Journal of Applied Ichthyology

J. Appl. Ichthyol. 27 (2011), 1322–1331

ISSN 0175–8659

Received: December 10, 2010
Accepted: June 25, 2011
doi: 10.1111/j.1439-0426.2011.01874.x

Reproductive biology of the armoured catfish *Loricariichthys castaneus* (Castelnau, 1855) in Lajes reservoir, southeastern Brazil

By I. D. Gomes[1], F. G. Araújo[1], W. Uehara[1] and A. Sales[2]

[1]*Laboratório de Ecologia de Peixes, Universidade Federal Rural do Rio de Janeiro, Antiga Rodovia Rio-São Paulo, Seropédica, RJ, Brazil;* [2]*Laboratório de Histologia e Embriologia Animal, Universidade Federal Rural do Rio de Janeiro, Antiga Rodovia Rio-São Paulo, Seropédica, RJ, Brazil*

Summary

Sex ratio, gonadal development, breeding season and fecundity of the armoured catfish *Loricariichthys castaneus* were described to assess its reproductive strategy in a Brazilian tropical reservoir. In total, 226 specimens (199 females and 27 males) were captured from September 2005 to August 2006 and examined in the laboratory. Females outnumbered males and achieved sizes larger than 330 mm TL. Oocyte development, determined by histological analysis, was asynchronous with oocyte size, ranging from pre-spawning (27–270 μm) to spawning (243–3460 μm), followed by a sharp decrease in the mean oocyte diameter postspawning (590–730 μm) as the spawning proceeded. Spawning occurred throughout most of the year, peaking in August–September and reaching a low in April–May, according to variations in GSI and frequencies of stages of gonadal development. Batch fecundity ranged from 242 to 833 vitellogenic oocytes (relative fecundity = 2.27 oocytes g^{-1}), averaging 483 oocytes, and was positively related to gonad weight (P = 0.00003). Oocyte diameters ranged from 0.027 to 5.59 mm, with vitellogenic diameters ranging from 2.08 to 5.59 mm. Continuous development of oocytes throughout the year suggests that *L. castaneus* presents indeterminate fecundity and is a batch-spawner. These attributes, associated with parental care and a wide reproductive period, correspond to an equilibrium strategy that has proved to be effective in the Lajes reservoir.

Introduction

Loricariichthys castaneus (Castelnau, 1855) is a native iliophagous / detritivorous species that has successfully adapted to the Lajes reservoir, being one of the most abundant species in this system (Duarte and Araújo, 2001). One of the oldest impoundments in Brazil, the reservoir dates back almost a century. The environment is oligotrophic, surrounded by well-preserved stretches of Atlantic rainforest, and with a low degree of physical habitat complexity.

Studies on the reproductive biology of species of the genus *Loricariichthys* are uncommon. Marcucci et al. (2005) reported general information on reproductive tactics of *Loricariichthys platymetopon* Isbrüker and Nijssen, 1979, in Capivara reservoir; the fish has quickly become one of the most abundant species in this system. Suzuki et al. (2000) also observed that species of *Loricariichthys* rank among the most abundant species in the lentic habitats of southeastern Brazil, suggesting that their reproductive strategy is effective due to the ability to replace broods rapidly under a regime of environmental changes associated with an unpredictable hydrology in a reservoir constructed for hydroelectric purpose. Fecundity, reproductive cycle and gonad morphology of *Loricariichtys spixii* (Steindachner, 1862) were reported for Lajes reservoir (Araújo et al., 1998; Duarte and Araújo, 2000; Duarte et al., 2007), but information is still lacking on the reproductive strategy of this species.

Species characterised by continuous oocyte development have often been described as indeterminate spawners (Hunter et al., 1985; Winemiller, 1989; West, 1990). These species have the ability to develop unyolked oocytes continually, adding them to the standing stock of advanced-yolked oocytes even after spawning begins. Spawning in multiple batches is a mechanism that increases the reproductive effort as well as the distribution throughout the reproductive period (Burt et al., 1988).

Fish spawning times vary little from year to year (Marraro et al., 2005). This means that the events of the reproductive cycle are locked to, and synchronised with, the environmental changes that occur on a seasonal basis. Indeterminate spawners usually spawn many times during the course of a prolonged breeding season. Batch spawning can be seen as a strategy to release eggs over a long period of time, increasing the survival probability of offspring (Lambert and Ware, 1984). Accordingly, it is believed that species of *Loricariichthys* have several attributes, such as parental care, asynchronic oocyte development and aseasonal reproduction, which appear to correspond to the equilibrium strategy reported by Winemiller (1989). This study aims to more fully test the hypothesis that *L. castaneus* exhibits equilibrium strategies, similar to those reported for most Siluriformes loricariids, and to describe the tactics the species uses for success in the Lajes reservoir. To this end, the following questions were posed: (i) Is the sex ratio well-balanced and parental care practised? (ii) Does oocyte size-frequency distribution vary among maturation stages or seasonally? (iii) Does spawning occur in batches with undetermined fecundity? (iv) Is the reproductive season protracted or is it restricted to a few months?

Materials and methods

The Lajes reservoir (22°42′–22°50′S, 43°53′–44°05′W) is a major impoundment in Rio de Janeiro State, and located 415 m above mean sea level in the upper slopes of the Serra do Mar (Sea Mountains) in southeastern Brazil. It has an area of c.a. 38.9 km^2, mean and maximum depth of 15 and 40 m, respectively, and a long residence time of 297 days. This

oligotrophic reservoir with high water quality has low concentrations of nitrogen (< 10 μg L^{-1}), phosphate (< 120 μg L^{-1}) and chlorophyll α (< 2.5 μg L^{-1}) and possesses a high water residence time (286 days) (Santos et al., 2004; Gomes et al., 2008). The reservoir altitude influences the tropical humid climate, resulting in two distinct seasons: a wet season, from late spring to early autumn; and a dry season, from late autumn to spring (Barbieri and Kronemberg, 1994). Surface water temperatures range from 15.3 to 30.6°C, pH between 6 and 8, dissolved oxygen is higher than 4.7 mg L^{-1} and Secchi transparency averages 2.30 m ± 0.05 standard error (Araújo and Santos, 2001). Fluctuation in rainfall combined with a regulated outflow results in water level peaks one or 2 months after the rainfall season (Santos et al., 2004), with differences among extremes of flood and drawdown events (Duarte and Araújo, 2001). Lajes reservoir experiences remarkable water level fluctuations dictated by rainfall and hydroelectric demands. Overall, the annual water level fluctuation averages nearly 3 m, but extreme differences between high and low water levels can reach up to 10 m (Duarte and Araújo, 2001; Santos et al., 2004). According to Santos et al. (2004), low water levels have negative impacts upon the structural habitat complexity and, consequently, on the ichthyofauna, with the most detrimental effects occurring in years of severe and prolonged drawdown. LIGHT Energy Services, the concessionary of the hydroelectric plant, provided data for rainfall, water temperature, and reservoir water level during the study period.

