C. piquiti} generally receive intensive parental care in nature (Lowe-McConnell 1991), \textit{B. orbignyanus} may help to decrease the density of this invasive species in natural ecosystems but is unlikely to eradicate it.

The effectiveness of \textit{B. orbignyanus} to prey on both \textit{I. punctatus} and \textit{C. piquiti} might be greater in natural systems with low levels of habitat complexity, such as in the main river channels and in those marginal lagoons in which macrophytes and other submersed structures are not excessively abundant. \textit{Brycon orbignyanus} is, however, a native species presently endangered following the IUCN criteria (Machado et al. 2007) and there are several pleas for implanting management actions to promote specifically its population recovery in the Paraná River basin. Fishery restrictions, flooding pulse rehabilitation and scientific stocking programs are amongst the primary management actions to be undertaken (Agostinho et al. 2003). If these management actions effectively succeed in restoring \textit{B. orbignyanus} populations, they would be an excellent opportunity to test whether (i) the results of our experimental trials scale up at very large scales (Lodge et al. 1998) and (ii) whether the recovery of endangered native predators reduces the success of invasive fish species in the Paraná River and other diverse and large freshwater ecosystems in South America.

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**References**


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