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Reproduction of the non-native fish *Lepomis gibbosus* (Perciformes: Centrarchidae) in Brazil

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Abstract: Minas Gerais is the fourth largest Brazilian state, and has an estimate of 354 native fish species. However, these fish species may be threatened, as this state has the highest rank of fish introductions reported for Brazil and South America. As one from the total of 85 non-native species detected, *Lepomis gibbosus* was introduced in the 60s to serve both as foragefish and to improve sport fishing. In this study, we evaluated the establishment of *L. gibbosus* in a shallow lake in the city of Ouro Preto, Doce River basin, state of Minas Gerais, Southeastern Brazil. We collected fish with fishing rods every two months from March 2002-February 2003. Fragments of gonads from a total of 226 females and 226 males were obtained and processed following standard histological techniques; then 5-7µm thickness sections were taken and stained in hematoxylin-eosin. Besides, for each specimen, the biometric measurements included the standard length (SL) and body weight (BW); and the sex ratio was obtained. The reproductive cycle stages were confirmed by the distribution of oocytes and spermatogenic cells. The type of spawning was determined by the frequency distribution of the reproductive cycle stages and ovarian histology. Based on the microscopic characteristics of the gonads, the following stages of the reproductive cycle were determined: one=Rest, two=Mature, three=Spawned for females or Spent for males; males and females in reproduction were found throughout the study period. Post-spawned ovaries containing oocytes in stages one (initial perinucleolar), two (advanced perinucleolar), three (pre-vitellogenic), four (vitellogenic) and post-ovulatory follicles indicated fractionated-type spawning in this species. The smallest breeding male and female measured were 4.6 and 4.9cm standard length, respectively, suggesting stunting. The sex ratio did not vary between males and females along the year and bimonthly, being 1:1. Moreover, *L. gibbosus* appears to be at stage three of biological invasion: establishment through reproduction. We suggest to deliver information about “non-native species” through lectures in schools, colleges/ universities, NGOs, government and environmental agencies in the cities and villages, in order to try to prevent environmental degradation by the introduction of non-native fish such as *L. gibbosus* in the region. We also recommend high fines for red-handed, and the import ban of non-native fish species to the region. Rev. Biol. Trop. 60 (3): 1327-1334. Epub 2012 September 01.

Key words: *Lepomis gibbosus*, biological invasions, sunfish, reproductive biology, Brazil.

The rate of fish introduction have rapidly increased in South America since the late 80s (Lima *et al.* 2010). The first period of introductions in Brazil began in the early 20th century, coinciding with the increase of fish farming (Latini *et al.* 2004). The second period occurred in the middle of the last century, when electric companies in the Southeast invested in induced breeding of fish, often non-native to basins where they were released

(Alves *et al.* 2007). The increasing demand for pond fee-fishing (‘fish-and-pay’) in most populous regions (Fernandes *et al.* 2003) and the aquarium trade (Magalhães & Jacobi 2008) contributed to a third period of introductions, which began at the end of the last century and is currently in progress (Vitule 2009).

Minas Gerais is the fourth largest Brazilian state, with an area of 586 528Km² embracing 17 drainage basins (Alves *et al.* 2007), and an

estimate of 354 native fish species (Vieira *et al.* 2009b). However, these fish species may be threatened, since Minas Gerais is at the top of the ranking of fish introductions in Brazil and in South America, with 85 non-native species detected (Magalhães 2010).

Lepomis gibbosus (Linnaeus 1758) is one of the 85 species of non-native fish found in inland waters of the state (is not found anywhere else in Brazil). This species is a centrarchid native to Canada and United States and was introduced in the city of Ouro Preto in the 60s to serve both as prey ('foragefish') to *Micropterus salmoides* (Lacépède 1802) and improve sport fishing in the region (Magalhães & Ratton 2005). It is also present in Venezuela and Chile (Welcomme 1988), but there is no data about its biology in these two countries.