Loricariichthys castaneus is widely distributed throughout the Lajes reservoir (Araújo and Santos, 2001). Fish were collected monthly from August 2005 to September 2006 by gill nets (30 m × 4 m, 3–12 cm stretched mesh size) at randomly chosen sampling sites whereby intentions were to encompass the entire reservoir area throughout 1 year, under various environmental conditions. A total of 226 specimens (198 females, 28 males) were examined. All individuals were sexed (males with enlarged lower lips) and total length (TL, mm) and the wet body weight of the eviscerated fish (BW, g) were recorded. Size structure of males and females was determined by total length frequency analysis. A chi-square (χ^2) test was used to assess differences in size structure between sexes and size classes.

The gonad classification was adapted from West (1990) and Nuñez and Duponchelle (2009). Gonads were weighed and preserved in 4% buffered formaldehyde for subsequent histological analyses and determination of oocytes size-frequency distributions. Gonads were assigned to developmental stages, based on form, size, weight, colour and vascularisation; however, gonads were ultimately classified as either immature (juveniles and inactive stages) or mature (maturing, ripe, spawn / spent and recovering stages) to reduce the chance of error in correctly identifying individual stages. The diameter of oocytes was measured in a stereomicroscope fitted with an ocular micrometric (precision 0.001 mm).

A cross-section from the gonads was dehydrated in ascending solutions of alcohol and embedded in paraffin; 5 μm sections were then cut using a microtome and followed by staining with haematoxylin and eosin. Oocytes were classified according to their morphology, their affinity to the dyes used, and the presence of specific inclusions (lipid droplets, yolk granules, yolk vesicles). In five fish randomly chosen from the monthly sample, the diameters of the first 50 oocytes and their nuclei were measured to the nearest 0.0001 mm. Histological identification of the various maturity stages were determined according to development of the ovary and testis and also by the presence / absence of different types of oocytes (i.e. whether organised by ovarian lamellae or not) and spermatocytes. Histological classification of ovaries was based upon the most advanced oocyte stage and on the occurrence of different stages of postovulatory follicles (POFs).

The annual gonadal cycle was determined by variations in the gonadosomatic index, $GSI = 100 \times (GW \times BW^{-1})$, where GW is the total gonad weight, and by size-frequency distribution of the gonadal maturity stages. Condition factor and hepatosomatic index (HSI) were calculated as indirect indices of energy status. The condition factor was calculated with a Fulton-type equation, $K = 100 \times (BW \times TL^{-3})$. The hepatosomatic index (HSI) was calculated according to the equation, $HSI = 100 \times (LW \times BW^{-1})$, where LW is the weight of the liver.

Batch fecundity was estimated by direct counts of fixed mature ovarian subsamples in 30 ovaries and calculated as: $F = (N \times GW) \times GWS^{-1}$, where F = fecundity, N = number of mature oocytes, GW = total gonad weight and GWS = gonadal subsample weight. Relative fecundity (number of eggs per gram of body weight) was calculated to remove the effect of body size. A linear regression analysis was performed to assess relationships between fecundity and total length, total body weight and gonad weight.

Results

Loricariichthys castaneus exhibits sexual dimorphism, with males having enlarged lower lips to carry fertilised eggs attached to the lower lip until the larvae hatch. This feature is more conspicuous during the reproductive period, but persists throughout the year. In post-larval stage individuals (TL = 15–20 mm) it was observed adhering to the anterior ventral surface of males (Fig. 1).

Sex ratio

Size ranged from 310 to 408 mm total length (TL) for females, and from 265 to 372 mm TL for males. Significant differences ($P < 0.05$) in the sex ratio were found for individuals larger than 330 mm TL (Table 1), with higher number of females than males. The overall sex ratio for *L. castaneus* in Lajes reservoir (all pooled data) was found to be significantly female-biased (7.1 : 1).

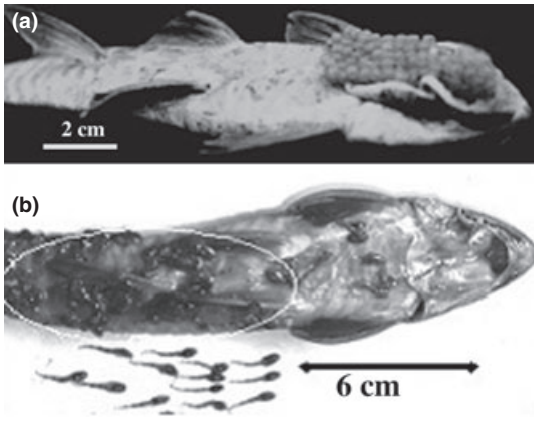

Fig. 1. *Loricariichtys castaneus*, Lajes reservoir: males carrying eggs on their lips (a); post-larvae eggs adhering to their ventral surface (b)

Table 1
Chi-square (χ^2) test for *Loricariichthys castaneus* sex ratio comparisons by size classes, Lajes Reservoir.

Size classes (TL, mm)	Females (n)	Males (n)	Sex ratio	Total	EF	χ^2
< 300	0	4	0.0 : 4.0	4	2	4*
300–330	9	12	1.0 : 1.3	21	10.5	0.4
331–360	100	10	10 : 1.0	110	55	73.6*
361–390	79	2	39.5 : 1.0	81	40.5	73.2*
> 390	10	0	10 : 0.0	10	5	10*
Total	198	28	7.1 : 1.0	226	113	127.9*

EF, expected frequency; TL, total length (mm); n, number of individuals.
*95% level of significance.

