



Establishment of non-native guppy *Poecilia reticulata* (Peters, 1859) (Cyprinodontiformes: Poeciliidae) in an Municipal Park located in Minas Gerais State, Brazil

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Abstract. We evaluated the establishment of the ornamental non-native guppy *Poecilia reticulata* bimonthly from November 2006 to October 2007 in an urban reservoir located in the city of Belo Horizonte, Southeastern Brazil. Females were found in reproduction during almost all the sampling period. The fecundity was always low. For the total period, we found more females than males, and the number of juveniles in all periods was higher than adults. There was no correlation between water temperature, rainfall and reproduction. Of the five stages of biological invasion, the species was at least in the stage three: establishment through reproduction and recruitment. Management recommendations, such as prohibition on sales of *P. reticulata* by local aquarium trade, and awareness campaigns with wholesalers, retailers and hobbyists showing that aquarium dumping is an environmental crime are suggested to avoid introductions of *P. reticulata* in the region.

Key words: aquarium dumping, biological invasions, poeciliids, reproduction, sex ratio

Resumo. Estabelecimento do peixe não-nativo guppy *Poecilia reticulata* (Peters, 1859) (Cyprinodontiformes: Poeciliidae) em um parque municipal localizado no estado de Minas Gerais, Brasil. Avaliamos bimestralmente de Novembro de 2006 a Outubro de 2007 o estabelecimento do peixe ornamental não-nativo guppy *Poecilia reticulata* em um reservatório urbano localizado na cidade de Belo Horizonte, sudeste do Brasil. Fêmeas foram encontradas em reprodução durante todo o período amostral, e a fecundidade foi sempre baixa. Para o período total, foram encontradas mais fêmeas que machos, e o número de juvenis em todos os períodos foi maior que o de adultos. Não houve correlação entre a temperatura da água, precipitação pluviométrica e a reprodução de *P. reticulata*. Dos cinco estágios de invasão biológica, *P. reticulata* estava pelo menos na fase três: estabelecimento através de reprodução e recrutamento. Recomendações como proibição de venda de *P. reticulata* pelo comércio local e campanhas de conscientização com fornecedores, lojistas e aquaristas mostrando que o abandono de peixes de aquário em ambientes naturais é considerado crime ambiental são sugeridas para evitar a introdução de *P. reticulata* na região.

Palavras chave: poecilídeos, invasão biológica, aspectos reprodutivos, Lagoa do Nado, sudeste do Brasil

Introduction

Among the Brazilian states, Minas Gerais is the fourth largest, with an area of 586,528 Km², and a growing population of about 20 million people living in 853 cities (IBGE 2002). The state is drained by 17 watersheds (IGAM 1998), with thousands of creeks and more than 2,000 dammed water bodies of different sizes (Alves *et al.* 2007). The native ichthyofauna of the state, comprising 354 species (Alves *et al.* 2007), of which 49 species are considered some of the country's most threatened fishes (Fundação Biodiversitas 2007). All these species are likely to be seriously endangered, since Minas Gerais State ranks first in fish introductions not only in Brazil but in South America, with 85 non-native species, 65 of which ornamental (Magalhães & Jacobi 2013).

The ornamental guppy *Poecilia reticulata* is one of the 85 non-native fish found in inland waters of the state. It is a small live-bearing (i.e., ovoviviparous) cyprinodontiform native to the Caribbean Islands (Netherlands Antilles, Trinidad and Tobago, Barbados, Windward and Leeward Islands), Venezuela, Guyana, and northern Brazil (Amapá and Pará States) (Lucinda & Costa 2007). Males grow about 3.5 cm in length and they have their anal fin modified into a gonopodium, while females are larger (up to 5.0 cm) (Froese & Pauly 2011). They feed on zooplankton, invertebrates and algae (Rocha *et al.* 2009) and prefer slow-flowing warmwater habitats (Froese & Pauly 2011). Guppies are among the most popular in the aquarium trade of Minas Gerais (Magalhães & Jacobi 2013), and they are introduced into water bodies in the city of Belo Horizonte through the aquarium dumping (Godinho *et al.* 1992, Chaves & Magalhães 2010).