Despite Minas Gerais has a high number of oviparous, non-native fishes, consistent studies about their reproductive biology are still incipient and restricted to few species, such as *Cichla ocellaris* (= *C. kelberi* Kullander & Ferreira 2006) (Magalhães *et al.* 1996, Normando *et al.* 2009) in the São Francisco River basin, *Cichla piquiti* Kullander & Ferreira 2006 in the Paranaíba River Basin (Vieira *et al.* 2009a), *Plagioscion squamosissimus* (Heckel 1840) (Godinho *et al.* 1997), *C. cf. ocellaris* (Schneider 1801), *Cichla monoculus* Spix & Agassiz 1831 in the Grande River basin (Gomiero & Braga 2004), and *L. gibbosus* in the Doce River basin (Magalhães & Ratton 2005).

Studies about the reproduction of oviparous fish outside their natural range provide the parameters for understanding their establishment in the invaded ecosystem. Thus, our aims in this paper were: i) to study the reproductive cycle and type of spawning; ii) to determine the minimum and maximum standard length and body weight; iii) to calculate the sex ratio; iv) to evaluate the actual phase of invasion of *L. gibbosus* in the region; and v) to suggest actions to prevent further fish introductions in the upper Doce River, state of Minas Gerais, Brazil.

MATERIALS AND METHODS

Study site: The Gambá Lake is a permanent, natural lake formed in a rupestrian ferruginous field ('canga'), with maximum depth of 3.3m and area of 6 700m². The native fish fauna of the lake is represented by *Gymnotus carapo* Linnaeus 1758, *Geophagus brasiliensis* (Quoy & Gaimard 1824), *Australoheros facetus* (Jenyns 1842). Non-native species include *Poecilia reticulata* Peters 1859, *Xiphophorus hellerii* Heckel 1848, *Tilapia rendalli* (Boulenger 1897) and *L. gibbosus* (Costa 2003).

Fish samples: We collected a total of 226 females and 226 males of *L. gibbosus* every two months, from March 2002-February 2003, using fishing rod and line (fishing gear allowed in the site) in the Gambá Lake (20°43'51.11" S - 43°30'0.63" W), city of Ouro Preto, state of Minas Gerais, Southeastern Brazil. The region is located in the headwaters of the Doce River (Magalhães & Ratton 2005). The fish were sorted by site collection, packed in plastic bags, fixed in 10% formalin, and subsequently transferred to 70% alcohol. Voucher specimen used in this study are deposited under their respective catalogue number (MCP28664) in the Museum of Science and Technology, Pontifical Catholic University of Rio Grande do Sul, Brazil.

Histological methods and stages of the reproductive cycle: We also collected fragments of gonads (ovaries and testes) of specimens and fixed them in Bouin's fluid for 12hours. Fragments were subjected to routine histological techniques as follows: embedding in paraffin, and staining in hematoxylin-eosin of 5-7µm thickness sections (Vazzoler 1996). Stages of the reproductive cycle were determined based on microscopic characteristics of the gonads. We determined spawning type by analyzing the frequency distribution of stages of the reproductive cycle and ovarian histology (Bazzoli 2003). After determining the stages of the reproductive cycle of *L. gibbosus*, we

calculated the bimonthly absolute and relative frequencies of females and males.

Standard length, body weight and sex ratio: From each individual the following biometric data were taken in the laboratory: standard length (SL), given in cm and body weight (BW) in g. Besides, we determined the sex ratio from the bimonthly absolute frequency and the total number of males and females of *L. gibbosus* using a chi-square (χ^2) test. We considered statistically significant differences

when $p < 0.05$. The analysis was performed using the Paleontological Statistics-Past (Hammer *et al.* 2009).

RESULTS

Histological methods and stages of the reproductive cycle: Based on the microscopic characteristics of the gonads, the following stages of the reproductive cycle were determined: one=Rest, two=Mature, three=Spawning for females or Spent for males (Fig. 1).