Stages of oocyte development

Oogonia (Fig. 2a). Spherical to slightly oval in shape. Diameter averaging 36.6 ± 1.5 μm (n = 50). Cytoplasm lightly stained. Very large nucleus (26.6 ± 0.1 μm, n = 50) with single very prominent nucleolus. Sited on periphery of the ovarian lamellae, isolated or forming cysts.

Chromatin nucleolar stage (Fig. 2a). Similar to oogonia, although somewhat larger (mean diameter = 60.2 ± 0.5 μm, n = 47). Large nucleus (48.1 ± 1.4 μm, n = 47), with single nucleolus. Reduced cytoplasm, with little or no affinity with dyes used.

Perinucleolar stage (Fig. 2a). Primary-growth oocyte at early stage of development, size increases (mean diameter = 88.5 ± 1.7 μm, n = 50). Cytoplasm with strong affinity for haematoxylin. Nucleus more evident (42.9 ± 1.1 μm, n = 50), with multiple nucleoli, generally peripheral, next to nuclear membrane. Yolk nucleus or Balbiani body (Wallace and Selman, 1981) present in cytoplasm. Follicular layer present but difficult to observe. Late stage exhibits rapid growth (mean diameter = 146.0 ± 4.3 μm, n = 49) and nucleus averages to 55.1 ± 1.4 μm, n = 49 (Fig. 2b). Progressive loss of affinity for haematoxylin. Disintegration of Balbiani body. Follicle or follicular layer easier to observe, and consisting of an internal layer (granular layer) and a further external layer (theca) (Hunter and Macewicz, 1985).

Yolk-vesicle formation (Fig. 2c). Secondary-growth oocytes in different stages of development. Small vesicles and allveol appear in the periphery of the cytoplasm. The contents of these vesicles have an ionic composition to enhance imbibing

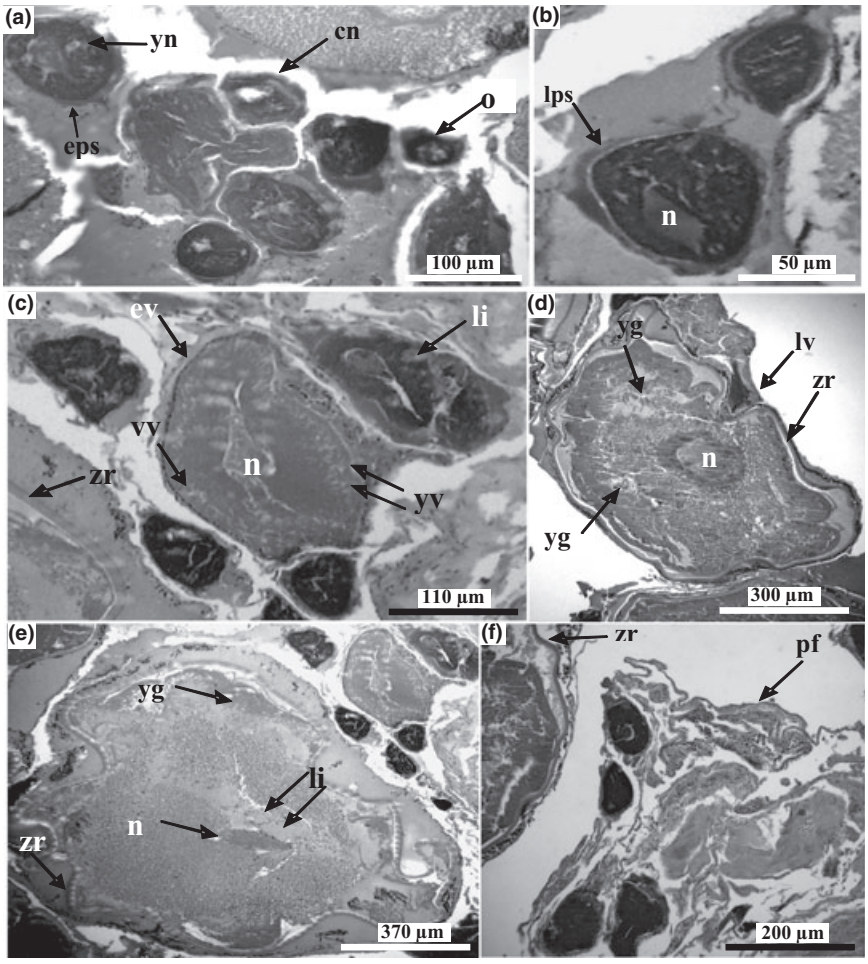

Fig. 2. Histological transversal sections, ovarian tissue of *Loricariichthys castaneus*: (a) Primary-growth oocytes at various stages of development; (b) larger primary-growth oocytes; (c) secondary-growth oocytes at various stages of development; (d) oocytes at vitellogenesis; (e) ripe and (f) postovulatory follicles. *o*, oogonia; *cn*, chromatin nucleolar stage; *eps*, early perinucleolar stage; *yn*, yolk nucleus; *lps*, late perinucleolar stage; *n*, nucleus; *ev*, early-vitellogenesis; *li*, lipid inclusions; *zr*, zona radiate; *lv*, late-vitellogenesis; *yg*, yolk granules; yv, yolk vesicles

of water during fertilisation so that the perivitelline space is formed. Mean diameter of oocyte 482.7 ± 18.8 μm, n = 49 and nucleus 215.4 ± 9.1 μm, n = 49. Progressive loss of affinity by cytoplasm for haematoxylin. Zona radiata visible, although not yet stained by eosin. Accumulation of lipid inclusions in cytoplasm has begun.

Vitellogenesis (Fig. 2d). In early stage, yolk granules small and numerous, also called yolk spheres or yolk globules containing yolk vesicles present, occupying the entire cytoplasm. Mean oocyte diameter 802.3 ± 10.4 μm (n = 50) and nucleus 266.9 ± 3.5 μm (n = 50). Yolk vesicles increase in size and gravitate towards the periphery as the yolk granules grow. Follicular layer and zona radiate (10–20 μm thickness) are visible, with the latter being dyed with eosin. Mean oocyte diameter 1250.0 ± 3.2 μm, n = 49 and nucleus 420.0 ± 0.9 μm, n = 49.

Ripe (Fig. 2e). Nucleus decreases in size. Lipid inclusions dispersed in the cytoplasm. Mean oocyte diameter 1537 ± 13.4 μm, n = 49. Yolk granules fused into homogeneous mass, creating 'hyaline oocyte' or 'hydrated oocyte' with mean diameter 2270 ± 37.7 μm, n = 49; nucleus often not visible due to disintegration of nuclear membrane and dispersion of its contents in the cytoplasm.