Despite introduced throughout Minas Gerais State (Alves *et al.* 2007), several features of the life-history of *P. reticulata* are not well-studied, including essential ones such as reproductive biology and population structure, which are needed to understand the invasion of this species in new environments. Thus, the aim of the present study was to help filling in this gap, with special focus on the reproductive cycle, fecundity, sex ratio, juvenile-to-adult ratio, reproduction and abiotic factors and the process of invasion of guppy in an urban reservoir located in the city of Belo Horizonte. We also suggest management actions to prevent further introductions in the region.

Material and Methods

Study area

The Lagoa do Nado (19°49'56"S; 43°57'34"W), is a small meso-eutrophic reservoir located in the city of Belo Horizonte, Minas Gerais State, Southeastern Brazil (Figure 1). The superficial area of the lake is 1.5 ha, and the mean depth is 2.7 m. The native fish fauna is represented by species from the São Francisco River Basin such as the piabas *Hyphessobrycon santae*, *Hasemanina nana*, chameleon cichlid *Australoheros facetum*, pearl cichlid *Geophagus brasiliensis*, cascarudo *Callichthys callichthys*, suckermouth catfish *Hypostomus* sp.. Non-native fishes include guppy *P. reticulata*, giant trahira *Hoplias cf. lacerdae*, and Nile tilapia *Oreochromis niloticus* (Braga 2010).

This reservoir is located at the Fazenda Lagoa do Nado Municipal Park, a unit of conservation created in 1994 with the purpose to increase the human interaction with natural areas (Mafia *et al.* 2012). Due to the proximity of Lagoa do Nado reservoir with two densely populated neighborhoods (Bairros de Belo Horizonte 2010), the introductions of *P. reticulata* was most likely caused by aquarium hobbyists discarding the species (Chaves & Magalhães 2010).

Sampling design

We collected a total of 1,536 juveniles (0.5-0.9 cm total length), 700 females (1.0-2.1 and 2.2-3.3 cm standard length) (a random subsample of 366 females only to study macroscopic/mesosopic developmental stages and the reproductive cycle), and 427 males (1.0-2.0 and 2.1-2.5 cm standard length) of *P. reticulata* every two months; from November 2006-October 2007 using sieves (50 cm long, 30 cm high, and 0.2 mm mesh) in a sampling site nearby the dam. The fishes were euthanized on an ice slurry, a method approved for field work by IACUC (2002), packed in plastic bags, fixed in 10% formalin, and subsequently transferred to 70% alcohol. In the laboratory, the species was sexed as juveniles were smaller than the smallest male, females in the case of the absence of gonopodia but were larger than the smallest male and as males if they possessed any evidence of a gonopodium (Fernandez-Delgado & Rossomanno 1997). Abiotic data were collected bimonthly, namely water temperature and rainfall (Table I). The first data were obtained at the times of the fish sampling. We measured water temperature in the field with a

thermometer to the scale 0° to 50°C. Rainfall data are from the database of the 5th Meteorological District (Instituto Nacional de Meteorologia-INMET) located in the city of Belo Horizonte.