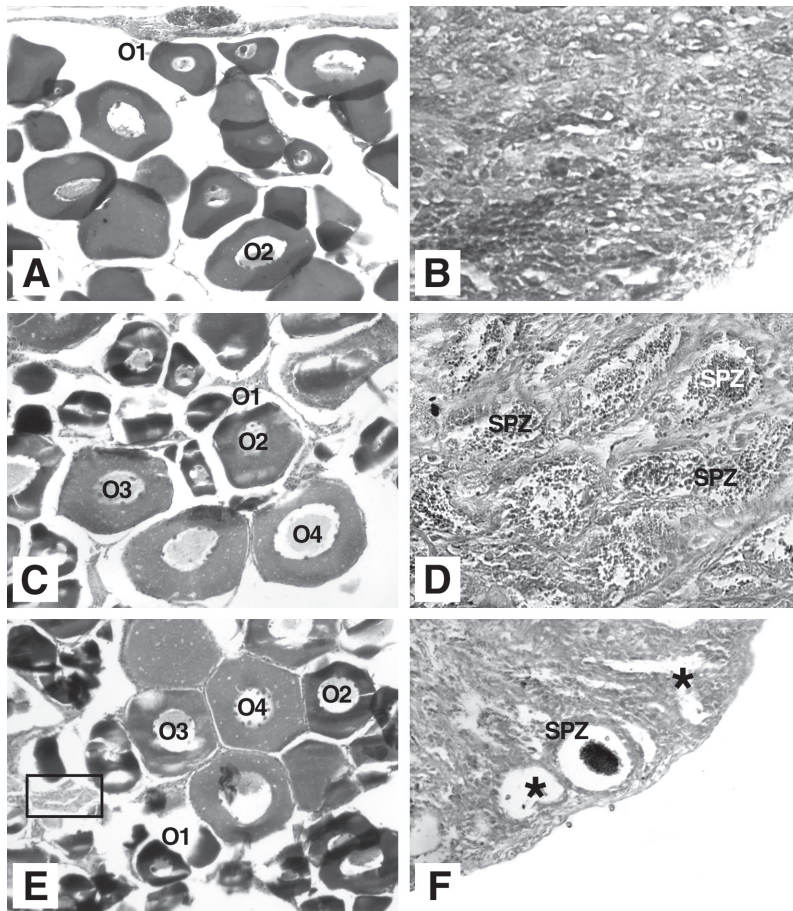


Fig. 1. Histological sections of *Lepomis gibbosus* ovaries (left column) and testes (right column) in different stages of reproductive cycle: (A)=Rest: only initial perinucleolar (O1) and advanced perinucleolar (O2) oocytes are present; (C)=Mature: O1, O2, pre-vitellogenic (O3) and various vitellogenic (O4) oocytes are present; (E)=Spawning: O1, O2, O3, O4 and post-ovulatory follicle (rectangle); (B)=Rest: the lumen of the seminiferous tubules is closed; (D)=Mature: lumen of seminiferous tubules is filled with spermatozoa (SPZ); (F): Spent: seminiferous tubule partially empty, few cysts of spermatocytes and spermatozoa (SPZ) associated with seminiferous tubules containing spermatogonia and empty lumen (asterisks). Hematoxylin-eosin stain. (A), (B), (D): 400 ×, (C), (E), (F): 100 ×.

Reproductive cycle and type of spawning: Males and females in reproduction (stages two and three) were found during all the sampling period (Fig. 2). The high frequency of females in stage two and three was associated with the histologic features of spawned ovaries containing oocytes in stages one (initial perinucleolar), two (advanced perinucleolar), three (pre-vitellogenic), four (vitellogenic) and post-ovulatory follicles, indicated that the species has a fractionated-type spawning.

Standard length, body weight and sex ratio: The smallest breeding female we found was at stage three (Spawned), measured 4.6cm SL and weighted 2g. The smallest breeding male we found was at stage two (Mature) and measured 4.9cm with a weight of 2g (Table 1).

We found no differences in the percentage frequencies between females and males of *L. gibbosus* for the whole study period (1:1, chi-square=0.00, $p<0.05$), except for September-October 2002, where we found more males than females (0.5:1, chi-square=5.12, $p<0.05$) (Table 2).