Postovulatory follicles (Fig. 2f). The structure of the follicle is recognisable by its disorganised structure, abundant vacuoles and a convoluted follicular wall surrounding an irregular cavity or lumen containing only a large cell. Mean oocyte diameter is 667.3 ± 5.1 μm, n = 50.

Stages of spermatocytes development

The spermatogenic cells appear in the interior of the seminiferous tubules at different stages during spermatogenesis (spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa), forming cysts (Fig. 3). Each cyst is bound by a layer of connective tissue and contains cells at the same stage of development. In mature testes, the seminiferous tubules are filled with spermatozoa.

Spermatogonia. Occur isolated on the periphery of the seminiferous tubules. Spherical in form and do not hold colour well when colouration techniques are applied. The appearance of spermatogonia is generally associated with tunica albuginea (Fig. 3).

Primary spermatocytes. Smaller than spermatogonia, from which they originate. The nucleus is strongly stained with haematoxilin and cytoplasm has little affinity for dyes.

Secondary spermatocytes. Little morphologic variation present and therefore are only slightly different from primary spermatocytes. They are somewhat smaller than primary spermatocytes and contain a weakly-stained nucleus.

Spermatids. Occur in cysts and in the interior region of the seminiferous tubules. The nucleus is denser and has more uniform chromatin.

Spermatozoa. The smallest germinative cells, not considering the length of the tail. They occur in the interior region of the seminiferous tubules and sperm duct and have a very basophilic nucleus and large eosinophilic tail.

Stages of sexual maturation

Ovaries and testes were assigned to one of five developmental stages according to macroscopic and histological traits. Macroscopic variations were related to gonadal morphology and histology according to the composition of the oocytes and spematogenic cells.

Immature. Ovaries slender, filiform, whitish and translucent, 30–45 mm long and weighing between 0.01 and 0.1 g. Presence of oocytes in oogonia, nucleolar chromatin and perinucleolar stages. Testes translucent, filiform formed by spermatogonia, not organised in tubules.

Maturing. Ovaries of pale cream colour to whitish-yellow, with subtle granulation visible to the bare eye, weightng 0.9–5.65 g, and measuring 22–48 mm. Presence of oocytes in nucleolar chromatin to cortical alveoli stages. Testes white to pale cream colour, and visible enlargement. Tubules with spermatogonia, spermatocytes and spermatids.

Ripe. Ovaries yellowish, voluminous with blood vessels, 42–65 mm long and weighing 5.8–22 g. Vitellogenic golden-yellow eggs with larger diameter than mature stage. Oocytes at all stages, with diameters ranging from 0.05 to 0.9 mm. Predominance of vitelogenic oocytes (diameter = 1–5.6 mm). Testes highly developed, cream coloured with seminiferous tubules filled with spermatozoa, which accumulate in different ducts next to gonadal wall, from which they will be expelled.

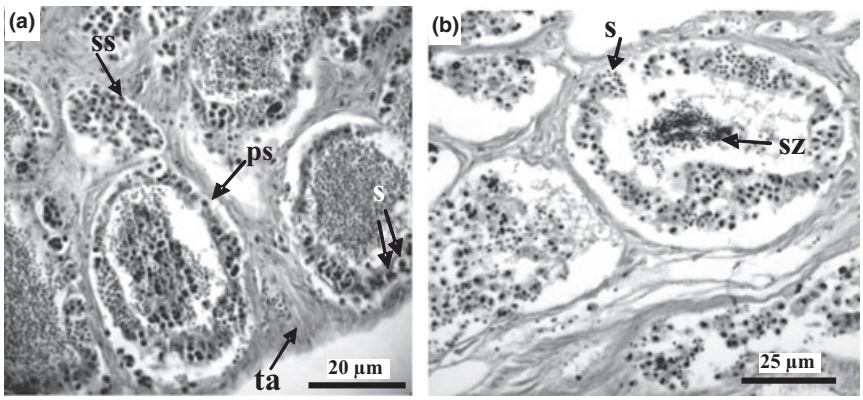

Fig. 3. Histological transversal sections, *Loricariichthys castaneus* testis tissue. (a) Tubules containing stages of spermatogenesis; (b) tubules containing stages of spermatogenesis; *s*, spermatogonia; *ps*, primary spermatocytes; *ss*, secondary spermatocytes; *st*, spermatids; sz, spermatozoa; ta, tunica albuginea

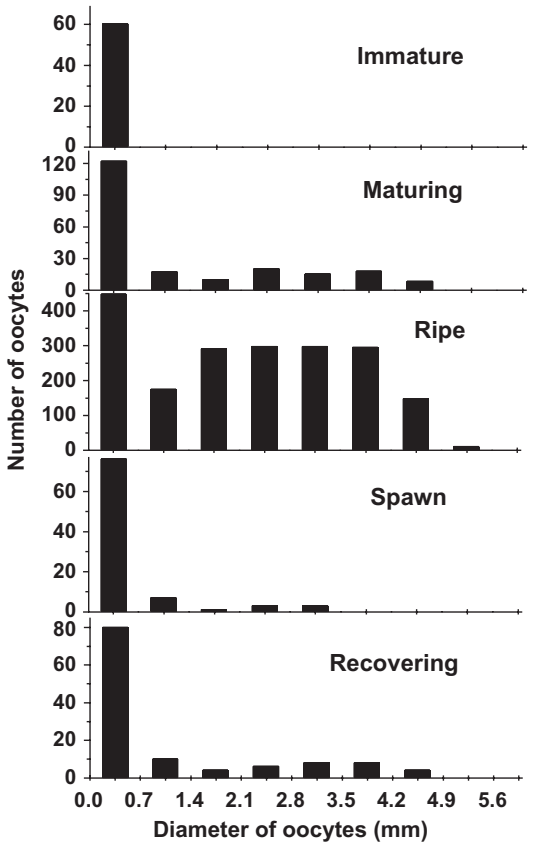

Fig. 4. Oocyte size-frequency distributions through subsequent maturation stages, *Loricariichthys castaneus*