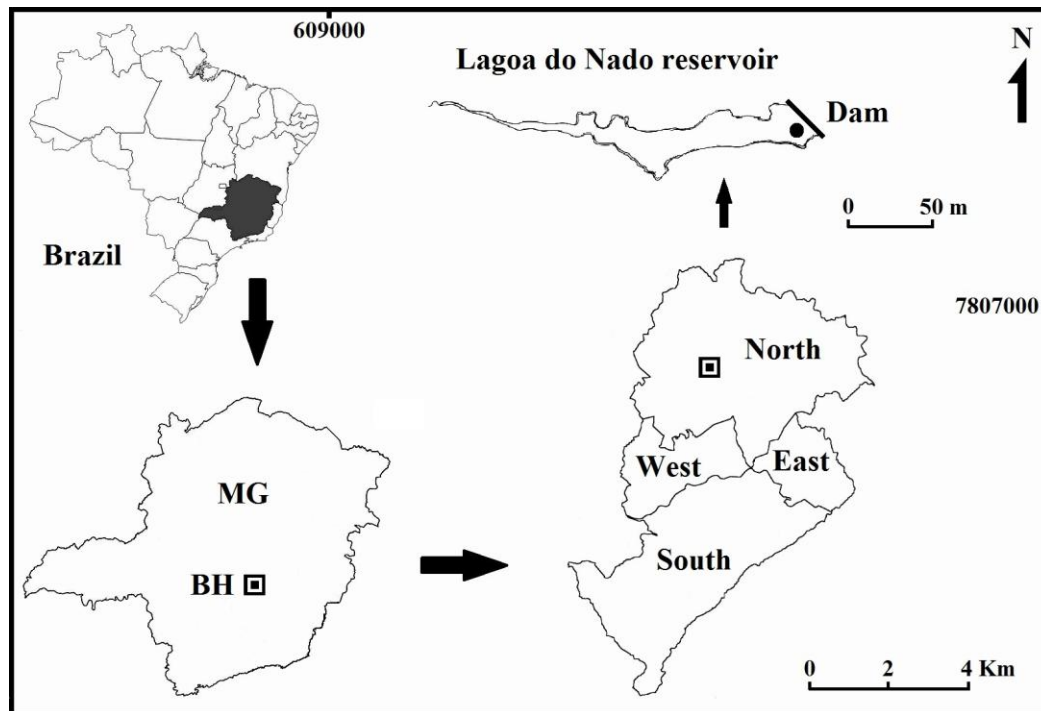


Figure 1. Map showing sampling location of non-native *Poecilia reticulata* in North region of the city of Belo Horizonte. MG= Minas Gerais State, BH= city of Belo Horizonte, ●= sampling site in Lagoa do Nado reservoir, UTM coordinates.

Developmental stages

Macroscopically, we classified as non-gravid females those without a conspicuously enlarged ventral region, absence of gravid spot near the base of the anal fin, eggs or embryos. Females with enlarged bellies, presence of gravid spots, eggs, or embryos seen with the naked eye were classified as gravid according to Winemiller (1989). We adapted the methods of Shahjahan *et al.* (2013) to determine under the binocular stereomicroscope the developmental stages of reproductive females. Stages were classified as follows: 1) Non-gravid

(ovarian tissue without ovum and/or embryos), 2) Early-yolked ovum (orange ovum in the process of yolking), 3) Early-eyed embryos (pigmented eyes, enlarged head compared to trunk, presence of caudal and pectoral fin buds, orange yolk), and 4) Mature embryos (yolk sac mostly or completely absorbed, elongate pectoral fins, presence of scales, embryo resembling a small adult) (Figure 2). After determining the developmental stages, the bimonthly absolute and relative frequencies of females were calculated.

Table I. Mean values of abiotic factors bimonthly in Lagoa do Nado reservoir between November 2006 and October 2007.

Bimesters	Water temperature (°C)	Rainfall (mm)
November-December	26.9	447
January-February	27.4	278.9
March-April	29.3	93.3
May-June	26.2	10.2
July-August	25.4	0
September-October	28.5	2