DISCUSSION

We found females and males of *L. gibbosus* in reproduction activity throughout the study period in the Gambá Lake. The same trend occurred with *L. gibbosus* introduced into a dam (Custódio's Dam) 20km always from

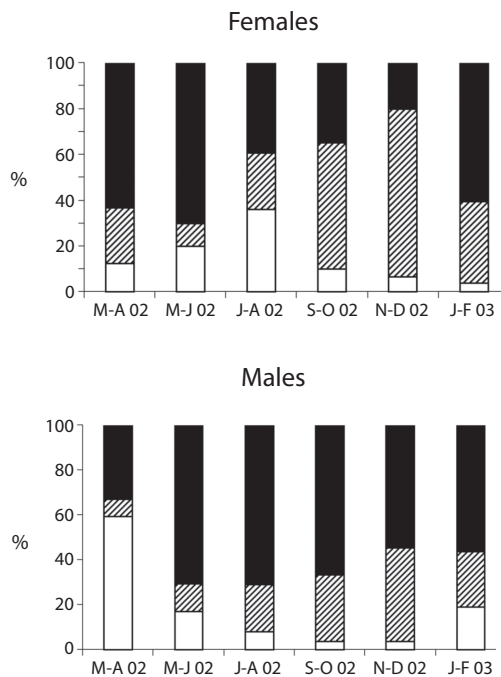


Fig. 2. Absolute frequency by stage of reproductive cycle in females and males of *Lepomis gibbosus* captured in the Gambá Lake, between March 2002 and February 2003. White=Rest, lines=Mature, black=Spawned/Spent.

Gambá Lake, where this species reproduced year-round during February 2001 to March 2002 (Magalhães & Ratton 2005). The longer reproductive period in an introduced population of *L. gibbosus* out of South America was recorded in Greece and Spain, where the species reproduced during four months of the

TABLE 1

Biometric data for *Lepomis gibbosus* females and males captured in Gambá Lake between March 2002 and February 2003

Measures	Females			Males		
	SRC ³			SRC ³		
	Rest	Mature	Spawned	Rest	Mature	Spent
SL ¹ -Minimum (cm)	4.8	4.9	4.6	5.5	4.9	5.2
SL ¹ -Maximum (cm)	5.7	6.5	9.1	9.5	10.0	12.1
BW ² -Minimum (g)	3	2	2	3	2	3
BW ² -Maximum (g)	5	13	22	26	32	53

¹ SL=Standard Length

² BW=Body Weight

³ SRC=Stage of the Reproductive Cycle

TABLE 2
Bimonthly and total sex ratio of *Lepomis gibbosus* captured in Gambá Lake between March 2002 and February 2003

Bimesters	Females	Males	Sex ratio	chi-square
	N	N	♀:♂	$\chi^2_{0.05}$
March-April 02	53	50	1.0:1	0.08
May-June 02	28	17	1.6:1	2.68
July-August 02	27	33	0.8:1	0.60
September-October 02	17	33	0.5:1	5.12*
November-December 02	48	57	0.8:1	0.76
January-February 03	53	36	1.4:1	3.24
Total	266	266	1:1	0.00

*Indicates the significant values for sex ratio (chi-square, $p < 0.05$, $\chi^2_{0.05} = 3.841$, d.f.=1).

year in these countries (Neophytou & Giapis 1994, Vila-Gispert & Moreno-Amich 1998). A prolonged reproductive period is a biological response of introduced fishes in countries with warm or mild climate, providing a mechanism for the reestablishment of population structure (Smith & Walker 2004).

Females having oocytes at all developmental stages, with different sizes and asynchronous growth, along with post-ovulatory follicles, indicate that the species has a fractionated-type spawning. Fractionated spawning is characteristic of this species both in his native range (Scott & Crossman 1973), and in places where it is introduced, such as South America (Magalhães & Ratton 2005), Europe, and Asia (Copp & Fox 2007). Reproductive features of species may influence its probability of establishment (Latini & Petrere 2004). Species with fractionated spawning have higher chance of establishment due to mainly two reasons: i) decrease competition for spawning site among females within populations with synchronous reproduction and ii) increased larval survival, since releasing oocytes in various postures let time for larvae from each spawning abandon the planktivorous phase and attain another ecological niche, reducing food competition (Nikolsky 1963).