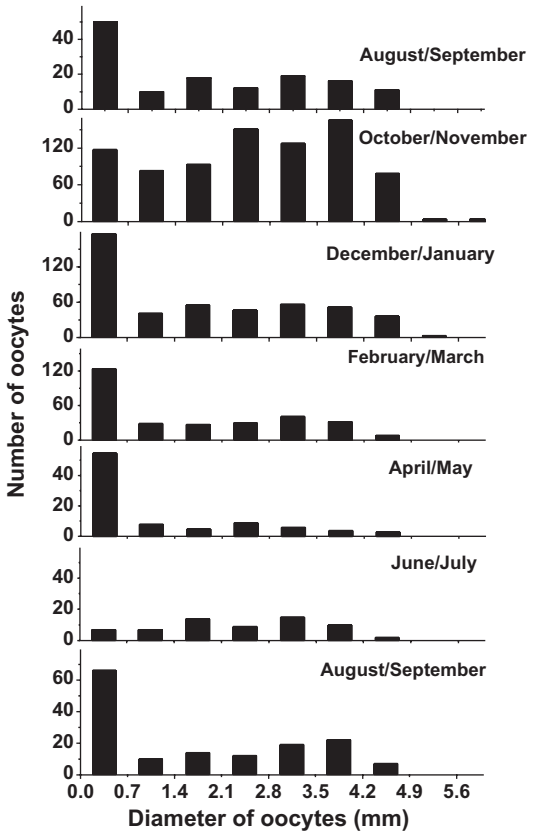

Fig. 5. Temporal oocytes size-frequency distribution, *Loricariichthys castaneus*

Spawn / Spent. Ovaries flaccid, wrinkled, with hemorrhagic appearance, 40–51 mm long, weighing 24–10.6 g. Heterogeneous oocytes, some residual or in degeneration. Presence of atresic and postovulatory follicles. Testes flaccid. Cells appear fused. Higher frequency of primary and secondary spermatocytes.

Recovering. Ovaries of pale cream colour, with enlargement of more than the anterior stage, weighing 2.8–4.07 g, and measuring 42–43 mm. Oocytes from oogonias to perinucleolar stages. Testes pale cream in colour, more consistent. Tubular reorganisation with spermatogonia, spermatocytes and spermatids in formation.

Ovarian development

The size-frequency distributions of *L. castaneus* oocytes show a defined pattern for each stage of maturation (Fig. 4). Reserve oocyte stock had a diameter <0.70 mm and was present in large numbers in all maturation stages; immature stages showed only this type of oocyte. In the maturing stage, oocytes ranged from 0.80 to 4.8 mm in diameter. The ripe stage had oocytes 2.08–5.59 mm in diameter. Spawn stages showed a small number of oocytes with diameters of 0.80–3.0 mm, in addition to a large number of reserve oocytes. The recovering stage had a pattern of oocyte distribution similar to the maturing stage, with diameters between 0.80 and 4.5 mm.

The frequency distribution of changes in oocyte diameter in the study is shown in Fig. 5. A large number of reserve oocytes and vitellogenic oocytes were found in all months, but the latter were more frequent in October–November. From October to November onward there was a decrease in the vitellogenic oocytes, attaining lower numbers in April–May.

Oocyte size distribution

Histological classification of ovaries was based upon different types of 50 oocytes from five fish per month. The oocyte size-frequency distribution was continuous throughout the phases of gonad development. The pre-spawning phase was characterised by previtellogenic oocytes with diameters <210 μm. No hiatus was observed between the previtellogenic and nuclear migration oocytes; a small hiatus was observed between the nuclear migration and hydrated oocytes. When hydration occurred just before ovulation, oocytes outgrew the standing stock of yolked oocytes and a separate mode of mature hydrated oocytes developed (1816–2790 μm). A mode of smaller-size oocytes (630 μm) was present in postovulatory follicles, which ranged from 590 to 730 μm (Fig. 6).

Spawning season

Maximum GSI values for females were recorded in August–September; lowest values were in April–May (Fig. 7). Females had higher GSI than males, but such between-sex differences in GSI decreased in April–May. Significant temporal differences in GSI were found for females, with higher values recorded from August to November compared with April–May (Table 2). On the other hand, males showed no significant differences in GSI among the bi-monthly periods. The highest average temperature (Fig. 8) was recorded in February–March (27°C) and the lowest in June–July (20°C). Rainfall had the

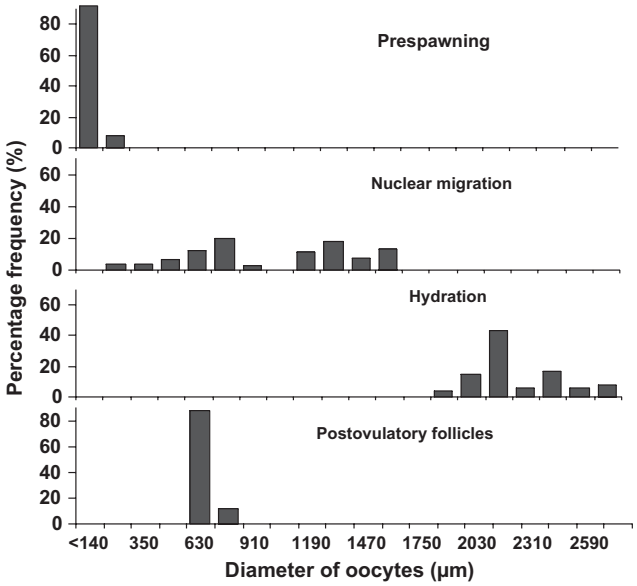

Fig. 6. Oocyte size-frequency distributions through subsequent phase of gonad development, *Loricariichthys castaneus*

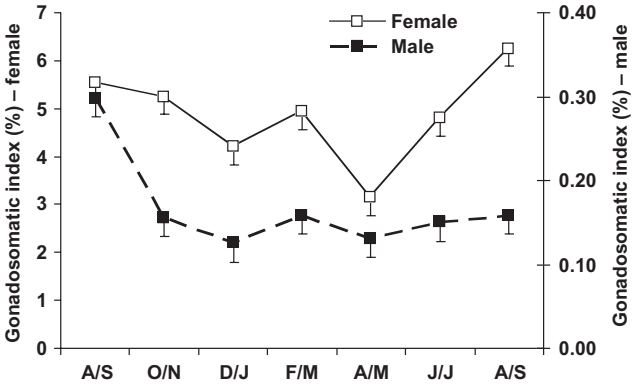

Fig. 7. Bi-monthly changes in gonadosomatic index (means + standard error), female and male *Loricariichthys castaneus*, August 2005–September 2006. A / S, August–September; O / N, October–November; D / J, December–January; F / M, February–March; A / M, April–May; J / J, June–July

