

Fish fauna disassembly after the introduction of a voracious predator: main drivers and the role of the invader's demography

Fernando Mayer Pelicice · João Dirço Latini ·
Angelo Antonio Agostinho

Received: 25 November 2013 / Revised: 27 April 2014 / Accepted: 12 May 2014 / Published online: 25 May 2014
© Springer International Publishing Switzerland 2014

Abstract The introduction of *Cichla kelberi* to the Rosana Reservoir (Paraná River basin, Brazil) was followed by a substantial loss of fish diversity in macrophyte patches, and this study investigated the hypothesis that *C. kelberi* was the driver of faunal disassembly via certain demographic dynamics (i.e. a pulse of juvenile fish). We analysed the variation in the structure of fish assemblages, the abundance of *C. kelberi* and habitat quality between 2003 and 2007, including time points that preceded and followed the introduction of the predator. A stepwise regression analysis showed that macrophyte biomass was positively correlated with assemblage attributes (richness and abundance), whereas *C. kelberi* density showed a strong negative correlation. Variables related to habitat quality were not included in the model, and exhibited little variation over the study years. As predicted, the density of small-bodied fish was negatively associated

with the pulse of juvenile *C. kelberi*, and a tethering experiment revealed that predation pressure increased in the macrophyte patches when young *C. kelberi* were abundant. This study therefore obtained strong evidence supporting the hypotheses that *C. kelberi* caused the fish fauna disassembly and that the predator's demography was the driver underlying the diversity loss.

Keywords Invasion · Alien fish · Diversity loss · *Cichla* · Reservoir · South America

Introduction

The introduction of freshwater fish has become a global issue. Fish species have been transferred between basins and continents, largely because many of these species are valuable in aquaculture and fishing activities (Cambray, 2003; Ruesink, 2005; Casal, 2006). Although numerous examples exist to show the negative consequences that follow the introduction of non-native fish (e.g. Rahel, 2007; Cucherousset & Olden, 2011; Simberloff et al., 2013), it has been difficult to unequivocally demonstrate the main effects associated with introduction events (Light & Marchetti, 2007; Leprieur et al., 2008, 2009). This difficulty may partly explain the existence of different points of view within the discipline (e.g. Gozlan, 2008; Vitule et al., 2009, 2012a; Davis et al., 2011; Schlaepfer et al., 2011; Lövei et al., 2012).

Introductions of freshwater fishes in South America have occurred under this scenario. In particular, studies

Guest editors: Sidinei M. Thomaz, Katya E. Kovalenko, John E. Havel & Lee B. Kats / Aquatic Invasive Species

F. M. Pelicice (✉)
Núcleo de Estudos Ambientais, Universidade Federal de Tocantins, Rua 3, Quadra 11 17, Jardim dos Ipês,
Porto Nacional, TO 77500-000, Brazil
e-mail: fmpelicice@gmail.com

J. D. Latini · A. A. Agostinho
Núcleo de Pesquisas em Limnologia, Ictiologia e
Aqüicultura (NUPELIA)/DBI, Universidade Estadual de Maringá, Maringá, PR, Brazil

have documented the introduction of a number of fish species (e.g. Agostinho et al., 2007; Júlio et al., 2009; Vitule et al., 2009; Britton & Orsi, 2012) and recorded declines or changes in native biodiversity after introductions (e.g. Latini & Petrere Junior, 2004; Pelicice & Agostinho, 2009; Attayde et al., 2011; Vitule et al., 2012b). However, most of these studies were unable to provide evidence showing that non-native species disturbed resident communities. An emblematic example of the gap between introductions and biodiversity losses is provided by the introductions of peacock bass species (genus *Cichla*), which are voracious predators native to the Amazon basin. These fish have been extensively introduced in different basins in South America, establishing populations in many hydroelectric reservoirs across Brazil (Agostinho et al., 2007; Espínola et al., 2010). Significant changes in resident fish populations have been reported (Latini & Petrere Junior, 2004; Fugi et al., 2008; Pinto-Coelho et al., 2008; Menezes et al., 2012), but no study has investigated in detail the connection between events. For different methodological reasons, these previous studies merely described changes in fish diversity, acknowledging the peacock bass as the driver of impacts.

The recent introduction of *Cichla kelberi* Kullander & Ferreira in the Rosana Reservoir (Parapanema River, Brazil) illustrates this situation. The introduction of this predator was followed by the progressive decline of small-bodied fish (Pelicice & Agostinho, 2009), which formed assemblages closely associated with patches of *Egeria*, a submerged macrophyte (Pelicice et al., 2005). Although this fish fauna disassembly was a tangible event, it was not possible to conclusively acknowledge the non-native predator as its main driver, particularly because the context supported alternative explanations. For example, fish communities in reservoirs are subject to sudden changes in environmental quality as a result of dam operation procedures (water level variations; Agostinho et al., 2007), human activities (Barrela & Petrere, 2003; Miranda et al., 2010) or intense colonisation by macrophytes (Miranda & Hodges, 2000; Van Nes et al., 2002). In such situations, fish assemblages may experience rapid and profound changes in their structure and organisation. Therefore, the introduction of *C. kelberi* in the Rosana Reservoir, as well as the introductions of *Cichla* species documented elsewhere, demand further investigation and sound evidence to allow a conclusion to be reached regarding

whether these predators are drivers or passengers (MacDougall & Turkington, 2005; Light & Marchetti, 2007; Thomaz et al., 2012) of the biotic changes in reservoirs. Furthermore, although *Cichla* species are voracious piscivores, it is largely unknown how these fish affect resident fauna, primarily because experimental studies have provided evidence against the prey naiveté hypothesis (Kovalenko et al., 2010a).

Under this scenario, this study provides a body of evidence that support the hypothesis that *C. kelberi* was the driver of faunal disassembly in the Rosana Reservoir. We analysed the variations in the structure of the fish assemblages, the abundance of *C. kelberi* and habitat conditions between 2003 and 2007, including time points that preceded and followed the introduction of the predator. In addition, considering that changes in the fish fauna structure occurred specifically during warm months (Pelicice & Agostinho, 2009), we provide evidence to support the hypothesis that the demographic dynamics of *C. kelberi* (i.e. the reproduction and consequent pulse of juvenile fish) were linked to the fauna disassembly. This study, therefore, provides the best evidence available to support the idea that *Cichla* invasion can disrupt fish assemblages in littoral areas of Neotropical reservoirs.