We found breeding males and females of *L. gibbosus* with less than 5cm SL and small body weight, which can indicate stunting. This phenomenon may be due to three reasons in the

Gambá Lake: i) intraspecific competition due to overcrowding, ii) low food availability and iii) increased survival rate due to the absence of top predators (Roff 1992). This is perhaps the lowest measurement found in reproductively active, introduced populations of *L. gibbosus*. In France, Spain, England, Greece and Romania, the minimum size reproductive individuals of this species ranged from 5.1cm-9.1cm SL (Copp *et al.* 2002). Moreover, the fishing gear used in this work (rod and line) did not capture immature fish, making it impossible to determine the size at sexual maturity by the method L50 (Santos 1978). This method estimates the length at which 50% of the population is mature and 50% is immature. Thus, the standard length at first sexual maturity was suggested based on the standard length of the smallest breeding female and male captured (Bazzoli 2003).

As expected, we found a sex ratio of 1:1 for *L. gibbosus* in the majority of bimesters and in the whole study period. The same proportion of females and males was also found for the species introduced in England (Cucherousset *et al.* 2009). That is advantageous for the species in terms of reproduction, because as the male build the nest during the breeding season (Klarr *et al.* 2004), there will always be at least one female available to mate, thus ensuring the establishment in the site.

According to Lockwood *et al.* (2007), the process of invasion by a non-native species happens in five stages: i) transportation from

their place of origin, ii) arrival of the species in the new environment, iii) establishing through reproduction, iv) expansion of its geographic range, v) impact (low or high), the moment when the receiving biota suffers consequences from the introduction. In this study, we confirmed that *L. gibbosus* is at stage three, since it is well adapted in the Gambá Lake, reproducing throughout the study period. This species have found in this habitat similar conditions to those of their places of origin, being considered as an established species. Moreover, this shallow lake has natural very low richness of native species such as *G. carapo*, *G. brasiliensis* and *A. facetum* and this may have been facilitating the establishment process of *L. gibbosus*. These results agree with the statement of Moyle & Light (1996) that the magnitude of the impact of introduced species in a natural environment can increase according to the specific characteristics of the environment. Therefore, systems with few native species, such as headwater regions, tend to have low environmental resistance to invasion by non-native species than ecosystems with high species richness.

We suggest that an important tool to prevent further introductions of fish in the Upper Doce River environments will be the dissemination of scientific information about “non-native species” through lectures in schools, colleges/universities, NGOs, government and environmental agencies in the cities and villages. Besides, to prevent the reproduction and dissemination of *L. gibbosus* and other species, the direction of Itacolomi State Park and State Forestry Institute of Minas Gerais (both located in the city of Ouro Preto) have recommend the selective fishing only of non-native species. In addition, to popularize the subject “non-native species” mentioned earlier, our specific recommendations to the authorities are: i) massive clarification to the public about the National Law 9605/1998 indicating that the release of non-native species is considered an environmental crime, ii) high value (>US\$5 000.00) fines if any offender is caught introducing non-native fish in the region, and iii) to restrict or prohibit the importation of *L. gibbosus*, and any

other non-native fish species considered pest to cities that are located in headwater regions.

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RESUMEN

Se analiza el establecimiento del pez introducido *Lepomis gibbosus* en una laguna natural de la ciudad de Ouro Preto, Cuenca del Rio Doce, provincia de Minas Gerais, región sureste de Brasil. Cada dos meses se realizaron muestreos con anzuelo y línea, entre marzo 2002-febrero 2003 y se capturaron 226 hembras y 226 machos. Se encontraron hembras y machos en actividad reproductiva durante todo el muestreo. Ovarios con signos de desove con oocitos de diferentes tallas y folículos post-ovulatorios indicaron la puesta parcial para *L. gibbosus*. La hembra y macho en reproducción más pequeños tenían entre 4.6cm y 4.9cm de longitud, que caracteriza el enanismo. La proporción sexual fue 1:1 y no presentó diferencias bimensuales ni anuales. De las cinco fases del proceso de bioinvasión, se confirmó que *L. gibbosus* se encuentra en la fase tres, llamada establecimiento a través de la reproducción. Se sugiere aclaración sobre la temática “especie foránea” para evitar la degradación del ambiente con la introducción de peces foráneos en la región.

Palabras clave: *Lepomis gibbosus*, invasiones biológicas, pez sol, biología reproductiva, Brasil.

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