Table 2
ANOVA results for temporal comparisons of gonadosomatic index (GSI), hepatosomatic index (HIS) and condition factor (K), male and female *Loricariichthys casteneus*

Parameters	Sex	*F*	P-values	*Post-hoc* comparisons
GSI	Female	4.2	.00049**	August–September; October–November > April–May
	Male	0.75	n.s	–
HSI	Female	3.4	0.035*	August–September > October–March
	Male	0.4	n.s	–
K	Female	1.75	n.s	–
	Male	1.89	n.s	–

n.s., non-significant difference ; *P < 0.05, **P < 0.01.

highest values between December and March and the lowest between June and September. The highest accumulated rainfall was in December–January (350 mm) and the lowest in June–July (50 mm).

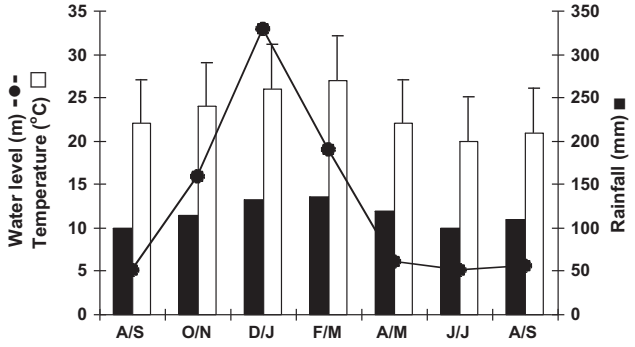

Fig. 8. Bi-monthly changes in water level (400 m + above sea level), surface temperature (°C, means + standard error), and accumulated rainfall (mm), Lajes reservoir, August 2005–September 2006. A / S, August–September; O / N, October–November; D / J, December–January; F / M, February–March; A / M, April–May; J / J, June–July

The percentage of ovaries in the ripe stage was between 56 and 100% in all months, with exception of April–May when only 42% of the oocytes were ripe (Fig. 9a). On the other hand, the percentage of testes in the ripe stage was between 58 and 100% during most periods of the year, with the exception of April to July, when no fish were recorded as ripe (Fig. 9b).

The hepatosomatic index (HSI) showed higher values in August–September, then decreased to the lowest values in February–March, followed by an increase in the subsequent months. Significant differences in HSI were found only for females, with higher values in August–September compared with lower values from October to March (Fig. 10, Table 2).

The values of the condition factor (K) had lower values in June–July. However, no significant differences were found in the study period for either males or females (Fig. 11, Table 2).

A significant correlation was found between GSI and HSI for females only ($r = 0.35$). All other combinations among these three indexes (GSI, HSI and K) were not significant for either sex.

Fecundity

Batch fecundity ranged from 242 to 833 (relative fecundity = 2.27 oocytes g^{-1}), averaging 483 vitellogenic oocytes in the 47 females examined, with a total of 1500 oocytes measured. Fecundity tended to increase linearly with total length, total weight and gonad weight, but the only significant relationship ($r^2 = 0.462$; $P < 0.05$) was found between the batch fecundity and gonad weight. The equations that relate these parameters are shown in Table 3.

Discussion

Sex ratio and parental care

The populations of *L. castaneus* showed an unbalanced sex ratio in Lajes reservoir, with females outnumbering and reaching larger sizes than males. This results are in accordance with the findings of Araújo et al. (1998) and Duarte et al. (2007), who found a predominance of females, especially in the largest size classes of *L. spixii*. Departure from the balanced sex ratio has been attributed to differences between the sex growth rate, behaviour and mortality. The ecological importance of the sex ratio is still not fully explained (Gross, 2005). The male predominance in oligotrophic environments reported by Nikolsky (1963) does not match our information for *L. castaneus* in the Lajes reservoir.

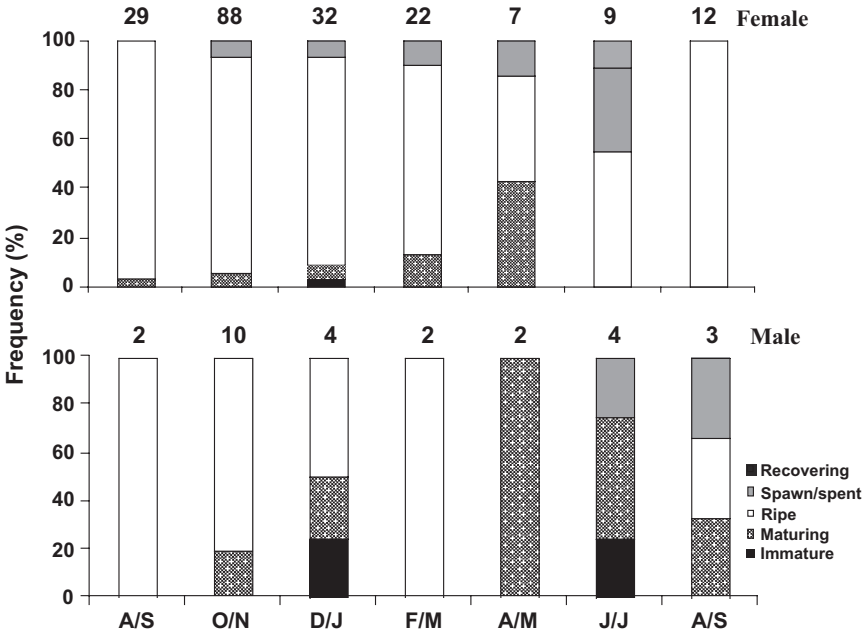

Fig. 9. Bi-monthly frequency distribution, *Loricariichtys castaneus* gonadal maturity stage. Numbers above each bar = sample size