Materials and methods

Study area

The Rosana Reservoir is the last of a series of impoundments that regulate the discharge of the Parapanema River, one of the main tributaries of the Upper Paraná River (22°36'S and 52°52'W). The dam was closed in 1986 for hydroelectric production and created a shallow reservoir with a surface area of 276 km². Submerged macrophytes, especially *Egeria densa* and *E. najas*, are very abundant in terms of their spatial distribution and coverage (depths <5 m). The introduction of *C. kelberi* in this reservoir is recent; local fishermen informed the researchers that this species began to appear in captures in 2004 (approximately), and the species was first sampled in 2005 (Pelicice & Agostinho, 2009). The dispersal of this species from the Capivara Reservoir (~150 km upstream), or even new clandestine fish releases, are possible causes of its introduction in this reservoir. *Cichla kelberi* is native to the Tocantins River Basin,

Table 1 Sampling schedule in the Rosana Reservoir, indicating the date, season and sample size (throw traps and seines) for each period

| Periods | Date | Season | Throw trap | Seine |
|---------|----------------|-------------|------------|-------|
| Before | Jan. 2003 | Summer | 19 | |
| After-1 | Mar.–Apr. 2005 | Late Summer | 20 | |
| After-2 | Nov. 2005 | Late Spring | 23 | 7 |
| After-3 | Mar.–Apr. 2006 | Late Summer | 20 | 9 |
| After-4 | Dec. 2006 | Late Spring | 21 | 9 |
| After-5 | Mar.–Apr. 2007 | Late Summer | 20 | 11 |

Periods: Before = prior to the introduction of *C. kelberi*; after = after the introduction

and we followed Kullander & Ferreira (2006) to identify this species (voucher specimen is deposited in the Ichthyological Collection of Nupélia, Maringá, Brazil, NUP6311).

This study was carried out in an arm of the transition zone of the reservoir (22°34′07″S; 52°33′34″W) upstream from the Euclides da Cunha Paulista, district in São Paulo state. This arm covers ~40 ha and presents littoral areas colonised by highly abundant *E. densa*. The site is surrounded by pasture lands and lacks riparian vegetation. See Pelicice & Agostinho (2009) for a map of the study area.

Sampling of fish among macrophytes

Sampling was carried out over six periods between 2003 and 2007 during warm months. The first sampling period was in the austral summer of 2003, when *C. kelberi* was absent from the Rosana Reservoir (hereafter referred to as Before). The other sampling periods began approximately 1 year after the introduction, extending from 2005 and 2007 during late summer and spring (hereafter referred to as After-1, 2, 3, 4, and 5) (Table 1).

Fish were collected in patches of *E. densa* (with some minor occurrence of *E. najas*) distributed in both margins of the arm. Sampling was conducted with a 1 m² throw trap (Pelicice et al., 2005), which was operated during the day (07:00 AM–06:00 PM). This trap has a rectangular aluminium frame (1.0 × 1.0 × 1.5 m) and small mesh covering all sides

(0.5 cm mesh) except for the top and bottom. A boat was positioned silently above *Egeria* patches and used as a platform for trap deployment. Immediately following boat positioning, the trap was quickly thrown into the water and pressed to the bottom. Macrophytes were then removed from the trap with a metal fork and were washed and weighed in the field (fresh mass, g). Next, the fish inside the trap were collected with a large sieve (0.90 × 1.5 m) and a dip net (49 × 49 cm), both with 0.5 cm mesh. We used both methods to ensure that all of the fish inside the trap were recovered. The sieve was redeployed until no fish had been captured in three procedures. In addition, the dip net was redeployed within the trap area until 10 successive hauls resulted in no additional captures. All fish were preserved in 10% formalin and transported to the laboratory, where they were identified, counted and measured (standard length, cm; SL). Species identification was based on Graça & Pavanelli (2007).

Each throw trap sample was considered an independent sampling unit. Approximately 20 samples were collected in each period. The samples were distributed at least 50 m apart to cover both margins of the arm. A total of 123 samples (throw traps) were obtained in the study area between 2003 and 2007 (Table 1).

We characterised the habitat condition in each sampled patch by measuring limnological variables prior to removing the fish from the trap. These variables were measured adjacent to each patch with portable field metres and included dissolved oxygen (mg/l), temperature (°C), pH, electrical conductivity (mS/cm) and depth (m). Water transparency (Secchi disk; cm) was measured once in each period in the middle of the arm.

Sampling of *C. kelberi*

Because the throw trap is biased towards small fish (Kushlan, 1981), we used seines (20 m long, 0.5 cm mesh between opposite knots) to estimate the density of *C. kelberi*. This methodology was also employed to characterise the fish assemblages associated with *Egeria* after the introduction of *C. kelberi*, specifically to investigate the influence of the predator's demography.

The seine was pulled manually from the shores to sample littoral areas covered by *Egeria*, enclosing an average area of 30 m². Sampling was conducted

during the day in four periods between 2005 and 2007 (Table 1). Approximately 10 samples were obtained in each period ($n = 36$). The samples were distributed at least 50 m apart and covered both margins of the arm. All fish were preserved and processed as described previously, and *C. kelberi* individuals were eviscerated to determine maturity state: juvenile (immature) or adult.

Predation experiment

Because the fish assemblages experience significant changes specifically during summer seasons (Pelicice & Agostinho, 2009), we performed a tethering experiment to investigate the variation in predation pressure in *Egeria* patches between seasons (late spring and summer).

Prey items were tethered to a main structure consisting of a rope (6 m) stretched perpendicular to the shore, maintained near the surface with the aid of buoys. A cotton line (0.2 mm thick, 80 cm long) was attached with a running knot (Owen, 1993) to the caudal peduncle of each prey fish and then tied to the main rope at 1-m interval, totalling five tethered preys per structure. *Roeboides descaltvadensis* Fowler was chosen as the prey species because it is small in size and abundant in the reservoir (Pelicice et al., 2005; Pelicice & Agostinho, 2009). The prey fish were captured at sites close to the arm where the experiment was conducted. Although the line may constrain or alter prey movements, we were cautious to tether each fish without provoking any injury; indeed, fish were able to swim whilst tethered, but they could not escape from the line.

The experiment was run during two time periods, and the contrasting densities of *C. kelberi* during these periods were used as treatment levels: *C. kelberi*₍₋₎ = 0.45 ± 0.45 ind./100 m² SE (in October 2006, spring) and *C. kelberi*₍₊₎ = 13.31 ± 3.60 ind./100 m² (in March 2007, summer). Predator density in each period was calculated from seine samples. A total of 12 structures (experimental replicates) were put in place in each period, distributed along the arm at least 100 m apart (6 on each shore), totalling 60 prey items per treatment level. The mean total prey length was 5.4 cm (± 0.9 EP; 4.1–7.3 cm) in *C. kelberi*₍₋₎ and 4.2 cm (± 0.9 EP; 2.7–5.6 cm) in *C. kelberi*₍₊₎. The prey remained exposed for 2 h, and the experiment was run between 14:30 and 18:30. An absence of prey after this period was interpreted as predation;

injured fish (e.g. bitten) were considered non-predated, to avoid the influence of other predators (e.g. piranha).

Data analysis

Main drivers

To investigate the variables potentially associated with the decline in the fish assemblages between 2003 and 2007, we analysed only *Egeria* patches with a biomass exceeding 100 g/m² (throw trap data). This procedure was employed to restrict the analysis to patches where fish assemblages occur with the greatest frequency, density and species richness (Pelicice et al., 2005).