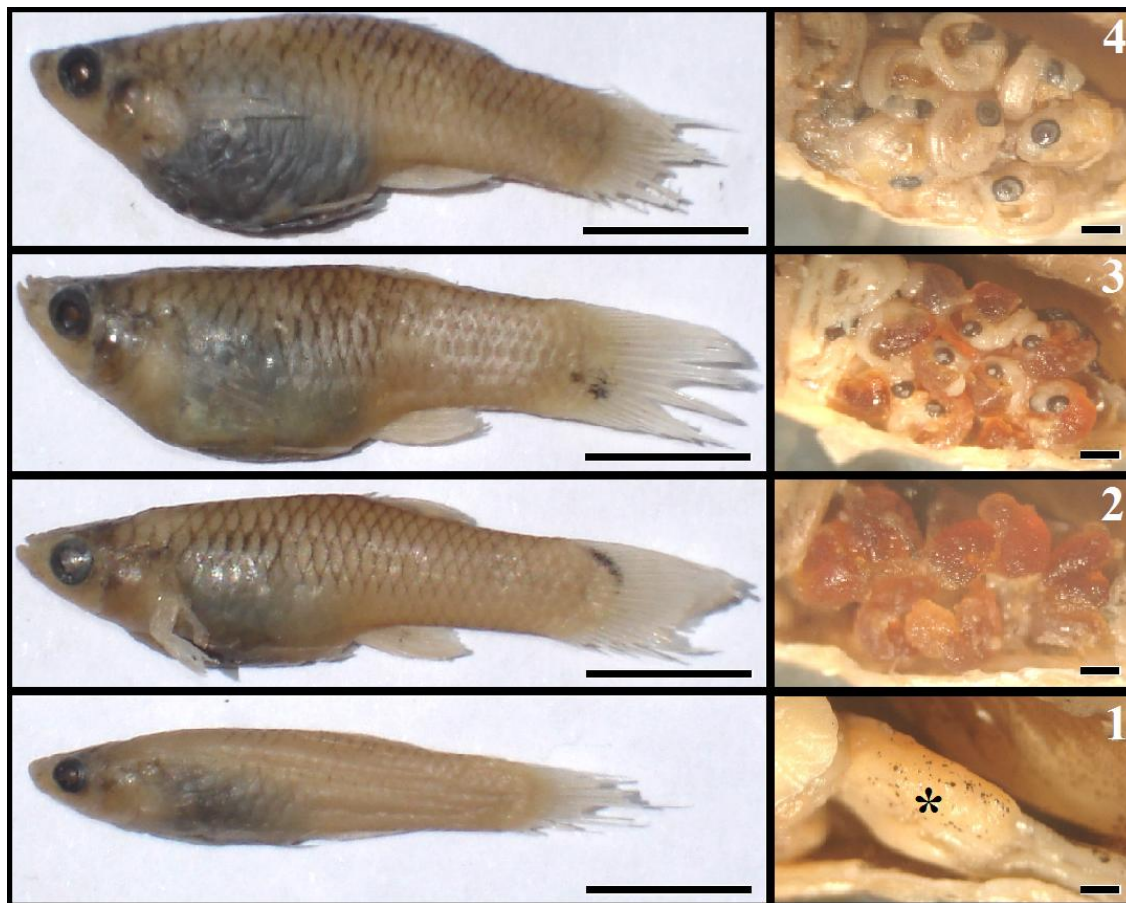


Figure 2. Macroscopic (left column) and mesoscopic (right column) developmental stages of *Poecilia reticulata* females. 1) Non-gravid (Asterisk: ovarian tissue without ovum or embryos), 2) Early-yolked ovum, 3) Early-eyed embryos, 4) Mature embryos. Scale bars (left column: 1 cm, right column: 20 \times).

Fecundity

We estimated the bimonthly and total mean values of fecundity (developmental stages 2, 3 and 4) of *P. reticulata* in the Lagoa do Nado reservoir. Fecundity was determined from early-yolked ovum or embryos in the ovaries, and they were counted under the binocular stereomicroscope according to Öztürk & Ikiz (2004).

Population structure

The sex and juvenile-to-adult ratio (Pope *et al.* 2010) were calculated from the bimonthly and total number of females, males and juveniles of *P. reticulata*.

Correlation between reproduction and abiotic factors

Following Andrade & Braga (2005), we grouped the bimonthly relative frequencies of gravid females (stages 2, 3, 4), which were then correlated with the average bimonthly water temperature and rainfall.

Statistical analysis

The fecundity values were compared using one-way ANOVA, followed when necessary by a Tukey pos-hoc test to determine significant

differences between mean values. Chi-square (χ^2 test) was used to check the differences in proportions between sexes and juveniles-to-adults, and the Spearman's rank correlation (r) to test a relationship between female reproduction and abiotic factors (Sokal & Rohlf 1995). Differences were considered significant for $P < 0.05$. All statistical analyses were performed using PAST-Paleontological Statistics (version 1.91) software (Hammer *et al.* 2009).