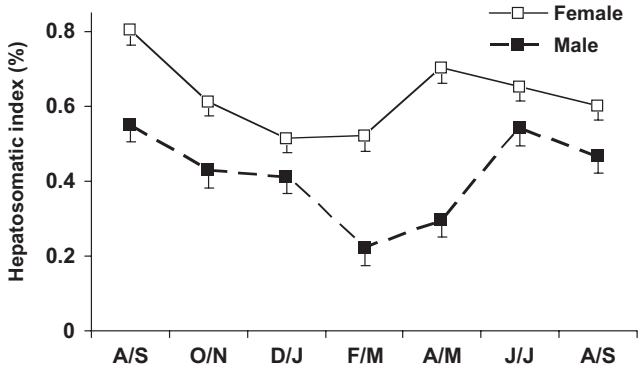

Fig. 10. Bi-monthly changes in hepatosomatic index (means + standard error), female and male *Loricariichthys castaneus*, August 2005–September 2006. A/S, August–September; O/N, October–November; D/J, December–January; F/M, February–March; A/M, April–May; J/J, June–July. Sample size as indicated in Fig. 9

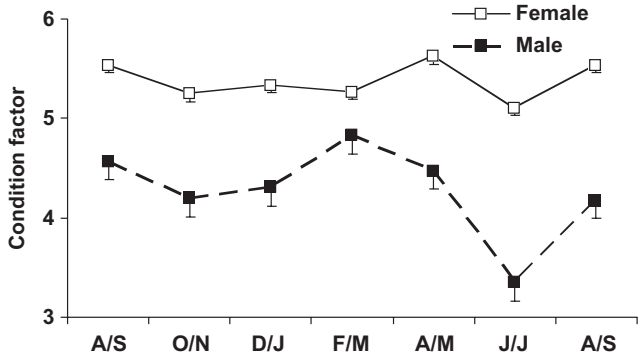

Fig. 11. Bi-monthly changes in condition factor (means + standard error), female and male *Loricariichthys castaneus*, August 2005–September 2006. A/S, August–September; O/N, October–November; D/J, December–January; F/M, February–March; A/M, April–May; J/J, June–July. Sample size as indicated in Fig. 9

Table 3
Regression parameters (*a*, *b*) re potential relationship between batch fecundity (*y*, dependent variable) and parameters of total length (TL), body weight (BW) and gonad weight (GW) (*x*, independent variables), *Loricariichthys castaneus* (n = sample size, r^2 = coefficient of determination)

Relationship	n	Range of *x*	*a*	*b*	r^2
Fecundity vs TL	30	360–402 mm	646.28	3.23	0.11
Fecundity vs BW	30	159–296 g	265.08	1.04	0.04
Fecundity vs GW	30	6–22 g	80.94	37.67	0.46

The smaller male size may in part be a consequence of selection for early male maturation and reproductive effort, which reduces male growth compared to that of females (Endler, 1983; Andersson, 1994). On the other hand, sexual competition favours the large male size in territorial fishes (Hardie et al., 2007), but hardly the case in *L. castaneus*. There is no evident predator of loricariids in the reservoir (Araújo and Santos, 2001), and males probably do not need to provide a physical defence from predators. Therefore, the male growth rate could be lower than in females due to feeding restrictions encountered while egg- and larvae-bearing, explaining why females reach larger sizes than males. Similar to *L. castaneus*, females also reach a larger size and weight in other egg-carrying loricariids, such as *L. platymetopon* (Suzuki et al., 2000) in southern Brazil.

Parental care is developed in *L. castaneus* in males, which carry the eggs while larvae adhere to their ventral surface. Males present a sexual dimorphism by showing elongated labial papillae. Thus far, *Loricariichthys* genera males have been reported to transport egg masses (e.g. Duarte et al., 2007); this is the first record of larvae transport for this species of *Loricariichthys*. In such species, the egg mass has a wedge form and is partially enclosed by the broad flap extending from the lower lip (Taylor, 1983; Machado-Allison, 1990). Males carrying larvae are likely to feature an adaptive advantage, allowing increased juvenile survival under varying environmental conditions. In the Lajes reservoir, changes in water level, dissolved oxygen and other environmental factors may pose constraints to survival (Santos et al., 2004) and could have led to this result in behaviour.

According to Gross and Sargent (1985), the male provides parental care in 61% of the loricariid species where information is available. The parental investment theory predicts that males will be the providers of parental care in fishes (Perrone and Zaret, 1979; Baylis, 1981). Empirical evidence supports this prediction and shows that paternal egg care is the predominant mode of care in fishes (Gross and Sargent, 1985). Furthermore, the prevalence of male parental care in teleost fishes cannot be explained by a greater benefit of the males providing the care, but rather that males pay a smaller future cost of parental care than would females. In fish, it is often the male that provides parental care, not because the male obtains greater benefits from this care, but probably because he pays fewer costs; however, this is a matter of debate.

Loricariichthys castaneus has a very thick zona radiata (10–20 μm) when compared to other Siluriformes. As reported by Suzuki et al. (2000), the eggs of *Loricariichtys* are exposed to abrasion, and this species has the thickest zona radiata (e.g. *L. platymetopon*, mean = 9.87 μm). However, other loricariids that deposit their eggs in bottom-excavated nests have a thin zona radiata, such as *Hypostomus ternetzi* (Boulenger, 1895) (mean zona radiata = 3.97 μm) and *Megalancistrus aculeatus* (Perugia, 1891) (mean zona radiata = 4.8 μm). Parental care is a reproductive tactic developed by fishes in systems that have changes in limnological conditions and water levels, such as reservoirs. This is an efficient adaptation to overcome competition for suitable bottom crevices for nest construction (Moodie and Power, 1982), reducing the impact of turbidity and low dissolved oxygen.

Gonadal development

The reasons for such differences in oocyte size between species of *Loricariichthys* are unknown, but are possibly due to inherent interspecific differences or selection to different types of systems, since these species come from different environments (lentic for *L. castaneus* and lotic for *L. platymetopon* and *Loricariichthys* sp.). Environmental stress in reservoirs, such as oxygen deficiencies (Potts, 1984), creates harder living conditions than in rivers, where oxygen levels are more stable. However, male care could provide sufficient ventilation to rapidly and continuously exchange the water interface at the egg surface and thereby assist in overcoming low oxygen periods in the water.