A multiple linear regression was used to investigate the influence of variations in (i) habitat condition and (ii) the density of *C. kelberi* (independent variables) on the fish density (ind./m²) and species richness (spp./m²) in *Egeria* patches. The habitat variables considered were macrophyte biomass (dry weight g/m²), dissolved oxygen, pH, temperature and depth. The dry mass of *Egeria* in each patch was estimated from the relationship between dry and wet mass using the equations presented in Pelicice et al. (2008). Electrical conductivity and Secchi depth were not considered in the regressions; the first was not measured in certain periods and would have significantly restricted the size of the data matrix; the second was measured once in each period and had, consequently, no variance. The average density of *C. kelberi* used in the regressions was obtained from seine data sampled between 2005 and 2007 (After-2 through After-5). The density in 2003 (Before) was considered zero because previous studies (Casatti et al., 2003; Pelicice et al., 2005) and intensive surveys conducted in the Rosana Reservoir (Agostinho A.A. unpublished data) did not record *C. kelberi* prior to 2003. The After-1 period (summer 2005) was not considered in the regression analysis, because seine samples were not collected in this period (Table 1).

To select the set of variables that best explain the decline of the fish assemblages, we used a stepwise regression based on the backward selection method. All of the independent variables were included in the model, and the best fit (least squares) was reached after the successive removal of variables showing a lower contribution. The importance of the predictors

selected was evaluated using the (i) semi-partial correlation coefficient ($R_{\text{semi-partial}}$, or the total variance explained by the predictor, controlling for the effect of other variables) and the (ii) standardised slope ($B\text{-std}$, or the slope of the linear relationship between $x * y$, standardised to a mean of 0 and a standard deviation of 1). The dependent variables (density and richness) were $\log + 1$ transformed to meet parametric assumptions.

Influence of the predator's demography

To investigate the influence of the predator's demography (e.g. the pulse density of juvenile fish), we analysed seine data collected between 2005 and 2007 (After periods, Table 1). First, we correlated the density of juvenile *C. kelberi* (independent variable) with the fish density and species richness. In this case, resident fish were grouped into two length categories: (i) <5 cm SL and (ii) >5 cm SL, considering that *Cichla* species usually consume prey smaller than this size (Jepsen et al., 1997; Novaes et al., 2004). The correlation in each category was assessed through Spearman correlation.

Second, a correspondence analysis (CA; Gauch, 1982) was used to investigate variations in the fish assemblage structure (composition and abundance) between periods. The abundance data were transformed to percentages, and rare species (those that contributed <0.1% of total abundance between 2005 and 2007) and *C. kelberi* were removed from the analysis. Only the first axis was interpreted because the other CA axes may present distortion (the arch effect; Gauch, 1982). We then correlated the scores on the first axis with the density of juvenile *C. kelberi* using Spearman correlation.

Predation experiment

To investigate the variation in predation pressure between periods, we calculated the mean percentage (\pm standard error) of fish predated at each treatment level. In addition, the incidence of predation in the arm was calculated as the percentage of experimental units showing predation during each period.

All analyses were conducted in Statistica 7.1 (Statsoft, 2005). Statistical differences implied $\alpha < 0.05$.

Results

Main drivers

A total of 553 individuals belonging to 25 species were captured in *Egeria* patches between 2003 and 2007. These fish were captured with the throw trap in patches with a dry biomass above 100 g/m² ($n = 97$). Six species contributed almost 85% of the total abundance: *Roeboides descaltvadensis* (29.9%), *Hyphessobrycon eques* (18.3%), *Serrassalmus marginatus* (14.4%), *Hemigrammus marginatus* (10.7%), *Satanoperca pappaterra* (5.7%) and *Metynnis lippincottianus* (4.8%). The collapse of the fish assemblages (declines in density and richness) over this period was investigated and described by Pelicice & Agostinho (2009).

A backward regression selected the density of *C. kelberi* and macrophyte biomass as the best variables to explain the variation in fish density over the study period ($R = 0.77$; $F_{2, 86} = 62.3$; $P < 0.00001$). Macrophyte biomass was positively correlated with fish density, whereas *C. kelberi* density was negatively and more strongly correlated with fish density (Table 2). Similarly, the density of *C. kelberi* and *Egeria* biomass represented the best variables to explain the variation in fish species richness ($R = 0.77$; $F_{2, 86} = 61.36$; $P < 0.00001$). Species richness showed a positive association with plant biomass and a negative association with *C. kelberi* (Table 2).

We recorded little variation in habitat conditions and no clear trend over the study years (Table 3). In addition, variables that could limit the fish

Table 2 Results of the stepwise regression between the fish assemblage attributes in *Egeria* patches (fish density and richness; $\log + 1$) and *C. kelberi* density (ind./m²) and variables related to habitat condition

| Factors | <i>B</i> -std | <i>R</i> -partial | <i>t</i> | <i>P</i> |
|---|---------------|-------------------|----------|----------|
| Fish density (ind./m ²) | | | | |
| <i>C. kelberi</i> | −0.59 | −0.59 | −8.29 | 0.000001 |
| <i>Egeria</i> biomass | 0.43 | 0.43 | 5.99 | 0.000001 |
| Species richness (spp./m ²) | | | | |
| <i>C. kelberi</i> | −0.59 | −0.59 | −8.01 | 0.000001 |
| <i>Egeria</i> biomass | 0.41 | 0.41 | 6.66 | 0.000001 |

The backward method was used to select the variables that explained the greatest amount of variation

B-std standardised slope, *R* semi-partial correlation coefficient

Table 3 Habitat characteristics in *Egeria* patches before and after the introduction of *C. kelberi* in the Rosana Reservoir

| Variables | Before | After-1 | After-2 | After-3 | After-4 | After-5 |
|--|----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Temperature (°C) | 28.8 (27.2–32.0) | 28.2 (26.7–29.2) | 26 (25.0–27.4) | 28 (26.4–29.7) | 28.1 (26.8–29.3) | 27.4 (26.4–29.3) |
| Oxygen (mg/l) | 6.7 (3.4–8.8) | 9 (6.2–10.9) | 7.2 (3.8–9.3) | 8 (6.4–9.8) | 7.6 (6.2–9.3) | 5.3 (2.6–8.7) |
| pH | 6.5 (5.9–7.2) | 7.1 (5.7–8.0) | 7.6 (6.6–8.7) | 6.4 (5.8–7.5) | 7.6 (7.1–8.8) | 6.9 (6.5–7.5) |
| Conductivity (µS/cm) | 45.4 (42.7–48.4) | – | – | 59.9 (56.2–62.4) | 54.9 (43.8–59.0) | 59.3 (46.8–65.8) |
| Depth (cm) | 95 (70.0–120.0) | 91.4 (75.0–125.0) | 85.4 (60.0–120.0) | 104.8 (85.0–128.0) | 82.4 (60.0–115.0) | 85.5 (65.0–110.0) |
| Secchi depth (cm) | 135.7 | 122.5 | 175 | 90 | 270 | 70 |
| <i>Egeria</i> biomass (g/m ²) | 207 (110.3–301.6) | 193.6 (135.8–294.2) | 199.1 (106.6–303.8) | 251.6 (123.8–499.7) | 217.6 (120.0–337.0) | 165.6 (108.5–315.8) |
| <i>C. kelberi</i> (ind./100 m ²) | 0 | – | 1.47 (0–6.2) | 18.55 (2.7–43.1) | 1.85 (0–5.7) | 13.31 (0–43.2) |

The table shows the mean, minimum and maximum values for each period

– absence of data

distribution, such as oxygen, pH and temperature, showed no extreme mean values (Table 3). Even electrical conductivity, which increased consistently in the After periods, did not show extreme high values (<70 mS/cm). However, the density of *C. kelberi* showed considerable variation between periods. The predator, which was absent in the Before period, showed a high mean density during the summer periods (After-3 and After-5) and a low density during the spring (After-2 and After-4) (Table 3).