Results

Females were found in several developmental stages (stages 2, 3, 4) during almost all the sampling period (Figure 3). Mean fecundity was low in all bimesters with no statistical differences while for the total period, there was a statistical difference between developmental stages 2 and 3. The lowest fecundities were observed in September-October 2007 with mean value of 3.5 (± 2.12), while for the whole study period the mean value was 6.22 (± 2.06) (Table II).

We found more females than males in November-December 2006, January-February 2007

and May-June 2007. Overall, females were more frequent than males ($\chi^2=66.12$, 1.64, $df=1$, $P<0.05$) (Table III). The number of juveniles in all periods was higher than adult females and males ($\chi^2=62.82$,

$df=1$, $P<0.05$) (Table IV). Water temperature and rainfall were not significantly correlated with reproduction in females ($r=0.486$, $P=0.33$; $r=-0.143$, $P=0.79$) (Table V).

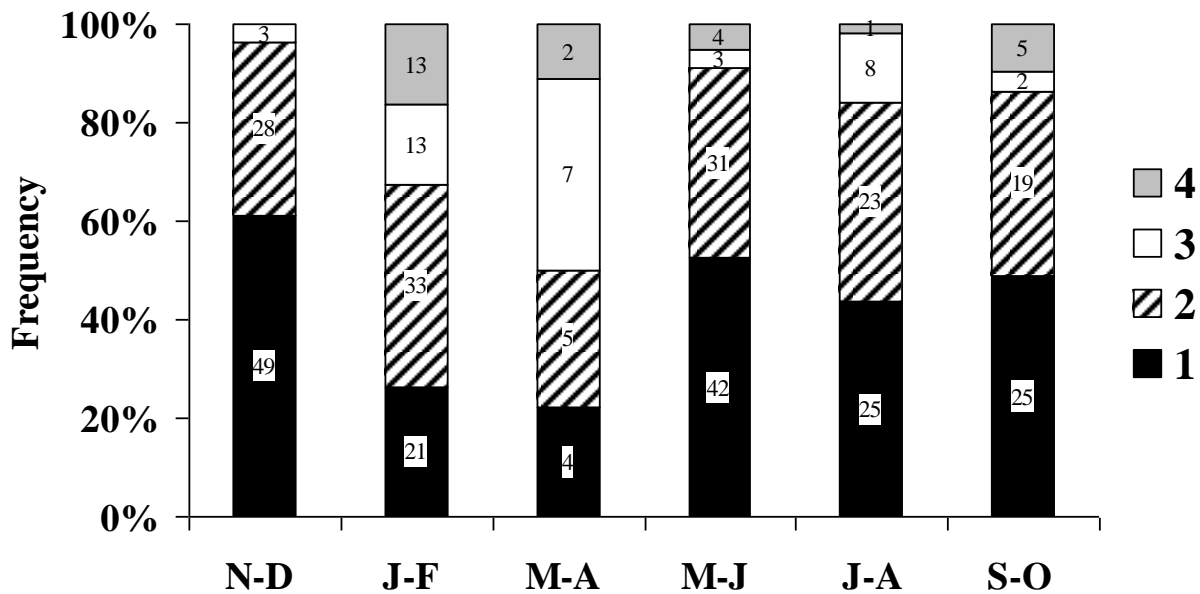


Figure 3. Relative bimonthly frequency of developmental stages in *Poecilia reticulata* females captured in Lagoa do Nado reservoir between November 2006 and October 2007. Developmental stages: 1) Non-gravid, 2) Early-yolked ovum, 3) Early-eyed embryos, 4) Mature embryos.

Table II. Bimonthly and total fecundity of *Poecilia reticulata* females captured in Lagoa do Nado reservoir between November 2006 and October 2007. Developmental stages: 2) Early-yolked ovum, 3) Early-eyed embryos, 4) Mature embryos. In a row, same letters indicate no significant differences ($P<0.05$).