According to the classification by Wallace and Selman (1981), *L. castaneus* show 'asynchronous ovaries', where oocytes of all stages are present without dominant populations. The different stages are a consequence of a continuous development process, since the cellular events of oocyte growth do not sequentially replace one another, but rather are initiated sequentially and remain active throughout oocyte development. The size of spawned eggs seems, to a large degree, to be decided during the very late oocyte development in the spawning season. According to Wallace and Selman (1985), up to one-third of the vitellogenic oocytes is included in the group of those to be released during the final maturation in the 2–3 days before the release of a batch. *L. castaneus* also undergoes this process during the spawning season, since hydrated oocytes almost overlap in size with nuclear migration. This species probably has the ability to continually develop unyolked oocytes and add them to the standing stock of advance-yolked oocytes even after the spawning season begins. It can be concluded that *L. castaneus* is a batch spawner with a continuous oocyte size.

Fecundity

Batch fecundity is developed in *L. castaneus*, as indicated by oocytes in different stages of development plus postovulatory follicles in a single ovary with an average of 483 oocytes. This number is very close to the batch fecundity found by Duarte and Araújo (2000) for *L. spixii* (average fecundity = 367 oocytes) caught in the Lajes reservoir between 1994 and 1997. Batch spawning can be seen as a strategy to release eggs over a long period of time, increasing the survival probability of offspring (Lambert and Ware, 1984). Furthermore, Murua and Saborido-Rey (2003) reported that batch spawning increases fecundity more than total spawning. Batch spawning for *L. castaneus* follows other patterns similar to *Loricariichtys* in other areas (Lopes et al., 2000; Suzuki et al., 2000), a probable strategy to increase fecundity in this lightly structured and changeable impounded system.

The continuous oocyte size-frequency distribution in spawning females throughout the year is typical of indeterminate fecundity species, as the recruitment of oocytes continues during the spawning season (West, 1990). Moreover, the decrease in mean diameter of advanced yolked oocytes throughout the spawning season is also considered as evidence for indeterminate fecundity. Such a decrease is due to the recruitment of newly formed small yolked oocytes into the stock of advanced vitellogenic oocytes. In the present study a larger number of hydrated oocytes was recorded in October–November, and a decrease in such large hydrated oocytes was recorded in December to March. This finding suggests that this species has indeterminate fecundity.

Spawning season

Seasonal variations in gonadal stage frequency and GSI bi-monthly changes indicate a broad spawning season, ranging from June to March. The macroscopic and histological data on the percentage of mature specimens, together with mean oocyte diameter and GSI, revealed that spawning mainly occurs August–September, coinciding with the low water level and rainfall; in this period there is a trend of increasing water temperature. In Lajes reservoir, the pause in spawning in April–May corresponds to the decreasing water level and temperature that occurs after the rainy season. *Loricariichthys platymetopon* showed peaks of spawning from September to January in Capivara reservoir (Marcucci et al., 2005), another large reservoir in southeast Brazil. Since information is only available on the spawning season of this species of *Loricariichtys*, we conclude that this species has a wide period of spawning encompassing spring and summer.

The GSI was significantly correlated to HSI for females only (r = 0.35). The HSI has been reported as related to the storage of reserves and the mobilisation of energetic reserves for vitellogenesis and the reproduction process. In *L. platymetopon* from the Uruguai River in southern Brazil, HSI was associated with the storage of winter reserves (Querol et al., 2002). In the *L. castaneus* females, the highest HSI was reported in August / September, the peak of the reproductive season, and the lowest in February-March, suggesting a weak correlation between these two indices.

The GSI and K peaks indicate an allocation of energy to somatic growth and reproduction (Gomes and Araújo, 2004). The condition factor for *L. castaneus* was at its lowest values in June–July, coinciding with high HSI and an increasing GSI. The worst condition occurred just after the reproductive paucity, when the fishes were at the beginning of another

spawning season. During this period (June–July) males were recording to be carrying larvae. Seasonal changes in conditions, with minimum values measured just after spawning, are often observed in fish species (Bengtsson, 1993). Higher GSI and K in the peak reproductive period suggest that the species is physiologically well prepared for reproduction during the spawning season, as with *L. castaneus* in Lajes reservoir. A non-significant correlation between K, GSI and HSI, in most cases, is evidence that the vitellogenesis and spermatogenesis are not directly related to the depletion of hepatic reserves or to the decrease in condition factor. These findings are in accordance with Chellappa et al. (1995), who reported the condition factor (K) and hepatosomatic index (HSI) as poor predictors of energy reserves in fish.

Loricariichthys castaneus uses the equilibrium strategy to be successful in Lajes reservoir. According to Winemiller (1989), the so-called equilibrium strategy is associated with sedentary local populations, relatively stable adult food resources, prolonged breeding seasons, and parental investment in individual offspring, which probably results in enhanced juvenile survivorship and reduced fluctuations in local population density. These features match the *L. castaneus* life history in Lajes reservoir, a relatively closed system, with a long residence time, little influence from large rivers, and few predators. Only the top piscivorous species, the characid *Hoplias malabaricus* (Bloch, 1794) and the cichlid *Cichla kelberi* Kullander and Ferreira, 2006, prey on fish species other than *L. castaneus* (Araújo and Santos, 2001). Therefore, this equilibrium strategy can be interpreted as being adaptive with respect to variation in abiotic environmental parameters and food availability, as no predation pressure seems to occur in Lajes reservoir on *L. castaneus*, which probably completes all life cycles in the reservoir, dwelling in shallow unconsolidated bottom areas; most other Siluriformes species use consolidated substrate in rocky or riparian areas in the littoral zone. *L. castaneus* is the most abundant species in the reservoir (Santos et al., 2004), a system that, although having broad seasonal water level fluctuations, seems to offer suitable environmental conditions to which this species has successfully adapted.

Acknowledgements

We thank all the technicians and undergraduate students from the Laboratory of Fish Ecology, University Federal Rural of Rio de Janeiro, for helping in field and laboratory work. We especially thank Dr. Luis Antonio Braga Grande, for encouragement and support with the research. We are also indebted to the Histological Techniques Laboratory staff, and, in particular, Thatiana Paz Ribeiro and Aparecida Alves Nascimento, for helping in histological techniques. This study was financed by the LIGHT Electricity S.A., concessionaire of the Lajes reservoir, through the Programme of Research and Development (P & D) Process number 4500179742.

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Author's address: Francisco G. Araújo, Laboratório de Ecologia de Peixes, Universidade Federal Rural do Rio de Janeiro, Km 47, antiga Rodovia Rio – SP, 23.851-970 Seropédica, RJ, Brazil.
E-mail: gerson@ufrrj.br