Influence of the predator's demography

A total of 2,682 fish belonging to 22 species were captured in *Egeria* patches between 2005 and 2007 (After periods) using seines. Five fish species contributed almost 90% of the total abundance: *H. marginatus* (44.4%), *R. descalvadensis* (24.6%), *M. lippincottianus* (9%), *S. marginatus* (7.5%) and *C. kelberi* (4.4%). Most fish were captured during the spring periods (After-2 = 36% of the total; After-4 = 53%), with a significant reduction observed during the summer periods (After-3 = 6%; After-5 = 5%). Juvenile *C. kelberi* also showed marked variation in abundance over time, with higher density values obtained during the summer periods (Table 4)—indicating that reproduction occurs during the warm months (November–

February). Juveniles were also captured during the spring periods, but at much lower densities (Table 4).

The assemblage attributes in the patches were closely associated with the pulse of juvenile *C. kelberi* (Fig. 1). For small fish (<5 cm), which represented 93% of the total abundance, we observed a strong negative correlation between the density of young *C. kelberi* and fish density (Spearman: $R = -0.61$; $t = -4.43$; $P < 0.0001$) and species richness ($R = -0.63$; $t = -4.71$; $P < 0.00005$). In contrast, young *C. kelberi* were not correlated with the density ($R = 0.19$; $t = 1.15$; $P < 0.2584$) or richness ($R = 0.16$, $t = 0.95$, $P < 0.3471$) of larger fish (>5 cm).

The CA explained 25.5% of the variability in the fish assemblage structure (axis 1; eigenvalue = 0.67). We observed a clear separation between the summer (After-3 and After-5) and spring periods (After-2 and After-4) (Fig. 2). The spring periods showed positive scores and were characterised by small-bodied species typically found before the introduction (e.g. *H. marginatus*, *H. eques*, *R. descalvadensis* and young *S. marginatus*; see Pelicice & Agostinho, 2009). In contrast, summer periods showed negative scores and were characterised by larger-bodied species or species with a low abundance (Table 5). The axis 1 scores were negatively correlated with the density of juvenile *C. kelberi* (Spearman: $R = -0.52$; $t = -3.42$;

Table 4 Mean density of juvenile *C. kelberi* (ind./100 m² ± standard error) over the study period, mean standard length (cm) and the relative contribution of juvenile fish to the total abundance (%)

| Periods | Density | | Length | | Juvenile (%) |
|---------|--------------|-----------|--------|------------|--------------|
| | Mean (SE) | Min.–max. | Mean | Min.–max. | |
| After-2 | 1.18 (0.89) | 0–6.2 | 4.43 | 2.50–6.79 | 75 |
| After-3 | 18.55 (5.57) | 2.7–43.1 | 6.81 | 4.50–12.70 | 100 |
| After-4 | 1.25 (0.83) | 0–5.7 | 5.65 | 5.50–5.8 | 66.67 |
| After-5 | 13.31 (3.60) | 0–43.2 | 6.34 | 3.00–7.70 | 100 |

$P < 0.0017$). In this case, the fish fauna structure during the spring was associated with low densities of *C. kelberi*, whereas the assemblages during the summer were correlated with high densities of the predator.

Predation experiment

The percentage of predated fish ranged between 0 and 60% among experimental units, but predation pressure tended to increase under *C. kelberi*₍₊₎. In this case, the mean percentage of predated fish for *C. kelberi*₍₊₎ was 2.5 times higher than for *C. kelberi*₍₋₎, increasing from 6.7 to 16.7% (Fig. 3). In addition, the incidence of predation increased under *C. kelberi*₍₊₎, from 25 to 58% of the replicates.