Bimesters	Developmental stages		
	2	3	4
November-December	6.54±7.00a	3.67±2.08a	-
January-February	4.79±2.04a	6.61±3.43a	4.85±3.53a
March-April	7.4±7.37a	12.71±13.63a	9.5±9.19a
May-June	4.19±3.39a	4.33±3.21a	6.25±3.59a
July-August	9.62±8.22a	14.67±6.93a	12.5±14.85a
September-October	4.79±2.53a	3.5±2.12a	5.0±2.24a
Total	6.22±2.06a	7.58±4.90b	6.35±4.29ab

Table III. Bimonthly and total sex ratio of *Poecilia reticulata* captured in Lagoa do Nado reservoir between November 2006 and October 2007. *Significantly different ($P < 0.05$), $\chi^2_{0.05} = 3.84$, $df = 1$.

Bimesters	Females	Males	Chi-square
November-December	272	146	37.98*
January-February	167	108	12.66*
March-April	35	47	1.76
May-June	163	70	37.12*
July-August	21	19	0.1
September-October	42	37	0.32
Total	700	427	66.12*

Table IV. Bimonthly and total juvenile-to-adult ratio of *Poecilia reticulata* captured in Lagoa do Nado reservoir between November 2006 and October 2007. Juv=Juveniles, Fem=Females, Mal=Males. *Significantly different ($P < 0.05$), $\chi^2_{0.05} = 3.84$, $df = 1$.

Bimesters	Juveniles			Females			Males			Chi-square (Juv-Fem+Mal)
	0.5 cm	0.9 cm	Total	1.0-2.1 cm	2.2-3.3 cm	Total	1.0-2.0 cm	2.1-2.5 cm	Total	
November-December	282	288	570	92	180	272	26	120	146	23.24*
January-February	146	138	284	34	133	167	30	78	108	0.14
March-April	20	107	127	10	25	35	26	21	47	9.68*
May-June	244	160	404	143	20	163	18	52	70	45.9*
July-August	33	7	40	5	16	21	9	10	19	0.00
September-October	81	30	111	11	31	42	23	14	37	5.38*
Total	806	730	1,536	295	405	700	132	295	427	62.82*

Table V. Spearman's rank correlation (r) between *Poecilia reticulata* females and abiotic factors in the Lagoa do Nado reservoir between November 2006 and October 2007.

Variables	r	P
Gravid females \times Water temperature	0.486	0.33
Gravid females \times Rainfall	-0.143	0.79

Discussion

Studies about the reproductive biology of *P. reticulata* outside their natural ranges are essential to assess their degree of establishment, spread and potential ecological impacts on native communities in Minas Gerais (Magalhães 2008). We found females of *P. reticulata* in reproductive activity during the 12 months of the study period. A prolonged reproductive period was also found for the same species in a small impoundment in Cuba and in concrete channels in Pará State, Brazil (Koldenkova *et al.* 1990, Montag *et al.* 2011). Frequent reproduction over an extended breeding season is an opportunistic life-history strategy adopted by guppies that permit effective colonization in human-altered habitats (Gratwicke 2000). The fecundity observed for the species was low. Low fecundity was also observed in *P. reticulata* introduced in Costa Rica (Hernández *et al.* 2004). This characteristic may be probably due to larger size of embryos, a common reproductive feature for this fish (Reznick & Endler 1982), which may result in a higher probability of survival in new habitats (Gordon *et al.* 2009).

The majority of fish populations are expected to maintain a 1:1 sex ratio since the probability of occurrence for each sex should be 50%. When this proportion does not hold, the reasons and their consequences for population growth should be investigated (Pope *et al.* 2010). A higher frequency of females (female-biased sex ratio), was found in *P. reticulata*. This trend was also found for this poeciliid in Colombia and São Paulo State, Brazil (Garcia *et al.* 2008, Andrade *et al.* 2008). In poeciliids, males are more susceptible to mortality from a variety of sources, including differential predation due to their bright colours, higher susceptibility to stressors such as extreme temperatures, overcrowding, hypoxia, and also accelerated aging, since they invest their energy exclusively on mating behaviors (Snelson 1989). The advantage of having more females in *P. reticulata* is that they do not depend on the constant presence of males to reproduce because they can store sperms for later fertilization and may produce 20-40 live young every four weeks (Froese & Pauly 2011), attributes that allow them to be able to colonize any environment (Deacon *et al.* 2011). The number of *P. reticulata* juveniles was higher than adults. The same characteristic was found for *P. reticulata* introduced in Rio de Janeiro State, Brazil (Araújo *et al.* 2009). A large ratio of juveniles to adults is an indication of successful recruitment, and a fish population with recruitment difficulties will be characterized by fewer juveniles relative to adults