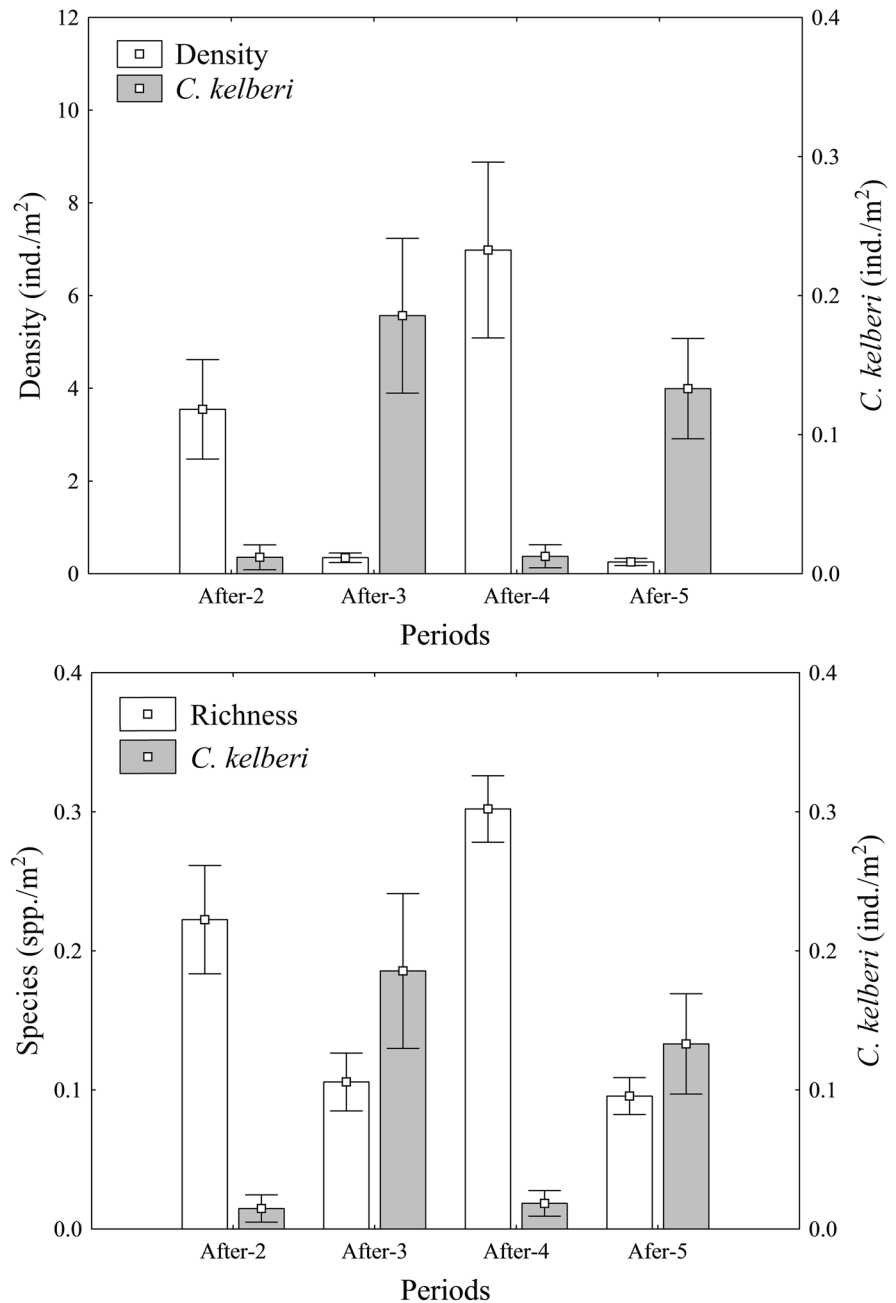
Discussion

The introduction of *Cichla kelberi* in the Rosana Reservoir (Paraná River Basin, Brazil) was followed by a striking and progressive loss of small-bodied fish (Pelicice & Agostinho, 2009), but demonstrating the connection between the introduction and subsequent impacts demanded sound evidence. In this sense, this study showed that the decline in fish diversity was not related to changes in habitat conditions, but correlated strongly with variations in *C. kelberi* abundance. More interestingly, we observed that demographic features of the invader (i.e. the pulse density of juvenile individuals during warm months) were correlated with the detected changes in fish fauna structure. We highlight the fact that the disturbance potential of *Cichla* species remained poorly investigated for decades—since the first studies suggested that *Cichla* may disrupt local communities and ecosystem organisation (e.g. Zaret & Paine, 1973). Subsequently,

other studies have provided indications of impacts (Latini & Petrere Junior, 2004; Fugi et al., 2008; Pelicice & Agostinho, 2009; Pinto-Coelho et al., 2008; Menezes et al., 2012), but this study is the first to provide a substantial body of evidence supporting the hypothesis that *C. kelberi* can affect the structure of biotic communities in reservoirs.

The episode documented in the Rosana Reservoir represents a special case, as there was information available about the fish assemblages in the reservoir at time points that preceded the introduction of the predator. The lack of such information is an important limitation in invasion studies (Thomaz et al., 2012). In addition, we initiated monitoring soon after the introduction of *C. kelberi*, simultaneously gathering data on the fish fauna, the invader and habitat conditions in *Egeria* patches. All of this information was essential to demonstrate that the fauna disassembly was not related to changes in habitat quality at small spatial scales. In fact, the limnological conditions and habitat structure showed little variation and no clear trend over the years (2003–2007); the recorded variation was probably associated with diel variation (e.g. Pelicice et al., 2005) and seasonal dynamics, in addition to casual inter-annual differences. Moreover, none of the measured variables showed extreme values that could restrict the fish distribution in the patches, a situation expected, for example, in cases of extensive colonisation of *Egeria*. Several studies have shown a decrease in water quality due to excessive aquatic plant coverage (Miranda & Hodges, 2000; Van Nes et al., 2002), but the present results indicate that *Egeria*, although abundant, has not created an inhospitable environment at the study site. In addition, the *Egeria* biomass was the only habitat variable that influenced the fish distribution, showing a positive correlation with fish diversity. This type of pattern has been described by previous studies

Fig. 1 Fish density, species richness and juvenile *C. kelberi* abundance in *Egeria* patches over the study period. *Points* are mean values and *bars* indicate standard errors

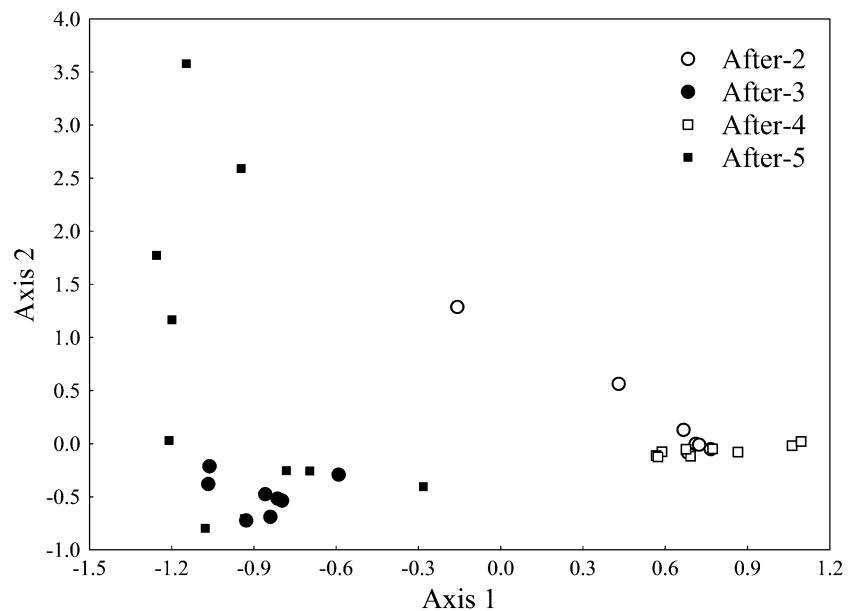


(Pelicice et al., 2005, 2008), in which plant biomass was found to substantially determine the structure of the fish fauna, reflecting the strong association between small-bodied fish and habitat structure.

Although habitat conditions showed little variation over the examined years and therefore did not explain the changes in fish diversity, the *C. kelberi* abundance was strongly correlated with the structure of the

resident fish assemblages. In this case, the *C. kelberi* density was the variable that best explained the decline in fish diversity (abundance and richness). Furthermore, we observed a close relationship between demographic aspects (the density pulses of young fish) and changes in abundance, richness and composition of small-bodied fish, which included some non-native prey species established in the area (e.g.

Fig. 2 Sample scores derived from a correspondence analysis (CA; first axis), applied to ordinate fish fauna samples in periods that followed the introduction of *C. kelberi*. See Table 1 for period codes



Serrassalmus marginatus and *Roeboides descalvagensis*; Júlio et al., 2009). Taken together, these data support the hypothesis that *C. kelberi* was the driver underlying the fish fauna disassembly. The presence of other non-native species in the reservoir, however, suggests alternative explanations. In this case, changes in fish assemblages may be associated with disturbances mediated by other invaders via predatory and competitive interactions. Studies have shown, for example, that competition may displace native species and facilitate predatory dynamics (Martin et al., 2010; Sanches et al., 2012). We highlight, however, that other non-native predators (e.g. *S. marginatus* and *Plagioscion squamosissimus*) have not been captured in *Egeria* patches, excepting for young piranhas *S. marginatus* (<5 cm); similarly, native predators (e.g. *Hoplias malabaricus*, *Acestrorhynchus lacustris*) were rare or absent in these habitats. In addition, before the introduction of *C. kelberi*, small-sized native and non-native fish were abundant and co-occurred in *Egeria* patches, where they coexisted with low competition for feeding resources (Pelicice et al., 2005; Pelicice & Agostinho, 2006). Finally, and more importantly, other non-native fish were introduced in the Rosana Reservoir decades ago (Agostinho et al., 2007; Júlio et al., 2009), so their presence cannot explain the sudden decrease in fish diversity recorded between 2003 and 2007. Even if these previous invaders have disturbed the resident fish fauna, the

rapid disassembly occurred precisely when *C. kelberi*, a top predator, invaded the system. This scenario, therefore, constitutes evidence that *C. kelberi* played an important or central role in the chain of events that led to the collapse.

We also began to clarify how this predator affects the resident fauna when it is introduced in reservoirs, i.e. via demographic dynamics. In the Rosana Reservoir, fish fauna disassembly occurred when the predator's abundance increased during the warm months (austral summer) as a consequence of the emergence of large shoals of juvenile *C. kelberi*. Other studies have found that intense reproduction occurs during warm periods in southeastern Brazil, with an increased abundance of smaller individuals found during the late summer and autumn (Souza et al., 2008). In the Rosana Reservoir, the emergence of small-bodied predators must increase the predation pressure upon smaller fish, primarily because juveniles of *Cichla* (>5 cm) include small-bodied fish in their diet (Jepsen et al., 1997; Novaes et al., 2004). We highlight that small-bodied fish (native and non-native) were the characteristic fauna associated with *Egeria* patches (Pelicice & Agostinho, 2009) and that juvenile *C. kelberi* were captured within macrophyte beds. Given that aquatic plants provide a limited refuge against *C. kelberi* (Kovalenko et al., 2010b), young predators most likely find a suitable habitat for foraging and protection within *Egeria* patches. The

Table 5 Species scores derived from a correspondence analysis (CA; first axis) applied to fish fauna data

| Species | Scores | Abundance (%) | Length (cm) | |
|-----------------------------------|--------|---------------|-------------|-----------|
| | | | Mean | Min.–max. |
| <i>Hemigrammus marginatus</i> | 0.92 | 44.41 | 2.8 | 1.3–3.9 |
| <i>Hyphessobrycon eques</i> | 0.85 | 1.23 | 2.83 | 2.2–3.2 |
| <i>Roeboides descavadensis</i> | 0.76 | 24.57 | 3.02 | 1.5–5.7 |
| <i>Oligosarcus pintoii</i> | 0.21 | 0.34 | 4.11 | 3.5–5.5 |
| <i>Serrassalmus marginatus</i> | 0.09 | 7.53 | 2.8 | 1.5–7.5 |
| <i>Acestrorhynchus lacustris</i> | 0.03 | 1.72 | 6.52 | 2.4–20.1 |
| <i>Schizodon borellii</i> | −0.39 | 0.11 | 19.33 | 12–24 |
| <i>Astyanax altiparanae</i> | −0.47 | 3.39 | 3.82 | 2.2–8.0 |
| <i>Hoplias malabaricus</i> | −0.58 | 0.11 | 25.25 | 22.5–28 |
| <i>Metynnis lippincottianus</i> | −0.89 | 8.99 | 4.25 | 1.6–9.2 |
| <i>Oxydoras eigenmanni</i> | −0.99 | 0.34 | 6.76 | 5.7–8.3 |
| <i>Satanoperca pappaterra</i> | −1.17 | 0.48 | 7.67 | 1.7–20.6 |
| <i>Schizodon nasutus</i> | −1.23 | 0.15 | 12 | 9.5–13.7 |
| <i>Steindachnerina brevipinna</i> | −1.26 | 0.37 | 4.04 | 1.9–6.5 |

The table also shows species relative abundance (%) and standard length (cm)

results from the tethering experiment supported these trends, as predation pressure (i.e. percentage of predated fish and incidence of predation along the shores) increased during the summer. Therefore, the mere presence of a non-native species (a voracious top predator, in this case) does not determine the extent and intensity of its impacts. Events that suddenly increase the population size of the invader, such as reproductive events, enhance its ecological effects. The reproductive activity of *C. kelberi* must therefore play an important role not only in the establishment of populations but also in mediating the extent of impacts.

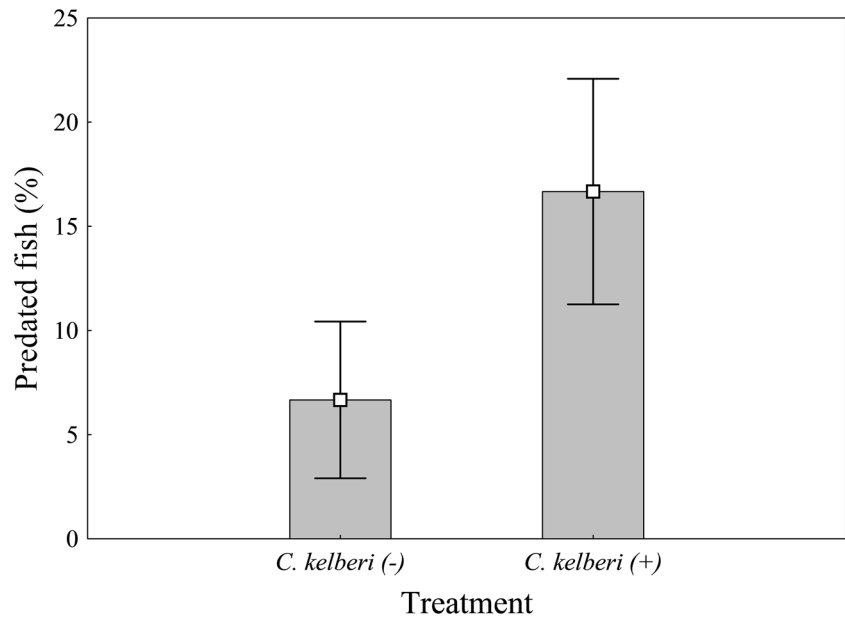
This finding, together with the results of other recent studies, begins to explain the process of *C. kelberi* invasion in reservoirs. Many aspects of this phenomenon are still unknown, but it is possible to outline a broad picture regarding invasion aspects and impacts. In short, we highlight that according to certain environmental conditions, some reservoirs present higher invasibility to *C. kelberi* (Espínola et al., 2010) and, once introduced, this species has a great potential to affect the resident fish fauna (Pelicice & Agostinho, 2009; this study). Other studies show that prey naiveté plays no role in the succession of impacts (Kovalenko et al., 2010a), and that aquatic plants offer limited protection against this predator

(Pelicice & Agostinho, 2009; Kovalenko et al., 2010b). We also know that feeding activity, the source of disturbance, increases during warm months (Villares Junior & Gomiero, 2010), and that demographic aspects (i.e. reproduction) mediate ecological impacts. A fundamental question that remains open is why *C. kelberi* and other *Cichla* species apparently do not disrupt the fish assemblages in Amazonian reservoirs, where they are native. In addition, the invasion process of *Cichla* in natural ecosystems might differ from the trends observed in reservoirs, which are environments that are already disturbed and may therefore facilitate invasions and enhance their impacts (Havel et al., 2005; Light & Marchetti, 2007; Johnson et al., 2008). The presence of other non-native species in reservoirs must also be considered, since previous invasions may facilitate the establishment of other invasive species (meltdown effects; Simberloff & Von Holle, 1999), or even enhance the impacts of top predators (Sanches et al., 2012). Future studies must, therefore, evaluate how the impoundment and other non-native species mediate the impacts (e.g. additive or emergent effects) caused by *Cichla* species in man-made reservoirs. These issues must receive particular attention in future studies so that we may improve the understanding of the environmental settings that cause *C. kelberi* to be a strong source of ecological change.