(Pope *et al.* 2010). The lack of correlation between abiotic factors was already expected. This has been explained by ovoviviparity, a common reproductive strategy of the studied species (Snelson 1989). Since eggs and embryos develop inside the female's body, they are less influenced by several external conditions such as temperature variations, rain, acidity, turbidity, salinity, diseases and predators attack (Nakatani *et al.* 2001).

According to Lockwood *et al.* (2007), the process of invasion by a non-native species occurs in five stages: 1) transportation from the place of origin; 2) arrival in the new environment; 3) establishment, when an immigrant population is maintained by reproduction and juvenile recruitment; 4) spread, when a population expands its geographic range; 5) low to high impact, when the receiving biota is affected by non-native species, and thus earn the name "invasive". The year-round presence of reproductive females and juveniles suggests that *P. reticulata* is established and probably expanding its population in Lagoa do Nado reservoir. Another evidence of establishment is the alteration of phenotype "store-bought type" (males are brighter and have larger colorful tails, females are plainer in color and have a smaller colorful tail) available in local aquarium trade to "wild-type". All of those that were collected in the present study resembled the "wild-type" in which adult males are grey with some coloured spots on the body and a translucent small tail, while females are dull brown (André Lincoln Barroso Magalhães, pers. obs.). These observations agree with the statement of Reznick & Bryga (1987) that when guppies are transplanted from one environment to another, the new phenotype evolves rapidly. Thus, and considering the highly competitive and destructive nature of this species worldwide and in Brazil (Courtenay & Meffe 1989, Vieira & Shibatta 2007, Cunico *et al.* 2009), this livebearer probably will reach the fifth stage of the process of biological invasion in this man-made lake. The nest-guarding cichlids such as *Australoheros facetum*, *Geophagus brasiliensis* and the calictiid *Callichthys callichthys* will probably be unaffected by it, but other native species that do not guard their eggs (e.g., *Hyphessobrycon santae*, *Hasemania nana*) could be adversely affected.

Due to the environmental problem detected in this study (i.e., establishment of a non-native fish), and the few strategies in Belo Horizonte and Minas Gerais to combat non-native aquatic species (Magalhães & Jacobi 2013), we recommend: a) the prohibition on sales of *P. reticulata* by local aquarium stores, b) a program for returning

unwanted fishes to aquarium stores can be effective in order to stop new aquarium-fish introductions, c) display warning informing about the negative ecological effects of aquarium dumping in all fish plastic bags and website of the aquarium store, and d) continuous awareness campaigns with wholesalers, retailers and aquarium hobbyists showing that abandonment of pets (i.e., aquarium dumping) is an environmental crime with a penalty of detention and fine, according to the Federal Law No. 9605 of 1998.

Concluding, these management recommendations are not intended to harm the activities of aquarium trade, given its undeniable economic importance to the city of Belo Horizonte. However, if these precautionary measures are not implemented, the propagule pressure due to releases of *P. reticulata* will continue, causing low resilience in Lagoa do Nado reservoir and permanently threatening native species in this ecosystem.

Acknowledgements

The authors are grateful to the Centro Universitário UNA (Institute of Education, Research and Extension) for financial support (BIC #5-2006/2007), to Instituto Estadual de Florestas de Minas Gerais (IEF-MG #068/2006) for granting permission to collect the fishes, to 5th Meteorological District (INMET) for rainfall data, and to Sérgio Augusto Domingues (Director, Fazenda Lagoa do Nado Municipal Park) for your assistance during this study.

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Received January 2014

Accepted May 2014

Published online May 2014