In view of the risk associated with introductions and the difficulty of predicting which species will become established, invade or cause impacts, a precautionary approach must prevail—especially because non-native consumers may cause more damage on prey populations than do native ones (Paolucci et al., 2013). It is our hope that the present results will inspire caution in handling *Cichla* and other non-native species, particularly in programs that promote aquaculture and fishery activities in developing countries (Pelicice et al., 2014). We also hope that scientific scepticism does not cause the precautionary approach to be relaxed, as taking a permissive approach towards commercial activities may further aggravate the traffic and establishment of non-native fish around the world.

Acknowledgements We thank Universidade Estadual de Maringá, PEA and Nupélia for creating ideal conditions for this research. We also thank the staff who helped with the field sampling and laboratory analyses. CAPES provided a scholarship for F.M.P. during his doctoral work, and CNPq provided a research grant for A.A.A and F.M.P. Finally, we

Fig. 3 Predation pressure in *Egeria* patches, measured as the mean percentage of fish predated in the tethering experiment, plotted against two different densities of *C. kelberi*: $C. kelberi_{(-)} = 0.45 \text{ ind./}100 \text{ m}^2 \pm 0.45$; $C. kelberi_{(+)} = 13.31 \text{ ind./}100 \text{ m}^2 \pm 3.60$. Bars indicate standard errors



thank the Editor and two anonymous reviewers for their valuable comments.

References

- Agostinho, A. A., L. C. Gomes & F. M. Pelicice, 2007. Ecologia e manejo de recursos pesqueiros em reservatórios do Brasil. Eduem, Maringá.
- Attayde, J. L., J. Brasil & R. A. Menescal, 2011. Impacts of introducing Nile tilapia on the fisheries of a tropical reservoir in North-eastern Brazil. *Fisheries Management and Ecology* 18: 437–443.
- Barrela, W. & M. Petreire Jr, 2003. Fish community alterations due to pollution and damming in Tietê and Paranapanema rivers (Brazil). *River Research and Applications* 19: 59–76.
- Britton, J. R. & M. L. Orsi, 2012. Non-native fish in aquaculture and sport fishing in Brazil: economic benefits versus risks to fish diversity in the upper River Paraná Basin. *Review in Fish Biology and Fisheries* 22: 555–565.
- Cambay, J. A., 2003. Impact on indigenous species biodiversity caused by the globalization of alien recreational freshwater fisheries. *Hydrobiologia* 500: 217–230.
- Casal, C. M. V., 2006. Global documentation of fish introductions: the growing crisis and recommendations for action. *Biological Invasions* 8: 3–11.
- Casatti, L., H. F. Mendes & K. M. Ferreira, 2003. Aquatic macrophytes as feeding site for small fishes in the Rosana Reservoir, Paranapanema River, southeastern Brazil. *Brazilian Journal of Biology* 63(2): 213–222.
- Cucherousset, J. & J. D. Olden, 2011. Ecological impacts of non-native freshwater fishes. *Fisheries* 36: 215–230.
- Davis, M. A., M. K. Chew, R. J. Hobbs, A. E. Lugo, J. J. Ewel, G. J. Vermeij, J. H. Brown, M. L. Rosenzweig, M. R. Gardener, S. P. Carroll, K. Thompson, S. T. A. Pickett, J. C. Stromberg, P. D. Tredici, K. N. Suding, J. G. Ehrenfeld, J. P. Grime, J. Mascaro & J. C. Briggs, 2011. Don't judge species on their origins. *Nature* 474: 153–154.
- Espínola, L. A., C. V. Minte-Vera & H. F. Júlio Jr, 2010. Invasibility of reservoirs in the Paraná Basin, Brazil, to *Cichla kelberi* Kullander and Ferreira. *Biological Invasions* 12: 1873–1888.
- Fugi, R., K. D. G. Luz-Agostinho & A. A. Agostinho, 2008. Trophic interaction between an introduced (peacock bass) and a native (dogfish) piscivorous fish in a neotropical impounded river. *Hydrobiologia* 607: 143–150.
- Gauch Jr, H. G., 1982. *Multivariate Analysis in Community Ecology*. Cambridge University Press, Cambridge.
- Gozlan, R. E., 2008. Introduction of non-native freshwater fish: is it all bad? *Fish and Fisheries* 9: 106–115.
- Graça, W. J. & C. S. Pavanelli, 2007. Peixes da planície de inundação do alto rio Paraná e áreas adjacentes. Eduem, Maringá.
- Havel, J. E., C. E. Lee & M. J. Vander Zanden, 2005. Do reservoirs facilitate invasions into landscapes? *BioScience* 55: 515–525.
- Jepsen, D. B., K. O. Winemiller & D. C. Taphorn, 1997. Temporal patterns of resource partitioning among *Cichla* species in a Venezuelan blackwater river. *Journal of Fish Biology* 51: 1085–1108.
- Johnson, P. T. J., J. D. Olden & M. J. Vander Zanden, 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. *Frontiers in Ecology and Environment* 6: 357–363.
- Júlio Jr, H. F., C. Dei Tos, A. A. Agostinho & C. S. Pavanelli, 2009. A massive invasion of fish species after eliminating a natural barrier in the upper Rio Paraná basin. *Neotropical Ichthyology* 7: 709–718.
- Kovalenko, K. E., E. D. Dibble, A. A. Agostinho & F. M. Pelicice, 2010a. Recognition of non-native peacock bass,

- Cichla kelberi* by native prey: testing the naïveté hypothesis. *Biological Invasions* 12: 3071–3080.
- Kovalenko, K. E., E. D. Dibble, A. A. Agostinho, G. Cantanhêde & R. Fugi, 2010b. Direct and indirect effects of an introduced piscivore, *Cichla kelberi* and their modification by aquatic plants. *Hydrobiologia* 638: 245–253.
- Kullander, S. O. & E. J. G. Ferreira, 2006. A review of the South American cichlid genus *Cichla*, with descriptions of nine new species (Teleostei: Cichlidae). *Ichthyological Exploration of Freshwaters* 17: 289–398.
- Kushlan, J. A., 1981. Sampling characteristics of enclosure fish traps. *Transactions of the American Fisheries Society* 110: 557–562.
- Latini, A. O. & M. Petrere Junior, 2004. Reduction of a native fish fauna by alien species: an example from Brazilian freshwater tropical lakes. *Fisheries Management and Ecology* 11(2): 71–79.
- Leprieur, F., O. Beauchard, S. Blanchet, T. Oberdorff & S. Brosse, 2008. Fish invasions in the world's river systems: when natural processes are blurred by human activities. *PLOS Biology* 6(2): 404–410.
- Leprieur, F., S. Brosse, E. Garcia-Berthou, T. Oberdorff, J. D. Olden & C. R. Townsend, 2009. Scientific uncertainty and the assessment of risks posed by non-native freshwater fishes. *Fish and Fisheries* 10: 88–97.
- Light, T. & M. P. Marchetti, 2007. Distinguishing between invasions and habitat changes as drivers of diversity loss among California's freshwater fishes. *Conservation Biology* 21(2): 434–446.
- Lövei, G. L., T. M. Lewinsohn & Invasions in Megadiverse Regions Network, 2012. Megadiverse developing countries face huge risks from invasives. *Trends in Ecology and Evolution* 27: 2–3.
- MacDougall, A. S. & R. Turkington, 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* 86(1): 42–55.
- Martin, C. W., M. M. Valentine & J. F. Valentine, 2010. Competitive interactions between invasive Nile tilapia and native fish: the potential for altered trophic exchange and modification of food webs. *PLOS One* 5(12): e14395.
- Menezes, R. F., J. L. Attayde, G. Lacerot, S. Kosten, L. C. Souza, L. S. Costa, E. H. Van Nes & E. Jeppesen, 2012. Lower biodiversity of native fish but only marginally altered plankton biomass in tropical lakes hosting introduced piscivorous *Cichla* cf. *ocellaris*. *Biological Invasions* 14: 1353–1363.
- Miranda, L. E. & K. B. Hodges, 2000. Role of aquatic vegetation coverage on hypoxia and sunfish abundance in bays of a eutrophic reservoir. *Hydrobiologia* 427: 51–57.
- Miranda, L. E., M. Spickard, T. Dunn, K. M. Webb, J. N. Aycock & K. Hunt, 2010. Fish habitat degradation in U.S. reservoirs. *Fisheries* 35(4): 175–184.
- Novaes, J. L. C., E. P. Caramaschi & K. O. Winemiller, 2004. Feeding of *Cichla monoculus* Spix, 1829 (Teleostei: Cichlidae) during and after reservoir formation in the Tocantins river, Central Brazil. *Acta Limnologica Brasiliensis* 16(1): 41–49.
- Owen, P., 1993. *Knots*. Running Press, Pennsylvania.
- Paolucci, E. M., H. J. MacIsaac & A. Ricciard, 2013. Origin matters: alien consumers inflict greater damage on prey populations than do native consumers. *Diversity and Distributions* 19(8): 988–995.
- Pelice, F. M. & A. A. Agostinho, 2006. Feeding ecology of fishes associated with *Egeria* spp. patches in a tropical reservoir, Brazil. *Ecology of Freshwater Fish* 15(1): 10–19.
- Pelice, F. M. & A. A. Agostinho, 2009. Fish fauna destruction after the introduction of non-native predator (*Cichla kelberi*) in a neotropical reservoir. *Biological Invasions* 11: 1789–1801.
- Pelice, F. M., A. A. Agostinho & S. M. Thomaz, 2005. Fish assemblages associated with *Egeria* in a tropical reservoir: investigating the effects of plant biomass and diel period. *Acta Oecologica* 27: 9–16.
- Pelice, F. M., S. M. Thomaz & A. A. Agostinho, 2008. Simple relationships to predict attributes of fish assemblages in patches of submerged macrophytes. *Neotropical Ichthyology* 6: 543–550.
- Pelice, F. M., J. R. S. Vitule, D. P. Lima Junior, M. L. Orsi & A. A. Agostinho, 2014. A serious new threat to Brazilian freshwater ecosystems: the naturalization of nonnative fish by decree. *Conservation Letters* 7: 55–60.
- Pinto-Coelho, R. M., J. F. Bezerra-Neto, F. Miranda, T. G. Mota, R. Resck, A. M. Santos, P. M. Maia-Barbosa, N. A. S. T. Mello, M. M. Marques, M. O. Campos & F. A. R. Barbosa, 2008. The inverted trophic cascade in tropical plankton communities: impacts of exotic fish in the Middle Rio Doce lake district, Minas Gerais, Brazil. *Brazilian Journal of Biology* 68: 1025–1037.
- Rahel, F. J., 2007. Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. *Freshwater Biology* 52: 696–710.
- Ruesink, J. L., 2005. Global analysis of factors affecting the outcome of freshwater fish introductions. *Conservation Biology* 19(6): 1883–1893.
- Sanches, F. H. C., C. A. Miyai, T. M. Costa, R. A. Christofoletti, G. L. Volpato & R. E. Barreto, 2012. Aggressiveness overcomes body-size effects in fights staged between invasive and native fish species with overlapping niches. *PLOS One* 7(1): e29746.
- Schlaepfer, M. A., D. F. Sax & J. D. Olden, 2011. The potential conservation value of non-native species. *Conservation Biology* 25(3): 428–437.
- Simberloff, D. & B. Von Holle, 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1: 21–32.
- Simberloff, D., J. L. Martin, P. Genovesi, V. Maris, D. A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, P. Pyšek, R. Sousa, E. Tabacchi & M. Vilà, 2013. Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution* 28: 58–66.
- Souza, J. E., E. M. Fragoso-Moura, N. Fenerich-Verani, O. Rocha & J. R. Verani, 2008. Population structure and reproductive biology of *Cichla kelberi* (Perciformes, Cichlidae) in Lobo Reservoir, Brazil. *Neotropical Ichthyology* 6(2): 201–210.
- Statsoft, 2005. *Statistica* (data analysis software system), version 7.1. StatSoft Inc, Tulsa.
- Thomaz, S. M., A. A. Agostinho, L. C. Gomes, M. J. Silveira, M. Rejmánek, C. E. Aslan & E. Chow, 2012. Using space-for-time substitution and time sequence approaches in invasion ecology. *Freshwater Biology* 57: 2401–2410.

- van Nes, E. H., M. Scheffer, M. S. van den Berg & H. Coops, 2002. Aquatic macrophytes: restore, eradicate or is there a compromise? *Aquatic Botany* 72: 387–403.
- Villares Junior, G. A. & L. A. Gomiero, 2010. Feeding dynamics of *Cichla kelberi* Kullander & Ferreira, 2006 introduced into an artificial lake in southeastern Brazil. *Neotropical Ichthyology* 8(4): 819–824.
- Vitule, J. R. S., C. A. Freire & D. Simberloff, 2009. Introduction of non-native freshwater fish can certainly be bad. *Fish and Fisheries* 10: 98–108.
- Vitule, J. R. S., C. A. Freire, D. P. Vazquez, M. A. Nuñez & D. Simberloff, 2012a. Revisiting the potential conservation value of non-native species. *Conservation Biology* 26(6): 1153–1155.
- Vitule, J. R. S., F. Skóra & V. Abilhoa, 2012b. Homogenization of freshwater fish faunas after the elimination of a natural barrier by a dam in neotropics. *Diversity and Distributions* 18: 111–120.
- Zaret, T. M. & R. T. Paine, 1973. Species introduction in a tropical lake. *Science* 182: 449–455.